

# **PHARMACOKINETICS AND SAFETY OF FIRST- AND SECOND-LINE ANTI-TUBERCULOSIS DRUGS IN CHILDREN**

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## Summary

The global burden of tuberculosis (TB) in children is high with a high morbidity and mortality, especially amongst young and HIV-infected children. The emerging epidemic of multidrug-resistant (MDR)-TB is a threat to children, while information on the use of second-line drugs in children is very limited.

By reviewing the literature on the first-line anti-tuberculosis agents it is shown that isoniazid (INH) and rifampicin (RMP) exhibit a dose-dependent activity against *Mycobacterium tuberculosis*. For effective anti-tuberculosis therapy, 2-hour serum concentrations of INH 3-5 $\mu$ g/ml, RMP 8-24 $\mu$ g/ml and pyrazinamide (PZA) >35 $\mu$ g/ml have been proposed. Although not optimal, the major tools at hand to determine desired serum concentrations of an anti-tuberculosis drug in children are comparative clinical data from adults and their pharmacokinetic “optimal” target values. In order to achieve serum concentrations in children comparable to those in adults and which are correlated with efficacy, the existing evidence advocates the use of higher mg/kg body weight doses of INH and RMP in younger children compared to adults. For PZA, similar mg/kg body weight doses lead to PZA maximum concentrations ( $C_{max}$ ) similar to those in adults. In 2009, the World Health Organization (WHO) increased their dosing recommendations and now advises giving INH at 10 mg/kg (range: 7-15 mg/kg), RMP 15 mg/kg (10-20 mg/kg) and PZA 35 mg/kg (30-40 mg/kg). Studies of the pharmacokinetics of the first-line agents in representative cohorts of children especially in younger ages and with different genetic backgrounds are limited; these needed to better define the doses appropriate for children.

I performed a pharmacokinetic study on the first-line agents INH, RMP and PZA in 20 children <2 years of age (mean age 1.09 years), following the previous and revised WHO dosing recommendations. Mean (95% confidence interval)  $C_{max}$ s [ $\mu$ g/ml], following previous/revised doses, were: INH 3.2 (2.4-4.0)/8.1 (6.7-9.5) $\mu$ g/ml, PZA 30.0 (26.2-33.7)/47.1 (42.6-51.6) $\mu$ g/ml, and RMP 6.4 (4.4-8.3)/11.7 (8.7-14.7) $\mu$ g/ml. The mean (95% confidence interval) area under the time-concentration curves (AUC) [ $\mu$ g·h/ml] were: INH 8.1 (5.8-10.4)/20.4 (15.8-25.0) $\mu$ g·h/ml, PZA 118.0 (101.3-134.7)/175.2 (155.5-195.0) $\mu$ g·h/ml, and RMP 17.8 (12.8-22.8)/36.9 (27.6-46.3) $\mu$ g·h/ml. This study

provides the first evidence for the implementation of the revised WHO guidelines for first-line anti-tuberculosis therapy in children younger than two years of age.

Because drug-resistant TB is increasing globally, pharmacokinetic studies to guide dosing and safe use of the second-line agents in children have become a matter of urgency. In this thesis, priority is given to the thioamides (ethionamide [ETH] and prothionamide [PTH]) and the 3 most frequently used fluoroquinolones, ofloxacin (OFX), levofloxacin (LFX) and moxifloxacin (MFX).

By reviewing the literature, I have demonstrated that ETH has shown to be effective in *in vitro* studies against *M. tuberculosis* and in combination with other drugs had good outcome in MDR-TB and tuberculous meningitis patients, including children. ETH/PTH exhibit dose-dependent activity and are bactericidal at higher doses, although dosing is limited mainly by gastro-intestinal adverse effects. During long-term ETH/PTH therapy hypothyroidism might also occur. An oral daily dose of ETH or PTH of 15-20mg/kg with a maximum daily dose of 1,000mg is recommended in children. No child-friendly formulations of the thioamides exist. Studies on dosing and toxicity of ETH and PTH in childhood TB are needed.

With the first study ever conducted on the pharmacokinetics of ETH in 31 children (mean age 4.25 years), supportive evidence for the current dosing recommendation of ETH 15-20mg/kg in children with TB is provided. Mean  $C_{max}$  was 4.14 $\mu$ g/ml (range 1.48 – 6.99 $\mu$ g/ml) and was reached within two hours (mean  $t_{max}$  1.29h, range 0.87 – 2.97h). Young children and HIV-infected children were at risk for lower ETH serum concentrations, but the mean drug exposure was still within range of the adult  $C_{max}$  reference target (2.5 $\mu$ g/ml).

In a retrospective study on 137 children (median age 2.9 years) receiving anti-tuberculosis therapy including ETH, abnormal thyroid function tests were recorded in 79 (58%) children. The risk for biochemical hypothyroidism was higher for children on regimens including *para*-aminosalicylic acid (PAS) and in HIV-infected children. This high frequency of thyroid function abnormalities in children treated with ETH indicates the need for careful thyroid function test monitoring in children on long-term ETH treatment, especially in case of HIV co-infection and concomitant use of PAS.

The literature review on the use of fluoroquinolones in childhood TB revealed that the strong bactericidal and sterilizing activity, favourable pharmacokinetics, and toxicity profile have made the fluoroquinolones the most important component of existing MDR-TB treatment regimens, not only in adults, but also in children. Proposed pharmacodynamic targets for fluoroquinolones against *Mycobacterium tuberculosis* are  $AUC_{0-24}/MIC >100$  or  $C_{max}/MIC$  8-10. *In vitro* and murine studies demonstrated the potential of MFX to shorten drug-susceptible TB treatment, but in multiple randomized controlled trials in adults, shortened fluoroquinolone-containing regimens have found to be inferior compared to standard therapy. Resistance occurs frequently via mutations in the *gyrA* gene, and emerges rapidly depending on the fluoroquinolone concentration. Fluoroquinolone resistance occurs in 4-30% in MDR-TB strains depending on the region/country and setting.

Emerging data from paediatric studies underlines the importance of fluoroquinolones in the treatment of MDR-TB in children. There is a paucity of pharmacokinetic data especially in children <5 years of age and HIV-infected children. Fluoroquinolone use has historically been restricted in children due to concerns about drug-induced arthropathy. The available data however does not demonstrate any serious arthropathy or other severe toxicity in children.

In order to fill the gap in knowledge on fluoroquinolone dosing in children with TB, prospective, intensive-sampling pharmacokinetic studies on OFX, LFX, and MFX including assessment of cardiac effects were conducted.

In the study on the pharmacokinetics of OFX and LFX, 23 children (median age 3.14 years) were enrolled; 4 were HIV-infected (all > 6 years of age) and 6 were underweight-for-age (z-score <-2). The median  $C_{max}$  [ $\mu\text{g}/\text{ml}$ ], median  $AUC_{(0-8)}$  [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ] and mean  $t_{max}$  [h] for OFX were: 9.67 (IQR 7.09-10.90), 43.34 (IQR 36.73-54.46) and 1.61 (SD 0.72); for LFX: 6.71 (IQR 4.69-8.06), 29.89 (IQR 23.81-36.39) and 1.44 (SD 0.51), respectively. Children in this study eliminated OFX and LFX more rapidly than adults, and failed to achieve the proposed adult pharmacodynamic target of an  $AUC_{0-24}/MIC >100$ . Nevertheless, the estimated pharmacodynamic indices favoured LFX over OFX. The mean corrected QT (QTc) was 361,4ms (SD 37,4) for OFX and 369,1ms (SD 21.9) for LFX, respectively and no QTc prolongation occurred.

In the study on MFX, 23 children (median age 11.1 years) were included; 6/23 (26.1%) were HIV-infected. The median (IQR)  $C_{max}$  [ $\mu\text{g}/\text{ml}$ ],  $AUC_{(0-8)}$  [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ],  $t_{max}$  [h] and half-life for MFX were: 3.08 (2.85-3.82), 17.24 (14.47-21.99), 2.0 (1.0-8.0); and 4.14 (IQR 3.45-6.11), respectively.  $AUC_{0-8}$  was reduced by  $6.85\mu\text{g}\cdot\text{h}/\text{ml}$  (95% CI 11.15-2.56) in HIV-infected children.  $t_{max}$  was shorter with crushed versus whole tablets ( $p=0.047$ ). In conclusion, children 7-15 years of age have low serum concentration compared with adults receiving 400mg MFX daily. MFX was well tolerated in children treated for MDR-TB. The mean corrected QT-interval was 403ms (SD 30ms) and as for OFX and LFX, no prolongation  $>450\text{ms}$  occurred.

In conclusion, my research identified and addressed critical gaps in the current knowledge in the management of children with both drug-susceptible and drug-resistant TB. I provided essential evidence on both the dosing and safety of first- and second-line anti-tuberculosis agents, informing international treatment guidelines for childhood TB. Nevertheless, more studies in a larger number of children with different genetic backgrounds, HIV co-infection nutritional status and with higher drug doses, novel treatment regimens and child-friendly formulations are needed to further optimize anti-tuberculosis treatment in children.

## Opsomming

Die globale lading van tuberkulose (TB) in kinders is hoog, met 'n hoë TB-verwante morbiditeit en mortaliteit, veral onder jong en MIV-geïnfekteerde kinders. Die toenemende epidemie van multimiddel-weerstandige (MMW)-TB hou 'n bedreiging in vir kinders, terwyl inligting oor die gebruik van tweede-linie middels in kinders tans baie beperk is.

Deur middel van 'n oorsig van die literatuur oor eerste-linie antituberkulose middels is aangetoon dat isoniasied (INH) en rifampisien (RMP) 'n dosisverwante aksie teen *Mycobacterium tuberculosis* uitoefen. Vir effektiewe TB behandeling is 2-uur serumkonsentrasies van INH 3-5µg/ml, RMP 8-24µg/ml en pirasienamied (PZA) van >35µg/ml voorgestel. Alhoewel nie optimaal nie, is die voor-die-hand-liggende manier om die verlangde serumkonsentrasies van 'n antituberkulose middel in kinders te bepaal die vergelykbare kliniese data in volwassenes en hulle farmakokinetiese "optimale" teikenwaardes. Om serumkonsentrasies in kinders gelykstaande aan dié in volwassenes en met ooreenstemmende effektiwiteit te bereik, toon die beskikbare data dat hoër mg/kg liggaamsmassa dosisse vir INH en RMP in jong kinders in vergelyking met volwasse dosisse gegee behoort te word. Met PZA sal soortgelyke mg/kg dosisse per liggaamsmassa in kinders lei tot soortgelyke maksimum konsentrasies ( $C_{max}$ ) in volwassenes. In 2009 het die Wêreld Gesondheidsorganisasie (WGO) hulle dosis-aanbevelings verhoog, en tans beveel die WGO INH teen 10mg/k (reikwydte 7-15 mg/kg), RMP 15mg/kg (10-20 mg/kg) en PZA teen 35mg/kg (30-40 mg/kg) aan in kinders. Studies oor die farmakokinetika van die eerste-linie antituberkulose middels in verteenwoordigende groepe van kinders, veral in die jonger ouderdomsgroepe en met verskillende genetiese agtergronde is beperk; sulke studies word dringend benodig om toepaslike dosisse vir kinders met TB beter te definieer.

Ek het 'n farmakokinetiese studie van die eerste-linie middels INH, RMP en PZA in 20 kinders <2 jaar oud (gemiddelde ouderdom 1.09 jaar) volgens die vorige en huidige WGO doseringsriglyne uitgevoer. Die gemiddelde (95% vertrouwe interval)  $C_{max}$  [µg/ml] volgens vorige/huidige doseringsriglyne was: INH 3.2 (2.4-4.0)/8.1 (6.7-9.5)µg/ml, PZA 30.0 (26.2-33.7)/47.1 (42.6-51.6)µg/ml, and RMP 6.4 (4.4-8.3)/11.7 (8.7-14.7)µg/ml. Die gemiddelde (95% vertrouwe interval) oppervlakte onder die tyd-konsentrasie kromme (AUC) [µg·h/ml] was: INH 8.1 (5.8-10.4)/20.4 (15.8-25.0)µg·h/ml, PZA 118.0



(101.3-134.7)/175.2 (155.5-195.0) $\mu\text{g}\cdot\text{h}/\text{ml}$ , and RMP 17.8 (12.8-22.8)/36.9 (27.6-46.3) $\mu\text{g}\cdot\text{h}/\text{ml}$ . Hierdie studie voorsien die eerste bewyse vir die toepassing van die hersiene WGO-riglyne vir eerste-linie antituberkulose behandeling in kinders jonger as twee jaar oud.

Omdat middelweerstandige TB wêreldwyd aan die toeneem is, het studies oor die farmakokinetika en veiligheid van die gebruik van tweede-linie middels in kinders 'dringend nodig geword. In hierdie verhandeling word voorkeur gegee aan die tioamiede (etionamied [ETH] en protionamied [PTH]) en die drie mees algemeen gebruikte fluorokwinolone, ofloksasien [OFX], levofloksasien [LFX] en moksifloksasien [MFX].

Deur 'n oorsig van die literatuur het ek aangetoon dat ETH in *in vitro* studies teen *M. tuberculosis* effektief is en in kombinasie met ander middels goeie uitkomst in MMW-TB en tuberkulose meningitis, insluitend kinders, het. ETH/PTH toon dosisverwante aktiwiteit en is bakteriedodend teen hoër dosisse, alhoewel dosering hoofsaaklik deur gastrointestinale newe-effekte beperk word. Tydens langtermyn behandeling met ETH/PTH kan hipotireose ook voorkom. 'n Daaglikse mondelingse dosis van ETH of PTH van 15-20mg/kg met 'n maksimum daaglikse dosis van 1,000 mg word vir kinders aanbeveel. Daar bestaan tans geen kindervriendelike formulering vir die tioamiedes nie.

Met die eerste studie ooit wat handel oor die farmakokinetika van ETH in 31 kinders (gemiddelde ouderdom 4.25 jaar), verleen ek ondersteunende bewys vir die huidige aanbevole dosis van ETH van 15-20mg/kg in kinders met TB. Die gemiddelde ETH  $C_{\text{max}}$  was 4.14 $\mu\text{g}/\text{ml}$  (reikwydte 1.48-6.99 $\mu\text{g}/\text{ml}$ ) en hierdie konsentrasie was binne twee ure (gemiddelde  $t_{\text{max}}$  1.29h, reikwydte 0.87-2.97h) bereik. Jong en MIV-geïnfekteerde kinders het geneig om laer ETH konsentrasies te toon, maar die gemiddelde middelblootstelling was steeds binne die reikwydte van die volwasse  $C_{\text{max}}$  teiken (2.5 $\mu\text{g}/\text{ml}$ ).

In 'n retrospektiewe studie van 137 kinders (gemiddelde ouderdom 2.9 jaar) wat antituberkulose behandeling insluitende ETH ontvang het, is abnormale tiroïedfunksietoetse in 79 (58%) kinders gedokumenteer. Die risiko vir biochemiese hipotireose was hoër in kinders op behandeling wat para-aminosalisielsuur (PAS) ingesluit het, asook in MIV-geïnfekteerde kinders. Hierdie hoë voorkoms van

tiroïedfunksie abnormaliteite in kinders wat ETH behandel ontvang het, dui op die belang van die versigtige monitering van tiroïedfunksietoetse in kinders op langtermyn ETH behandeling, veral in die geval van MIV ko-infeksie en met meegaande gebruik van PAS.

Die literatuuroorsig oor die gebruik van fluorokwinolone in kindertuberkulose het dit duidelik gemaak dat die sterk bakteriedodende effek, gunstige farmakokinetika en toksisiteitsprofiel die fluorokwinolone die belangrikste deel van die huidige MMW-TB behandeling gemaak het, nie alleen in volwassenes nie, maar ook in kinders. Voorgestelde farmakodinamiese teikens vir die fluorokwinolone teen *M. tuberculosis* is  $AUC_{0-24}/MIC >100$  of  $C_{max}/MIC$  8-10. *In vitro* en muisstudies het die potensiaal van MFX om die behandeling van middelsensitiewe TB te verkort, aangetoon, maar in veelvuldige ewekansig-gekontroleerde studies in volwassenes het verkorte fluorokwinoloon-bevattende regimens egter geblyk om minderwaardig te wees in vergelyking met huidige standaardbehandeling. Weerstandigheid kom dikwels via mutasies in die *gyrA*-gene voor en kom vining na vore afhangend van die fluorokwinoloonkonsentrasie. Fluorokwinoloon-weerstandigheid kom voor in 4-30% van MMW-TB stamme, afhangend van die konteks en streek.

Data van kinders wat na vore kom versterk die belang van die fluorokwinolone in die behandeling van kindertuberkulose. Daar is veral 'n tekort aan farmakokinetiese data in kinders <5 jaar oud en in MIV-geïnfekteerde kinders. Die gebruik van fluorokwinolone in kinders is geskiedkundig beperk as gevolg van besorgdheid oor middel-geïnduseerde gewrigsaantasting. Die beskikbare inligting dui egter nie op enige erge gewrigsaantasting of enige ander erge toksisiteit in kinders nie.

Ten einde die gaping in kennis oor die dosering van fluorokwinolone in kinders met TB te vul, is 'n prospektiewe, intensiewe-monsterneming farmakokinetiese studies oor OFX, LFX en MFX, insluitend evaluering van kardiotoxisiese effekte, uitgevoer.

In die studie oor die farmakokinetika van OFX en LFX is 23 kinders (mediane ouderdom 3.14 jaar) ingesluit; 4 was MIV-geïnfekteer (almal >6 jaar oud) en 6 was ondergewig-vir-ouderdom (z-telling <-2). Die mediane  $C_{max}$  [ $\mu\text{g}/\text{ml}$ ], mediane  $AUC_{0-8}$  [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ] en gemiddelde  $t_{max}$  [h] vir OFX was: 9.67 (interkwartielreikwydte IKR 4.69-8.06), 43.34 (IKR 36.73-54.46) en 1.61 (SD 0.72); vir LFX: 6.71 (IKR 4.69-8.06), 29.89 (IKR 23.81-36.39) en 1.44 (SD 0.51), onderskeidelik. Kinders in hierdie studie het OFX en LFX

vinniger as volwassenes uigeskei en het nie daarin geslaag om voorgestelde volwasse farmakodinamiese teikens van  $AUC_{0-24}/MIC >100$  te behaal nie. Nogatans was die berekende farmakodinamiese indekse ten gunste van LFX bo OFX. Die gemiddelde gekorrigeerde QT-interval (QTc) was 361.4ms (SD 37.4) vir OFX en 369.1ms (SD 21.9) vir LFX, onderskeidelik, en geen verlenging van QTc-interval het voorgekom nie.

In die studie oor MFX was 23 kinders (mediane ouderdom 11.1 jaar) ingesluit; 6/23 (26.1%) was MIV-geïnfekteerd. Die mediane (IKR)  $C_{max}$  [ $\mu\text{g}/\text{ml}$ ],  $AUC_{0-8}$  [ $\mu\text{g}\cdot\text{h}/\text{ml}$ ],  $t_{max}$  [h] en half-lewe van MFX was: 3.08 (2.85-3.82), 17.24 (14.47-21.99), 2.0 (1.0-8.0) en 4.14 (3.45-6.11), onderskeidelik. Die  $AUC_{0-8}$  was met 6.85 $\mu\text{g}\cdot\text{h}/\text{ml}$  (95% vertrouensinterval 11.15-2.56) verminder in MIV-geïnfekteerde kinders. Die  $t_{max}$  was korter met fyngemaakte teenoor heel tablette ( $p=0.047$ ). Ter samevatting, kinders 7-15 jaar oud het lae serumkonsentrasies in vergelyking met volwassenes wat 400mg MFX per dag ontvang, getoon. MFX was goed verdra in kinders met MMW-TB. Die gemiddelde QTc-interval was 403ms (SD 30ms). Soos in die geval van OFX en LFX, het geen verlenging  $>450\text{ms}$  voorgekom nie.

Ter samevatting spreek my navorsing kritiese gapings in die huidige kennis oor die hantering van kinders met middelsensitiewe en middelweerstandige TB aan.. Ek verskaf belangrike bewyse oor beide die dosering en veiligheid van eerste- en tweede-linie antituberkulose middels, wat internasionale behandelingsriglyne vir kindertuberkulose toegelig het. Nogatans is verdere studies met groter getalle kinders uit verskillende genetiese agtergronde, MIV ko-infeksie, voedingstatus, en met hoër doserings van antituberkulose middels, nuwe behandelingsregimens en kindervriendelike formulerings nodig om die behandeling van tuberkulose in kinders verder te verbeter.

## **Dedication**

I dedicate this research to my esteemed colleague and friend, Dr. Klaus Magdorf, *in memoriam*.

I also dedicate this work to my family, and would like to thank them deeply for their support and understanding.

## List of abbreviations

AFB	Acid-fast bacilli
ANOVA	Analysis of variance
ART	Antiretroviral therapy
AUC	Area under the time-concentration curve
BHCD	Brooklyn Hospital for Chest Diseases
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
C <sub>max</sub>	Maximum serum concentration
CNS	Central nervous system
CSF	Cerebrospinal fluid
CYP450	Cytochrome P450
DR	Drug-resistant
DS	Drug-susceptible
DST	Drug susceptibility testing
EBA	Early bactericidal activity
ECG	Electrocardiogram
EMB	Ethambutol
ETH	Ethionamide
FMO	Flavin-containing monooxygenase
h	Hour
HIV	Human immunodeficiency virus (type 1)
HPLC	High performance liquid chromatography
HPLC/MS	High performance liquid chromatography/mass spectrometry
INH	isoniazid
IPT	Isoniazid preventive therapy
IQR	Interquartile range
k <sub>e</sub>	Elimination coefficient
LFX	Levofloxacin
<i>M. tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
MBC	Minimum bactericidal concentration
MDR	Multidrug-resistant
MXF	Moxifloxacin
MIC	Minimum inhibitory concentration
NAT2	N-acetyltransferase 2
NCA	Noncompartmental analysis
NTP	National tuberculosis program
OFX	Ofloxacin
PAS	<i>Para</i> -amino salicylic acid
PD	Pharmacodynamic
PK	Pharmacokinetic
PTH	Prothionamide
PZA	Pyrazinamide
RMP	Rifampicin
SD	Standard deviation
SM	Streptomycin
t <sub>1/2</sub>	Half-life
TB	Tuberculosis
TBH	Tygerberg Hospital
TBM	Tuberculous meningitis
t <sub>max</sub>	Time to C <sub>max</sub>
WHO	World Health Organization
XDR	Extensively drug-resistant

## Chapter 1

### Introduction

#### Burden of childhood tuberculosis

Tuberculosis (TB) remains a major global health problem, particularly in the developing countries of sub-Saharan Africa and Asia. The World Health Organization (WHO) estimated that in 2013 there were 550,000 new cases of TB in children <15 years of age and 80,000 deaths from TB in HIV-negative children (1). Children account for up to 15-20% of TB cases in high-burden countries and this proportion may reach 40% in some communities (2). South Africa has one of the highest TB notification rates worldwide with 328,896 cases registered in 2013 and an estimated notification rate of 860 per 100,000 (1). WHO estimates for paediatric TB likely underestimate the true burden of childhood TB due to diagnostic challenges and poor recording and reporting of TB in children (3, 4). Using a mathematical model based on the 22 WHO-classified high-burden TB countries in 2010, Dodd et al. estimated that approximately 7.6 million children became infected with *Mycobacterium tuberculosis* and that 650,000 children developed TB disease with an estimated case detection rate of only 35% in these countries (5). In the Western Cape Province, South Africa, childhood TB (0-14 years of age) contributed to approximately 14% of the total disease burden in 2004, rising to 17.3% in 2008, with an annual notification rate of 407 versus 620 childhood TB cases per 100,000 respectively (6) (unpublished Data, Western Cape Department of Health) with emerging drug-resistant (DR)-TB amongst children as an important additional challenge.

The global threat of TB is further aggravated by the spread of DR-TB. Multidrug-resistant (MDR)-TB is defined as *M. tuberculosis* resistant to at least the first-line drugs isoniazid (INH) and rifampicin (RMP), while extensively drug-resistant (XDR)-TB involves additional resistance to any fluoroquinolone and any of the second-line anti-tuberculosis injectable drugs. WHO estimated that 480,000 patients developed MDR-TB in 2013, with only about 20% (97,000) of cases receiving appropriate treatment (1). Failure to treat infectious (adult) MDR-TB cases facilitates ongoing transmission and exposes vulnerable young children to infection with MDR-TB strains (7). In contrast to

adults, in whom drug resistance results from both acquisition and transmission, children with MDR-TB usually have transmitted (primary) resistance, as it is more difficult for children to acquire drug resistance due to the paucibacillary nature of TB disease in children. In addition, the practical challenges of obtaining respiratory specimens from young children add to the typical low bacteriologic (culture/molecular tests) yield achieved in children with pulmonary TB of 20-40% (8). Without bacteriological confirmation, drug susceptibility testing (DST) cannot be performed and confirmed MDR-TB is therefore infrequent in children (9). Model-based estimates suggest that 32,000 children had MDR-TB in 2010 (3). New molecular diagnostic tools, such as the Xpert MTB/RIF may increase the number of MDR-TB cases detected in adults and children, increasing the number of children needing MDR-TB treatment. In Southern Africa, the spread of MDR-TB and outbreaks of XDR-TB have caused considerable concern and drug resistance is now also a significant problem amongst children. In a recent surveillance study of children 0-13 years with culture-confirmed TB from Cape Town, MDR-TB was found in 7.1% of all patients; 21.9% of the children were also HIV-infected (10).

Despite available treatment, TB is amongst the 10 major causes of childhood mortality in developing countries as young children have an increased risk of severe, rapidly progressive forms of TB, a tendency exacerbated by the epidemic spread of HIV infection (6, 11, 12).

Without preventive therapy intervention, infants (<12 months of age) have a risk of up to 50% of developing TB disease following primary infection with *M.tuberculosis*, with a high proportion of disseminated forms of disease, such as miliary TB or tuberculous meningitis (TBM) even in the absence of HIV infection (13). Adult-type disease with cavities and a high bacterial load is a phenomenon that appears around puberty (from about 8 years of age), probably due to inappropriate containment of a recent primary infection (14).

#### *HIV infection and tuberculosis*

HIV infection not only increases the risk of acquiring infection with *M. tuberculosis* after exposure, but also the risk to progress rapidly from primary infection to TB disease and to develop re-activation of latent TB infection (15). Compared to HIV-uninfected children, HIV-infected children have greater morbidity and mortality from TB, especially

in the absence of antiretroviral therapy (ART) (12, 16-18). Initiation of ART reduces the number of TB cases in children substantially (12).

Reduced plasma concentrations of several anti-tuberculosis drugs have been reported in HIV-infected adults and children and have been attributed to malabsorption caused by drug-drug interactions, diarrhea and/or concurrent gastro-intestinal infections (19-24). Reduced drug exposure has been associated with worse treatment outcome and the development of drug resistance in adult studies (23, 24).

Plasma concentrations of several antiretroviral agents are reduced if co-administered with RMP. RMP is a potent inducer of CYP450 system and P-glycoprotein resulting in decreased plasma concentration of protease inhibitors and non-nucleoside reverse transcriptase inhibitors. RMP also leads to an upregulation of UDP-glucuronosyltransferase, an enzyme metabolizing integrase inhibitors (25, 26). In adult HIV-infected patients with TB, co-trimoxazole prophylaxis was associated with an increase in INH-half-life, possibly due to competitive interactions between INH and sulphamethoxazole in the N-acetyltransferase pathway (27).

#### *Preventive anti-tuberculosis therapy*

Following infection with DS-TB, INH preventive therapy (IPT) given for 6-9 months is the most commonly recommended preventive regimen (28, 29). It reduces the risk for TB disease in exposed children by at least two-thirds, probably by more than 90% in the presence of good adherence (30). Based on the high risk of TB disease progression following infection with *M. tuberculosis*, the WHO and the South African National TB programme (SANTP) recommend contact investigation and treatment of *M. tuberculosis* exposure/infection in children less than 5 years of age and all HIV-infected children irrespective of age in contact with an infectious TB case (28, 29). Alternatively, a combination therapy of INH and RMP given for 3 months has shown comparable efficacy in the prevention of disease after infection with *M. tuberculosis* (31). A once weekly administration of rifapentine and INH as preventive treatment in children 2 to 17 years of age has very recently been investigated and showed non-inferiority compared to 9 months of INH only (32).



### *Therapy of drug-susceptible and drug-resistant tuberculosis*

The first-line anti-tuberculosis drugs INH, RMP and pyrazinamide (PZA) with or without ethambutol (EMB) form the backbone of anti-tuberculosis treatment in all types of DS-TB and are routinely prescribed in children with TB disease (28, 33). The overall treatment success rate (cure or treatment completion) in children with TB is reported to be between 72-93%, while young age, extrapulmonary TB and HIV infection are related with poor treatment outcome (34-36).

While there is extensive knowledge on the mode of action, efficacy and safety of these first-line agents, information on their pharmacokinetics in paediatric TB, especially in young and HIV-infected children, is lacking. Therefore TB treatment guidelines for children are largely inferred from adult data (37, 38).

I hypothesized that there is insufficient data to guide the appropriate use of first-line anti-tuberculosis agents in HIV-infected and -uninfected children with TB. In order to address this question, I performed a literature review on their use in childhood TB focusing on pharmacokinetics and safety (chapter 2).

### *Pharmacokinetic considerations of anti-tuberculosis therapy in children*

For optimal dose finding, characteristics particular to children have to be considered which may have an influence on pharmacokinetics. During growth, children undergo profound developmental changes in absorption, distribution, metabolism and excretion of a drug (39-41). These changes are greatest within the first year of life (39, 42). Only by the age of 8 years, organ function and body composition approximate that of young adults (40). Dosing according to body surface area has been suggested, but never been studied in a larger paediatric population (43, 44). Allometric scaling has also been proposed to predict clearance in children, but has shown substantial potential for error in children less than 5 years of age (45).

Because of the complexity of current drug regimens against TB, it is challenging to evaluate efficacy against the serum concentration of a single drug. In children, evaluation is even more complicated, because of the lack of reliable parameters to measure microbiological and clinical outcome. In adults, reduction of the bacterial load

in sputum and/or culture negativity is used as surrogate markers for treatment success. However, these parameters are not feasible in children, due to the paucibacillary nature of most TB disease in children. Thus, the major tools at hand to determine desired blood concentrations of an anti-tuberculosis drug in children are comparative clinical data from adults and their pharmacokinetic “optimal” target values. The validity of the currently proposed targets is a subject of ongoing debate, especially for RMP (46, 47) and doses of the latter might be increased in the foreseeable future. Additionally, different TB disease types (e.g. TB meningitis) may, however need different (higher) doses to achieve adequate drug concentrations at the site of infection (48, 49). Notwithstanding these limitations, there is now good evidence that using the same mg/kg body weight doses of some first-line agents leads to children being exposed to considerably lower concentrations of anti-tuberculosis agents compared to adults, and that doses of anti-tuberculosis drugs in children need to be increased to yield the same exposure and drug concentrations as in adults (44, 50-55). Based on a recent systematic literature review and expert consultation, the WHO has issued revised dose recommendations in September 2009 for the dosing of children with first-line TB drugs (56). These recommended doses are considerably higher than previously recommended for TB treatment in children, and are as follows, according to body weight: INH 10 versus 5 mg/kg/day, RMP 15 versus 10 mg/kg/day, PZA 35 versus 25 mg/kg/day and EMB 20 versus 15 mg/kg/day. There is no data whether these recommendations on higher doses are also appropriate in children less than 2 years of age, as the maturation of enzyme systems is still ongoing in the first two years of life (especially infants i.e. younger than 12 months of age). Serum concentrations in this age group may therefore be different (higher or lower) than in older children or adults receiving the same mg/kg body weight dose. In order to create evidence for optimal dosing of first-line agents in this age group, I performed a prospective pharmacokinetic study in HIV-infected and -uninfected children less than 2 years of age receiving INH, RMP and PZA at previously and currently recommended doses as per WHO TB treatment guidelines (chapter 3).

#### *Therapy of drug-resistant tuberculosis in children*

In contrast to the relatively good body of evidence for the management of DS-TB in children, there are still major gaps in our knowledge on management of children with DR-TB. Currently, due to an absence of data, there is no consensus about the

management of children exposed to infectious MDR-TB cases and recommendations for preventive therapy in international guidelines vary widely. After ruling out TB disease, the WHO suggests to only follow up contacts of infectious MDR-TB cases without recommending a specific drug regimen, while South African guidelines recommend to give high-dose of INH (15mg/kg) to children <5 years of age (29, 57). In a consensus statement, the American Thoracic Society, the Infectious Diseases Society of America, and the US Centers of Disease Control and Prevention advocate that preventive therapy including two drugs to which the source case's isolate is susceptible should be given (58). A combination of either PZA plus EMB, or PZA plus a fluoroquinolone according to the DST result, are recommended (58). Ofloxacin (OFX) and sparfloxacin are the fluoroquinolones recommended in these guidelines for adults, but not for children. In a more recent report on management of children exposed to MDR-TB, the use of the fluoroquinolones OFX or levofloxacin (LFX) are suggested (59). Fluoroquinolone-based treatment regimens (OFX or LFX) have provided evidence suggesting that fluoroquinolones may prevent progression from TB infection to disease in adults and children (60, 61). Further second-line drugs suggested for preventive therapy are ethionamide (ETH) or prothionamide (PTH) (59). Future drugs that might be suitable for preventive treatment against MDR-TB are bedaquiline (TMC 207), delamanid (OPC 67683) or pretomanid (PA-824) (62). These drugs are in phase 3 trials in adults and some have already been provisionally licensed for the treatment of MDR-TB in adults.

The management and outcome of children with MDR-TB have only been reported as case series and a single meta-analysis (63-66). Children should be treated according to their DST results or, if not available, to the DST results of the source case. Three or preferably four drugs to which the isolates are susceptible or naïve should be included in an MDR-TB treatment regimen. In individualized treatment, a treatment regimen is built from different drug groups according to WHO classification (see table 1), including first-line anti-tuberculosis drug(s) to which the organism is still susceptible, a second-line injectable agent, a fluoroquinolone, and one or more oral second-line drugs, to a total of four active drugs (67, 68). If these drugs are not sufficient to build an effective regimen of four active drugs, then drugs from group 5 (agents of uncertain value) should be added (67). Two cohort studies of children with confirmed or probable MDR-TB gave an overview on the treatment regimens used in Western Cape province (63, 69). In the majority of cases, high-dose INH (15-20mg/kg) was used to overcome resistance in

isolates with an *inhA* promoter region mutation and an expected low-level INH resistance. Amikacin for up to 6 months was most frequently used as the injectable agent, substituted by capreomycin if resistance to amikacin was detected. In these studies, OFX was used from the fluoroquinolone group. Further drugs used in this cohort were: ETH, *para*-aminosalicylic acid (PAS), terizidone, amoxicillin/clavulanic acid, clarithromycin and linezolid. Favourable treatment outcome was seen in 82% in the first study including only children with culture-confirmed MDR-TB (n=111) (63) and in 92% of children with confirmed or probable MDR TB (n=149) (69). Nevertheless, death still occurred in a small proportion of children and was associated with HIV infection, malnutrition and extrapulmonary involvement (63, 69).

Following recent studies showing efficacy of newer fluoroquinolones in adults for the treatment of MDR-TB, the treatment policy of MDR-TB has been changed in South Africa, also for children, from OFX, to LFX or moxifloxacin (MFX) (70, 71), depending on the age of the child.

Data on the use of second-line anti-tuberculosis agents in children are urgently needed. Priority might be given to ETH and the fluoroquinolones; ETH, because it is not only frequently used in treatment of MDR-TB, but also for the treatment of DS-TB meningitis or miliary TB due to its good penetration into the cerebrospinal fluid (CSF) and its potential bactericidal activity (72); and fluoroquinolones because they form the backbone of MDR-TB preventive therapy and treatment of MDR-TB not only in current regimens, but will most likely also be used in future regimens with novel compounds, both for DS-TB and DR-TB.

I performed a scoping literature review on the thioamide (ETH and PTH) and the fluoroquinolones (OFX, LFX, MFX) to identify the existing evidence on their use in childhood TB (chapter 4). To better define the optimal dosage of the second-line drugs, pharmacokinetic and safety studies of these agents in children are a matter of urgency. To address this gap in current knowledge, I performed the first ever pharmacokinetic studies of ETH, OFX, LFX and MFX in children with TB (chapter 5).

Dosing does not only depend on efficacy, but also on safety, and for second-line anti-tuberculosis agents, the margin of efficacy and toxicity is much narrower than for the first-line anti-tuberculosis drugs. I therefore assessed specific adverse effects of these agents (chapter 6). Beside mainly gastro-intestinal intolerance, ETH may cause

hypothyroidism during long-term therapy; this has never been studied in children. Changes in thyroid function tests were therefore assessed in a cohort of children with MDR-TB receiving ETH as part of their MDR-TB regimen (Chapter 6.1.).

Fluoroquinolone use has been traditionally restricted in children due to safety concerns, especially drug-induced arthropathy. Their use has also been associated with prolongation of the QT interval in adults, not previously investigated in children. Further evaluation of QT prolongation in children is warranted, given that in future MFX may be combined with novel TB drugs also known to cause QT prolongation, such as bedaquiline, delamanid and pretomanid.

I therefore assessed adverse effects of OFX, LFX, and MFX including electrocardiogram-related cardiotoxicity in children on MDR anti-tuberculosis therapy, as part of the pharmacokinetic studies completed.

Taken together, paediatric TB has become a public health problem of special significance not only because it is a marker of recent transmission of TB (also DR-TB), but also because it is a major cause of disease and death in children from areas endemic for TB. Children with TB or exposed to *M. tuberculosis* urgently require optimized treatment to prevent disease after infection, to prevent paediatric morbidity and mortality, as well as to reduce the future burden of TB. Knowledge of the optimal use of the existing drugs is also required to guide the evaluation of novel and treatment shortening regimens for DS- and DR-TB in children.

The overall objective of the proposed research thesis was to generate robust evidence which would contribute to the optimal dosing of relevant first- and second-line anti-tuberculosis drugs in children.

**Table 1. WHO classification of anti-tuberculosis drugs**

Group	Group Name	Drugs
1	First-line oral agents	isoniazid, rifampicin, ethambutol, pyrazinamide (rifabutin, rifapentine)
2	Injectable agents	kanamycin, amikacin, capreomycin, streptomycin
3	Fluoroquinolones	moxifloxacin, levofloxacin, ofloxacin
4	Oral bacteriostatic second-line agents	ethionamide, prothionamide, cycloserine, terizidone, <i>para</i> -aminosalicylic acid
5	Agents with unclear efficacy or concerns regarding usage	clofazimine, linezolid, amoxicillin-clavulanic acid, thiacetazone, imipenem/cilastatin, high dose isoniazid, clarithromycin

## Chapter 2

### **The use of isoniazid, rifampicin and pyrazinamide in children with tuberculosis: a review of the literature**

#### **2.1. Methods**

In order to review the current evidence base on the use of first-line anti-tuberculosis agents in children, a structured descriptive review of the available published literature was performed. For the initial search, Pubmed was used. Additionally, the reference lists of identified articles were reviewed for further relevant reports. An extensive review of the first-line anti-tuberculosis agents is beyond the scope of this thesis, and therefore I focused on pharmacokinetics and safety in children with TB. The first-line drugs isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA) were included in this review. Where data on childhood TB were limited, literature on adults with TB and on the agents' use in conditions other than TB has also been consulted.

#### **2.2. Isoniazid**

INH plays an important role in the treatment of TB disease as well as of *Mycobacterium tuberculosis* infection. It is valued for its good early bactericidal activity (EBA) as well as for its ability to prevent the development of resistance in companion drugs in the intensive phase of anti-tuberculosis treatment (73).

##### **Mode of action of isoniazid**

INH has a bactericidal effect on rapidly dividing mycobacteria but has a bacteriostatic effect if the bacteria are slow growing. INH is a pro-drug that is converted by a mycobacterial catalase-peroxidase to an active metabolite. Following activation, INH inhibits the biosynthesis of mycolic acids in the mycobacterial cell wall (74).

The activating catalase-peroxidase is encoded by the *katG* gene. Mutations in this gene region are a common cause for INH resistance (75-77). Activated INH binds to the product of the *inhA* gene which is involved in the mycolic acid biosynthetic pathway correlating with cell death (76). In a systematic review, mutations in the *katG* gene were underlying in 64% of all observed phenotypic INH resistance worldwide and mutations in the *inhA* promoter region in about 20% (77). Regional variation in the frequency of individual mutations exists. In the Western Cape Province, South Africa, *inhA* mutations are found in about 60% of INH-resistant *M. tuberculosis* isolates (10, 78). Mutations in the *katG* gene usually confer high-level INH resistance and those in the *inhA* gene, low-level INH resistance that can be overcome by giving INH at higher doses (15-20mg/kg) (79). The latter mutation also confers ethionamide (ETH) resistance, limiting its use in the presence of *inhA* mutations.

### **Clinical efficacy of isoniazid**

Since its discovery in 1952 (80), INH has been the cornerstone of TB chemotherapy. In adults, the standard daily recommended dose is 5 mg/kg with a maximum of 300 mg. International treatment recommendations for children vary widely. While the WHO and Centers for Disease Control and Prevention (CDC) recommend a dose of 10-15mg/kg (81, 82), the British Thoracic Society recommends the same dose of 5 mg/kg for adults and children (although these guidelines are currently being updated) (83).

In very early studies, INH as a single drug showed a high potential in acute TB disease with a reduction in number of deaths due to TB and increase in sputum culture conversion (84). Since its potential to prevent progression from *M. tuberculosis* infection to disease has been evaluated, INH as a single drug has been widely used for preventive therapy (85).

The therapeutic efficacy of INH is determined by the exposure to the drug (86). INH causes rapid decrease in the number of bacilli in sputum in the first two days of treatment and thereafter shows a comparable bactericidal activity to other anti-tuberculosis drugs (73). The EBA increases proportionally to the INH dose until a maximum effect is reached (EBA 0.54 log<sub>10</sub> cfu/ml sputum/d) at an INH dose of 600mg



(73). A further INH EBA study showed that a maximum decline in colony forming units is obtained at a INH serum concentration of 2-3 $\mu$ g/ml and that a further increase of the concentrations does not increase INH EBA (87). An early report by the East African/British Medical Research Councils showed that increasing the INH dose, when used in combination with thiacetazone, from 200 mg daily to 300 mg improved the clinical response, but that a further increase to a single daily dose of 450 mg left the response unchanged (88). Whether these findings are still valid today in the context of high prevalence of HIV infection and an increased average body weight compared to the weight of the study participants in these early studies, leading to a lower mg/kg dose, is subject of ongoing debate (47).

Inactivation of INH occurs mainly by acetylation in the liver and the small intestine and N-acetyltransferase type 2 (NAT2) has been identified as the responsible enzyme. The activity of the NAT2 is genetically determined and a trimodality in elimination of INH has been demonstrated (51, 89, 90). Therefore individuals may be slow, intermediate or fast acetylators. Distribution of each phenotype differs between different populations. For example, Japanese and the Inuit population are predominately fast acetylators (91), while in the Scandinavian population slow acetylators are more common than fast acetylators (92).

In an early study, patients who were fast acetylators of INH responded less favourably to INH-containing treatment regimens (93). In patients receiving once weekly treatment of INH and rifapentine, failure and relapse were also associated with fast acetylator status (94). This has been attributed to a reduced area under the curve (AUC) and associated diminished post-antibiotic effect of INH (73, 94). This is consistent with *in vitro* findings, identifying the AUC/minimum inhibitory concentration (MIC) as the pharmacokinetic/pharmacodynamics index associated with microbial killing and development of resistance (95). In adult TB, low drug AUC<sub>0-24</sub> were predictors of poor long-term outcome (as microbiological failure, death or relapse) (23). The proposed AUC for INH was 52mg·h/L (23). In a pharmacokinetic study on Indian children, decreased maximum serum concentration ( $C_{max}$ ) of INH and RMP were associated with unfavourable treatment outcome (death, default) (96), while in a further study from India treatment outcome was not influenced by  $C_{max}$  of INH, RMP, PZA or ethambutol (EMB) (97). In children with TB, it was shown, that the 2-hour serum concentration as well as the  $C_{max}$  of INH were poor predictors of INH AUC<sub>0-24</sub> (98), probably due to



differences in elimination depending on the acetylator status. Because of the lack of a gold standard for measuring treatment success in children with TB, pharmacokinetic/pharmacodynamic studies investigating AUC/MIC in children may have the potential for surrogate markers to predict clinical outcome. However, this implies that the AUC concentrations needed for an optimal effect are identical in children and adults. Given the differences in bacterial burden and immune response (especially in young children) between adults and children, some uncertainty regarding this assumption exists (37).

### **Pharmacokinetics of isoniazid**

In adults, absorption of INH from the intestinal tract is almost complete when administered on an empty stomach but reduced when given with food (99). For children no difference of resulting INH serum concentrations after oral or intramuscular administration of INH was found (100). A  $C_{max}$  of 3-5 $\mu$ g/ml is reached 1 to 2 hours after an oral standard dose of 300mg in adults and are associated with efficacy (86, 99, 101, 102). More recent studies have proposed the  $AUC_{0-24}$  as the primary pharmacokinetic index, with a target for INH of 52mg·h/L (23).

Several studies have demonstrated that INH serum concentrations are lower in children than in adults following the same mg/kg dose (44, 51, 103, 104). INH is distributed throughout the body water and diffuses rapidly into all the tissue and body fluids including cerebrospinal fluid (CSF) without being appreciably bound to plasma protein. Thus, serum concentrations in infants and young children are expected to be lower than in older children and adults following the same mg/kg body weight doses because of age-dependent changes in body composition with higher relative volumes of body water early in life (105). Also, an age-dependent elimination of INH has been demonstrated, with younger children eliminating INH faster than older children and children as a group faster than adults (51, 96, 104, 106). This has been ascribed to the relatively greater mass of the liver in proportion to total body weight in young children. However, very long half-lives of up to 20 hours have been reported in neonates probably due to immature enzyme function (101, 107, 108), indicating that caution should be used when dosing this paediatric group.

INH is eliminated by NAT2-acetylation predominantly in the liver, and then mainly excreted by the urine (86, 101).

There is evidence that maturation of enzymes responsible for acetylation occurs (106, 109). In a study on 44 children with sequential testing of INH serum concentration, 12/30 children phenotypically classified as slow acetylators initially, were classified as fast acetylators over time (109). It was proposed, that NAT2 maturation reaches a plateau at 4 years of age (109). In order to separate the effects of body size change and enzyme maturation on the clearance of INH, a pharmacokinetic model was applied to data from 151 perinatally HIV-exposed infants receiving INH in the first 24 months of life (110). It was shown that after the age of 3 months, the apparent clearance increases in the fast and intermediate acetylator groups with no significant change in the slow acetylator group (110). Due to the strong influence on pharmacokinetic parameters, the acetylator status of the children should be known for comparison of study results.

To date, there has been no association found between sex or HIV infection and INH pharmacokinetic parameters in paediatric studies (51, 104). Reports on the influence of malnutrition on INH pharmacokinetic have shown no or moderate influence on INH serum concentrations (96, 111, 112). The extent of malnutrition differed between the studies, possibly accounting for some of the differences in the study results.

Although INH is the anti-tuberculosis drug most extensively studied, in previous studies, the age of children was often not documented, there was inadequate inclusion of young children (especially below the age of 2 years), NAT2 status was not known, or doses differed from the current WHO recommendation (100, 103, 113-116).

Prior to the study described in chapter 3, there were only two reports of INH pharmacokinetic in children younger than 3 years of age (except neonates), one dating from 1978, the other from 2001, where INH was given at a similar dose (15mg/kg and 10mg/kg respectively) as currently recommended by the WHO (7-15mg/kg) (116, 117). In these studies targeted  $C_{max}$  of INH 3-5 $\mu$ g/ml were achieved while information on the AUC was not provided (116, 117).

In the study completed in children younger than 2 years of age (chapter 3), we could confirm that an INH dose of at least 10mg/kg is needed in children younger than two

years of age to achieve adequate serum concentrations independently of acetylator status or co-infection with HIV (118).

Several studies provide further evidence for the implementation of the revised WHO dosing recommendations of INH 10mg/kg in infants and children (97, 119, 120). Although in children older than eight years of age organ function and body composition approximate that of young adults (40), low INH serum concentrations compared to adults were also found in children up to 13-16 years of age (51, 97, 120). Even with increased doses of INH, subtherapeutic serum concentrations have been reported, not only illustrating the large inter-patient pharmacokinetic variability but also the possible influence of demographic, genetic and clinical factors (98). Nevertheless, care needs to be taken in low-birth weight and premature babies. In a recent study in this population, an INH dose of 10mg/kg led to targeted serum concentrations but prolonged half-life and reduced elimination of INH were noted (108).

In order to administer recommended anti-tuberculosis drug doses adequately to paediatric patients, child-friendly drug formulations are required. A common practice is to crush or split tablets where child-friendly formulations are not available. None of the studies above have assessed the influences of different drug formulations (e.g. tablets versus syrup), or the crushing of INH tablets, which is common clinical practice, especially in young children, given the lack of child-friendly scored fixed dose combinations consistent with WHO dosing recommendations.

### **Adverse effects of isoniazid**

In adults, the incidence of adverse effects of INH treatment is estimated to be 5.4% (121). The most frequent adverse effects are rash, fever, hepatotoxicity and peripheral neuritis.

Hepatotoxicity is the most serious adverse effect related to INH. Subclinical, asymptomatic elevated liver enzymes were found in 5-10% of children receiving INH preventive therapy, with a higher incidence in adolescents (122-127). Large studies including more than 2,000 children receiving 10-20mg/kg INH for preventive therapy did not report a single case of discontinuation due to hepatotoxicity (126, 127). Contrarily, a very recent report found elevated transaminases to be common (41%) in

children on preventive therapy for TB, with a higher incidence in children younger than 9 years of age (128). INH had to be discontinued in 4/277 children due to hepatotoxicity (128). Severe hepatotoxicity possibly leading to liver failure has occasionally been reported previously in children receiving INH at a dose of 10mg/kg or less (129-132). In adults, slow acetylator status has shown to be associated with a higher risk of drug-induced hepatotoxicity in different populations (133-136). While others could not identify acetylator status as a risk factor for drug induced hepatotoxicity (137, 138). Different study populations with different treatment regimens, co-morbidities and additional risk factors are possible explanations for the different findings on the impact of the acetylator status. In children, the influence of NAT2-status on INH toxicity has not been systematically assessed (122). In a cohort of children with TB, the risk of raised liver enzymes tended to be increased in those receiving higher doses of INH, although this may have been confounded by disease related factors (104). A literature review on anti-tuberculosis drug-induced hepatotoxicity in children indicated that increased INH-doses as well as being slow-acetylator, infection versus disease and disease severity as well as combination therapy with RMP increases the risk for hepatotoxicity (122).

Peripheral neuritis and central nervous system (CNS) adverse effects of INH are caused by pyridoxine depletion, interfering with the synthesis of synaptic neurotransmitter (139). Resulting dose-related neurotoxicity can present as peripheral neuropathy, seizures, psychosis, ataxia or optic neuritis. It is reversed (or prevented) by pyridoxine administration (140). Even at doses of up to 20mg/kg, children receiving INH are less susceptible to developing INH-related neurotoxicity than adults (141). In a study on 38 children receiving INH as part anti-tuberculosis therapy, 5 children (13%) showed pyridoxine deficiency on assay, with higher incidence in children receiving more than INH 10mg/kg, but no child had clinical symptoms of pyridoxine deficiency (142). Similar results were reported from a study from Zaire (143) and from Cape Town (144). No signs of neurologic disorder were found in 85 children on INH therapy, regardless of whether they received additional pyridoxine supplementation or not (143). Therefore, pyridoxine supplementation is not routinely used in children, but supplementation is recommended in HIV-infected and/or malnourished children treated for TB, or those receiving high-dose INH (15-20mg/kg) as part of MDR-TB treatment (81, 144) not being based on level 1 evidence.

Slow acetylators are also more likely to develop polyneuropathy during INH therapy and pyridoxine supplementation should be considered in these patients (145). In a systematic review on the role of pyridoxine administration on polyneuropathy during anti-tuberculosis therapy the authors concluded that it appears prudent to support the co-administration of pyridoxine with INH in the light of its potential benefit and low costs (146).

### **Conclusions of isoniazid literature review**

INH has dose-dependent activity against *M. tuberculosis*. To achieve serum concentrations in children comparable to those in adults correlated with efficacy, the existing evidence advocates for the use of higher doses of INH in younger children compared to adults, although the optimal pharmacokinetic/pharmacodynamic target(s) in children still need to be established. The effect of different formulations or crushing/splitting of tablets on pharmacokinetic measures in children is not known. In children, severe hepatotoxicity or peripheral neuropathy is rare. The influence of NAT2-acetylator status on efficacy and toxicity of INH in children in different populations warrants further study.

### **2.3. Rifampicin**

RMP is active against a wide range of Gram-positive and many Gram-negative bacteria, but it has principally been developed for use in the treatment of TB. RMP has a moderate EBA against *M. tuberculosis* but in combination with INH, its sterilizing ability is unique (147). With the introduction of RMP into anti-tuberculosis treatment regimens together with PZA, it has allowed for the shortening of the duration of treatment from the conventional 9 to 12 months to the so-called “short- course treatment” of 6 months.

#### **Mode of action of rifampicin**

RMP inhibits DNA-dependent RNA synthesis by inhibiting RNA polymerase of bacteria by binding its  $\beta$ -unit, leading to suppression of initiation of chain formation (148). This

halts mRNA transcription, therefore preventing translation of proteins. Resistance to RMP is almost exclusively associated to mutations in the *rpoB* gene encoding the RNA polymerase  $\beta$ -subunit (149, 150). The mutations reduce binding of RMP to the polymerase. Eucaryotic cells are unaffected by RMP as it does not bind to their nuclear RNA polymerase. Efflux pump mediated drug resistance has been identified as an alternative mechanism in RMP drug-resistant isolates of *M. tuberculosis* (151).

In *in-vitro* as well as in *in-vivo* studies it has been demonstrated that RMP efficacy depends on concentration of RMP rather than time (147, 152, 153). In an *in-vitro* pharmacokinetic/pharmacodynamic model RMP AUC/MIC has been identified as the primary pharmacodynamic index for predicting efficacy of RMP, while the suppression of resistance has been associated with the free  $C_{max}$ /MIC ratio (152).

### **Clinical efficacy of rifampicin**

After its introduction, a series of randomized trials established that RMP-containing regimens achieved a high cure rate even when given intermittently (154). RMP is seen as the main contributor to the shortening of anti-tuberculosis treatment to 6-months caused by drug susceptible organisms. The standard daily RMP dose in adults is 600mg. There is evidence against treatment regimens using RMP only during the intensive phase (first 2 months of therapy), as these have been shown to be inferior to regimens that use RMP throughout a 6-month course of therapy (155).

In clinical trials using different doses of RMP, it was shown that a drop in colony forming units (CFU) of *M. tuberculosis* in the sputum of patients with pulmonary TB was higher when higher doses of RMP were given (147, 156). The EBA of a 1,200mg RMP dose was almost double that found after a dose of 600mg in adults (147, 157). Two studies show a direct correlation between higher RMP plasma concentrations and an increased fall in cfu (158, 159). A literature review concluded that there is little evidence for the currently recommended dose of RMP 600mg daily (46). It has been proposed that higher RMP doses of up to 1,200-1,800mg should be studied in the context of current four-drug-regimens (46, 47, 160) in adults with TB. Clinical outcome, as manifested by a lower rate of sputum conversion and a higher rate of treatment failure, was found to be inferior in adult TB patients treated with doses of 450mg RMP per day than in patients

treated with 600mg per day (153). In adult TB patients it was shown that a RMP  $AUC_{0-24} \leq 13\text{mg}\cdot\text{h/L}$  as well as low AUCs of INH and PZA predict failure to convert sputum culture at 2 months as well as long-term poor treatment outcome (23). Preliminary data from a recent phase IIb study shows that high-dose RMP results in faster killing of TB bacilli during treatment, compared to the current standard treatment (PanACEA MAMS-TB-01, NCT01785186). Over 12 weeks of treatment, high-dose (35mg/kg) RMP, in combination with standard dose of INH, PZA, and EMB, led to a significant shortening of time to culture conversion (hazard ratio of 1.75, 95% CI 1.21-2.55).

To our knowledge there are no published studies assessing the use and dose of RMP in relation to treatment outcome in children with TB. In the case of latent TB infection, RMP alone or in combination with INH has been shown to efficaciously prevent progression to disease in children and adults (161-163).

### **Pharmacokinetics of rifampicin**

In adults, following oral ingestion, RMP is well absorbed from the intestinal tract (164). There is evidence that absorption might be significantly reduced in children (165).  $C_{\text{max}}$  is achieved within 2 hours when RMP is given on an empty stomach, but delayed and incomplete when ingested with food (166). Following a single dose of different RMP oral formulations as preventive therapy against *Haemophilus influenzae*, co-administration of apple juice resulted in a reduced AUC of RMP in a paediatric cohort (167).

Despite protein binding of 80% RMP is distributed throughout the body (164). Effective concentrations can be found in many organs and body fluids although penetration into the CSF can be poor (168-170). RMP is extensively metabolized in the liver to 25-O-desacetyl-RMP, which is also active against *M. tuberculosis*. The main route of elimination is biliary excretion, and to a much lesser extent, renal excretion. RMP induces its own metabolism leading to a progressively shortened half-life within the first 14 days of treatment (164, 171, 172). Because RMP is a strong inducer of the cytochrome P450 (CYP450) system, concomitant treatment results in decreased half-life for a number of compounds, including HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors (26). RMP also activates the pregnane X receptor, an important component of the body's adaptive defense mechanism against toxic



substances including xenobiotics (173). Activation of pregnane X receptor leads to the induction of CYP3A4, an important phase I oxidative enzyme that is responsible for the metabolism of many drugs, as well as to up-regulation of phase II conjugating enzymes and p-glycoprotein, an efflux transporter (173). It has been noted for adults and children, that inter-individual variability in RMP pharmacokinetics is high (174, 175). Possible reasons include raw material characteristics, changes in the crystalline habit of the RMP, excipients, manufacturing and/or process variables (176, 177), degradation in the gastro-intestinal tract, influence of co-administration of food (166) and inherent variability in absorption and metabolism. In children, tablets are often grinded or split to be administered with unknown effect on bioavailability. Recently a polymorphism in the *SLC01B1* gene was identified. Low RMP exposure was associated with the polymorphism of the *SLC01B1* c,463C>A gene (178). Furthermore, the ontogeny of p-glycoprotein may also play a role in the variable absorption in children (179).

In anti-tuberculosis treatment in adults it has been suggested that RMP concentrations of less than 8µg/ml 2-hours post administration should be regarded as low and values less than 4µg/ml as very low (180, 181). In children, lower serum concentrations than in adults have been documented in several reports regardless of the presence of HIV co-infection (55, 98, 120, 172, 182-187). In 2009, the WHO revised the paediatric TB treatment guidelines, recommending an increased oral daily dose of RMP 15mg/kg with a range of 10-20mg/kg daily (56).

Many of the pharmacokinetic studies of RMP in children were performed after a single dose of RMP and not after anti-tuberculosis therapy had been established, at so-called "steady-state". Due to self-induction of RMP metabolism, these findings might show higher serum concentrations than would actually be present during established anti-tuberculosis therapy. In 1974, a study was undertaken in children from 2 months to 5 years of age after a dose of 15mg/kg; low maximum serum concentrations of 5.2µg/ml were found (188). We conducted the first study on pharmacokinetics of the first-line agents in children younger than 2 years of age (see chapter 3) (118). These data provide supportive evidence for the revised WHO guidelines recommending a dose of RMP 15mg/kg in children younger than two years of age; we also showed that previous recommendations of RMP 10mg/kg indeed lead to insufficient concentrations of RMP (118). In a study from India on HIV-infected children with TB, receiving thrice-weekly anti-tuberculosis therapy, a RMP dose of 10mg/kg also led to sub-therapeutic RMP



serum concentrations in 97% of children (185). In children <5years of age, RMP drug exposure was even lower than in children older than 5years (185). Based on a pharmacokinetic model it could be confirmed that a RMP dose of at least 15mg/kg is required for adequate RMP serum concentrations (174). Ongoing studies provide preliminary evidence that higher RMP doses (35mg/kg) might optimize TB treatment and pending on the final results, higher doses need to be evaluated in children as well.

In a group of children with TB (median age of 2.3 years), even weight banded drug doses according to new WHO recommendations led to insufficient serum concentrations (98). It has been noted that inter-patient variability in RMP pharmacokinetics is high (118, 174). There is very limited information on the pharmacokinetics of RMP in newborns and infants (<12 months). It is therefore important to further evaluate optimal RMP dosing strategies in children, especially given future planned treatment shortening trials in children with DS-TB.

### **Adverse effects of rifampicin**

RMP is generally well tolerated. When given at a standard dose of 10mg/kg, significant adverse effects are seen in less than 4% of adult patients. The most common adverse effects are rash (0.8%), fever (0.5%) and nausea and vomiting (1.5%) (189). Hepatotoxicity rarely occurs and has been observed mainly in patients with underlying liver disease. Also the combination of RMP plus PZA used for preventive therapy in HIV-uninfected patients has been associated with severe and sometimes fatal hepatotoxicity (190). In a study on RMP dose of 600mg in adult TB patients, mild hepatotoxicity occurred in about half of the patients, but no patient developed severe hepatotoxicity (191). In an ongoing study investigating RMP at a dose of 35mg/kg, the preliminary analysis of safety events showed no increase in adverse events compared to control arms (PanACEA MAMS-TB-01, NCT01785186).

There are indications that RMP toxicity is rarely dose-related but idiosyncratic (160). In intermittent therapy a “flu-like” syndrome might occur (160, 189). There is evidence that adding RMP to an INH containing regimen increases the risk of INH-induced hepatotoxicity, especially in patients being slow acetylators (122, 192).

Reports on adverse effects in children are rare and even higher doses seem to be

tolerated well (122, 193-195). In a study of children receiving RMP alone as preventive therapy (n=25), no adverse events occurred (196). In a larger study on 157 adolescents, mild adverse effects were reported in 41 children (26%), but only in one child did RMP need to be discontinued, because of increasing liver enzymes (197). In a literature review on anti-tuberculosis drug-induced hepatotoxicity, elevated liver enzymes were abnormal in 380/3855 children evaluated (9.9%) and jaundice occurred in 75 children (0.83%) (122). In this review, it has also been concluded that RMP increases the risk of hepatotoxicity if added to INH, regardless of the RMP dose used and that the risk of hepatotoxicity is increased in severe TB disease (122).

### **Conclusions of rifampicin literature review**

RMP has a dose-dependent activity against *M. tuberculosis* with  $AUC_{0-24}$  being identified a surrogate marker for efficacy, while RMP  $C_{max}$  is associated with the prevention of resistance. As for INH, several paediatric studies have shown that higher doses of RMP may be needed to achieve RMP serum concentrations comparable to those associated with efficacy in adults. For adults, even higher doses than currently used have been proposed and are currently being evaluated to optimize RMP efficacy; corresponding doses should therefore in future also be evaluated in children. Evidence regarding the pharmacokinetics in young children, especially newborns and infants, is very limited. As for INH, the effect of different RMP formulations or crushing/splitting of tablets and opening of capsules on pharmacokinetic measures is not known. RMP is well tolerated in children and RMP-related toxicity does not seem to be dose-related and occurs very rarely in children. More research on optimal RMP dosing strategies is needed, especially in young and in HIV-infected children.

## **2.4. Pyrazinamide**

Pyrazinamide (PZA) has important sterilizing activity against semi-dormant *M. tuberculosis* and is essential in the intensive phase of anti-tuberculosis treatment. PZA is bactericidal against intracellular mycobacteria in acidic medium, and is highly specific

for *M. tuberculosis* (198).

### **Mode of action of pyrazinamide**

Although one of the most frequently used anti-tuberculosis drugs, the mechanism of action of PZA is not fully understood (199, 200). The bactericidal activity of PZA increases as the metabolism of the bacteria decreases, e.g. in the dormant growth phase (200).

PZA is a pro-drug that is converted to its active form, pyrazinoic acid, by pyrazinamidase, in the bacterial cytoplasm (200). Pyrazinamidase is encoded by the *pncA* gene. Mutations in the *pncA* gene often underly PZA resistance (201, 202). Pyrazinoic acid diffuses slowly extracellularly. In an acidic environment, pyrazinoic acid diffuses again through the cell wall as the protonated molecule and accumulates inside the mycobacterium. Protonated pyrazinoic acid is toxic to the mycobacterial cell wall (203). Recent studies have shown that both PZA and pyrazinoic acid have several different targets interfering with diverse biochemical pathways, especially in the NAD(+) and energy metabolism (204). PZA displays a pH-dependent minimal inhibitory concentration (MIC) against *M. tuberculosis*. In an in-vitro study, a sharp decline in viable mycobacteria occurred at a PZA concentration of 50 µg/ml in broth in an acidic environment (pH 4.8-5.0) (205).

### **Clinical efficacy of pyrazinamide**

The unique sterilizing activity of PZA at a dose of 30-40mg/kg was shown in early studies on PZA and RMP containing regimens in adults with pulmonary TB (206-209). These studies also showed that continuing PZA beyond the first two months of treatment had little advantage (210). Today PZA is an important component in the intensive phase of short-course multiple-drug therapy for drug-susceptible TB in both adults and children.

Following a PZA dose of 40mg/kg, little activity of PZA was evident in EBA studies. However, during the 10 following days, the bactericidal activity of PZA at this dose matched that of other drugs evaluated (147). For anti-tuberculosis therapy there are

only few studies directly linking PZA pharmacokinetics to efficacy. Target PZA serum concentrations of 20-60µg/ml 1-2 hours post-dosing have been suggested (180); a serum concentration below 20µg/ml has been identified as low and <10µg/ml as very low (211). In a study on HIV-infected and HIV-uninfected TB patients, poor outcome (treatment failure or death) was associated with a PZA maximum serum concentration <35µg/ml (22). In a more recent study, low PZA AUC over 24 hours was the main predictor for poor long-term clinical outcome (23).

PZA has recently been investigated as a potent companion to new treatment shortening drug regimens for drug-susceptible and drug-resistant TB (212-214). Different combinations of moxifloxacin, pretomanid, bedaquiline, clofazimine and pyrazinamide showed comparable bactericidal activity to the standard first-line therapy (INH, RMP, PZA, EMB) (212-214).

### **Pharmacokinetics of pyrazinamide**

In adults, PZA is readily absorbed from the gastrointestinal tract after oral intake, is modestly protein bound (10-20%) and, thus, distributed throughout the body. Comparable concentrations are reached in serum, CSF and body tissue (215-217). The apparent PZA volume of distribution of 0.57-0.84 l/kg suggests distribution in a space comparable to total body water (42). In adults, maximum serum concentrations are achieved within 1 hour after oral ingestion (218). In different studies it could be shown that PZA serum concentrations increase linearly with dose in volunteers as well as in adults and children with TB (215, 219, 220). There is little intra-individual variation in absorption or excretion in adults of the same study population (215). Following PZA dosages of 30mg/kg,  $C_{max}$  range from 40-55µg/ml in adult TB patients and from 30-45µg/ml in patients receiving a dosage of 20-<30mg/kg (220).

Food might reduce absorption, therefore administration of PZA on an empty stomach is preferred (221, 222). In adults  $t_{1/2}$  is 10 hours therefore PZA is suitable for once daily dosing. PZA is hydrolysed in the liver to pyrazinoic acid and subsequently hydroxylated to 5-hydroxypyrazinoic acid, the main excretory product (215). It is then excreted primarily by glomerular filtration. Only 4% of PZA is excreted unchanged in the urine (215). HIV infection does not appear to be associated with reduced PZA serum

concentrations (20, 211).

There are several published reports on studies investigating the pharmacokinetics of PZA in children (19, 97, 120, 219, 223-226). In two studies prior to our study (chapter 3), PZA was used at a dose of 35mg/kg, the dose that is currently recommended by the WHO; in the first study from India, conducted in children between 6-12 years of age (n=10), a mean  $C_{max}$  of PZA of 41.2 $\mu$ g/ml was found, while in the second study from Malawi, maximum PZA serum concentrations were 36.6 $\mu$ g/ml (19). In the Malawian study (n=27, median age 5.7 years) PZA was given thrice weekly (19). In four further studies on the pharmacokinetics of PZA in children from India, using doses of 15mg/kg, 25-30mg/kg and 30-35mg/kg (mean 31.9mg/kg), surprisingly high PZA serum concentration of approximately 39 $\mu$ g/ml, 42 $\mu$ g/ml and 43-49 $\mu$ g/ml, respectively, were reported (185, 223, 225, 227). In the study by Roy et al. (227), PZA was given as a single dose to two treatment groups at a mean dose of 28.1mg/kg or 31.9mg/kg before starting standard anti-tuberculosis therapy. An increase in PZA serum concentrations following the start of treatment occurs, probably due to the amount of PZA still present 24 hours after dosing (50). Therefore PZA serum concentrations of this study cohort might have been even higher during continuous therapy. These difference compared to other paediatric studies might possibly be due to underlying genetic factors or different sampling schemes (223). Additionally, a relatively low volume of distribution of PZA compared to previous paediatric studies has been reported in the study by Gupta et al. (223). Because PZA is a moderately lipophilic small molecule (228), differences in body fat might possibly influence the volume of distribution with lower fat mass being associated with lower volume of distribution.

Following daily PZA doses ranging between 20-30mg/kg, a PZA  $C_{max}$  of 30-40 has been reported in children with TB from Germany, South Africa, Venezuela and Malawi (120, 186, 219, 224), similar to the findings in adult TB patients (215). Whether there are true regional differences in the metabolism of PZA possibly due to underlying genetic factors or if these differences are only due to high inter-individual variability has never been investigated.

Absorption seems to be delayed in children, with a  $t_{max}$  of 2-3.5hours (19, 224, 226). Although a tendency towards lower PZA serum concentrations has been reported, clinical co-variates including HIV infection or malnutrition are not significantly associated with PZA  $C_{max}$  (19, 219).

Very few children younger than 2 years of age were included in the pharmacokinetic studies completed to date. In our study on children <2 years of age (chapter 3) we provide further evidence that a daily oral PZA dose of 35mg/kg is needed in young children to achieve the proposed adult drug target of >35µg/ml. HIV infection, malnutrition, gender and type of disease (pulmonary versus extrapulmonary TB) did not seem to influence the PZA pharmacokinetic measures substantially in children <2 years (118). In another study from South Africa in children with TB (mean age 2.3 years), targeted PZA 2h serum concentration of 30µg/ml were only achieved in 55% (17/31) children following the revised WHO dose recommendations (98). Simulation in a population based pharmacokinetic model gave further evidence that younger children would achieve relatively low exposures to PZA compared to adult data (174). Because PZA distributes in the whole body water compartment, lower PZA serum concentrations in younger children would be expected (42).

All of the above studies used PZA tablets administered either as a whole or split/crushed tablet depending on the ability of the child to swallow tablets. As for INH and RMP, there is no information available on the influence of this formulation manipulation on PZA bioavailability. A suspension formulation of PZA is available in some countries, but has not been evaluated to our knowledge.

In an extensive literature review on the pharmacokinetics of PZA, Donald concluded that similar mg/kg body weight dosages of PZA in children lead to PZA serum  $C_{max}$  similar to those in adults (220). Some evidence exist that children younger than 5 years of age may be exposed to lower PZA serum concentrations, but the data are limited (120, 185, 219). In our study on children <2 years of age, similar PZA serum concentrations as reported in adult TB patients following a similar mg/kg dose were found (118, 215).

### **Adverse effects of pyrazinamide**

The most serious adverse effect in PZA therapy is dose dependent liver injury. In adults, after an oral dose of 40 to 50mg/kg, symptoms of hepatic disease appear in about 15% of patients (121). Rarely, hepatic failure and death attributed to PZA occurs (229). Contrarily, a literature review concluded that PZA induced hepatotoxicity is idiosyncratic rather than dose-dependent, and that doses of 30-40mg/kg shown to be

efficacious in early clinical trials are safe to use in anti-tuberculosis therapy (206-209, 230). There are a limited number of reports on PZA adverse effects in children. Transient elevation of liver enzymes in children treated with an anti-tuberculosis regimen including PZA was reported occasionally, but reports of severe hepatotoxicity are rare (231-235). In these reports on children a daily PZA dose of <30mg/kg was typically used, while for intermittent therapy, doses of up to 50-70mg/kg were prescribed (236). Studies in children comparing lower doses in daily anti-tuberculosis treatment regimen including PZA compared to higher doses of anti-tuberculosis agents given 2-3 times weekly, did not find an increase in adverse effects with the use of higher doses (237-239). There is no evidence to assume an increase in hepatotoxicity in children with the current WHO-recommended dose of 35mg/kg (230).

Hyperuricaemia is commonly found in PZA therapy as excretion of urate is inhibited by PZA; this is sometimes associated with arthralgia in adults, but rarely in children (216, 240). Acute episodes of gout following treatment with PZA have been reported in adults (241, 242). Hyperuricemia found in children was mostly mild and asymptomatic. Increased serum uric acid levels returned to normal after cessation of PZA therapy (231, 232). Other less severe adverse effects include gastrointestinal disturbances, pruritus and skin rashes.

### **Conclusions of pyrazinamide literature review**

A PZA dose of 30-40mg/kg resulting in a  $C_{max}$  of 40-55 $\mu$ g/ml has shown high sterilizing activity in anti-tuberculosis treatment of DS-TB in adults. For children, similar mg/kg body weight dosages of PZA lead to PZA  $C_{max}$  similar to those in adults, although evidence in children younger than 5 years of age remains limited. As for INH and RMP, the effect of different formulations or crushing/splitting of tablets on pharmacokinetic measures is not known. Regional differences in PZA pharmacokinetics might exist. Mildly elevated liver transaminases and transient hyperuricemia during PZA therapy occur, but severe adverse effects in children even at higher PZA doses are rare. Studies of PZA pharmacokinetics in a larger number of children of different ages and genetic background may be needed to better define the PZA doses appropriate for children across different ages.



## Chapter 3

### **Pharmacokinetics of isoniazid, rifampicin and pyrazinamide in children younger than two years of age with tuberculosis: evidence for implementation of revised World Health Organization recommendations**

The aim of this study was to evaluate the pharmacokinetic parameters of isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA) at the previous and current WHO-recommended doses in the young (<2 years) paediatric population.

The current recommended doses are considerably higher than previously recommended for TB treatment in children, and are as follows: INH 10 versus 5 mg/kg/day, RMP 15 versus 10 mg/kg/day, PZA 35 versus 25 mg/kg/day and EMB 20 versus 15 mg/kg/day (28, 81). In the absence of pharmacodynamic data in children, optimal TB therapy in children should aim to produce the targeted serum concentrations 2-hour post-dose that have been determined in adult pharmacokinetic and pharmacodynamic studies. These are as follows: INH 3-5 $\mu$ g/mL (72, 180), RMP 8-24 $\mu$ g/ml and PZA 20-60 $\mu$ g/ml. RMP serum levels (2 hours post-dose) below 8 $\mu$ g/ml are considered low and below 4 $\mu$ g/ml very low (160, 243). Poor treatment outcome of pulmonary TB in adults was associated with PZA serum concentrations <35 $\mu$ g/ml (22).

I designed and implemented a prospective, hospital-based, observational intensive sampling pharmacokinetic study on children treated for TB at Brooklyn Hospital for Chest Diseases (BHCD), utilizing a cross-over design. Resulting serum concentrations following the previous and revised doses were assessed on two days one week apart. Blood samples were taken at baseline (pre-dose) and at 0.5, 1.5, 3 and 5 hours after dosing. The serum concentrations were determined by high performance liquid chromatography (HPLC) and ultraviolet (UV) detection, RMP serum concentrations by HPLCS/MS technology. Pharmacokinetic measures were calculated using noncompartmental analysis (NCA) for each study drug. A secondary aim of this study was to determine the influence of the polymorphism in INH acetylation on the INH serum concentrations.



Of the twenty children included (mean age 1.09 years) 11 children had pulmonary and 9 extrapulmonary TB, 5 children were HIV-infected. Following the previous/revised WHO recommended doses the mean (95% confidence interval) pharmacokinetic measures were as follows:  $C_{max}$ : INH 3.2 (2.4-4.0)/8.1 (6.7-9.5) $\mu\text{g/ml}$ , PZA 30.0 (26.2-33.7)/47.1 (42.6-51.6) $\mu\text{g/ml}$ , and RMP 6.4 (4.4-8.3)/11.7 (8.7-14.7) $\mu\text{g/ml}$  and the AUC: INH 8.1 (5.8-10.4)/20.4 (15.8-25.0)  $\mu\text{g}\cdot\text{h/ml}$ , PZA 118.0 (101.3-134.7)/175.2 (155.5-195.0) $\mu\text{g}\cdot\text{h/ml}$ , and RMP 17.8 (12.8-22.8)/36.9 (27.6-46.3) $\mu\text{g}\cdot\text{h/ml}$ . Therefore, in children less than 2 years of age, the target concentrations of first-line anti-tuberculosis agents were only achieved using the revised WHO dose recommendations. For INH, this was irrespective of the acetylator status. HIV-infected children had lower serum concentrations of all of the first-line drugs tested, but only for PZA did this difference reach statistical significance. Inter-patient variability of the pharmacokinetic parameters was high, leading to sub-therapeutic concentrations in some children while in others concentrations exceeded the recommended therapeutic range (esp. INH  $C_{max}$ ). This has possible implications on efficacy and toxicity, respectively. The study is limited by the modest sample size and the inability to correlate the pharmacokinetic parameters with treatment outcome. This study provided the first evidence for the implementation of the revised WHO guidelines for first-line anti-tuberculosis therapy in children younger than two years of age (118).

## Pharmacokinetics of Isoniazid, Rifampin, and Pyrazinamide in Children Younger than Two Years of Age with Tuberculosis: Evidence for Implementation of Revised World Health Organization Recommendations<sup>∇</sup>

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The World Health Organization (WHO) recently issued revised first-line antituberculosis (anti-TB) drug dosage recommendations for children. No pharmacokinetic studies for these revised dosages are available for children <2 years. The aim of the study was to document the pharmacokinetics of the first-line anti-TB agents in children <2 years of age comparing previous and revised WHO dosages of isoniazid (INH; 5 versus 10 mg/kg/day), rifampin (RMP; 10 versus 15 mg/kg/day), and pyrazinamide (PZA; 25 versus 35 mg/kg/day) and to investigate the effects of clinical covariates, including HIV coinfection, nutritional status, age, gender, and type of tuberculosis (TB), and the effect of NAT2 acetylator status. Serum INH, PZA, and RMP levels were prospectively assessed in 20 children <2 years of age treated for TB following the previous and the revised WHO dosage recommendations. Samples were taken prior to dosing and at 0.5, 1.5, 3, and 5 h following dosing. The maximum drug concentration in serum ( $C_{max}$ ), the time to  $C_{max}$  ( $t_{max}$ ), and the area under the concentration-time curve (AUC) were calculated. Eleven children had pulmonary and 9 had extrapulmonary TB. Five were HIV infected. The mean  $C_{max}$  ( $\mu\text{g/ml}$ ) following the administration of previous/revised dosages were as follows: INH, 3.19/8.11; RMP, 6.36/11.69; PZA, 29.94/47.11. The mean AUC ( $\mu\text{g} \cdot \text{h/ml}$ ) were as follows: INH, 8.09/20.36; RMP, 17.78/36.95; PZA, 118.0/175.2. The mean  $C_{max}$  and AUC differed significantly between doses. There was no difference in the  $t_{max}$  values achieved. Children less than 2 years of age achieve target concentrations of first-line anti-TB agents using revised WHO dosage recommendations. Our data provided supportive evidence for the implementation of the revised WHO guidelines for first-line anti-TB therapy in young children.

Isoniazid (INH), rifampin (RMP), and pyrazinamide (PZA) are routinely used to treat tuberculosis (TB) in children (23, 44). Recommendations for pediatric dosages are based on a small number of pharmacokinetic studies, few of which included children younger than 2 years of age. During early life, children experience significant changes in the relative sizes of their body compartments and their ability to absorb, metabolize, and excrete drugs (5, 17). These changes are greatest within the first 2 years of life (4). Most published studies on first-line anti-TB drugs in children have not analyzed differences between older and younger children or the effect of HIV coinfection. The pharmacokinetics of INH are further complicated by genetic polymorphisms of *N*-acetyltransferase type 2 (NAT2) in the metabolic pathway of INH, which influences INH concentrations (18, 26, 46).

In the absence of pharmacodynamic data for children and therefore data that demonstrate an association between serum drug concentration and clinical outcome, optimal anti-TB therapy should aim to produce the targeted serum drug concentrations that have been determined in adult pharmacokinetic and pharmacodynamic studies. For INH, the proposed optimal maximum serum drug concentration ( $C_{max}$ ) for therapy is 3 to 5  $\mu\text{g/ml}$  (15, 27). Target serum RMP concentrations in adults after a standard oral dose of 600 mg are in the range of 8 to 24  $\mu\text{g/ml}$ ; serum RMP concentrations below 8  $\mu\text{g/ml}$  are considered low, and those below 4  $\mu\text{g/ml}$  are considered very low (28, 29). There is more uncertainty regarding the optimal therapeutic serum PZA concentration. In adults, serum PZA concentrations are targeted at 20 to 60  $\mu\text{g/ml}$  (11, 28). However, in a recent study of adults, poor treatment outcome of pulmonary TB was associated with serum PZA concentrations of <35  $\mu\text{g/ml}$  (8).

Optimal anti-TB therapy is important in all children but particularly in young children (<2 years of age) and those HIV infected, where there is a high risk of progression to severe

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TABLE 1. Demographic, diagnostic, and clinical features of 20 children with TB

Demographic, diagnostic, or clinical feature	Value
<b>Demographic features</b>	
Mean age, yr (SD)	1.09 (0.49)
No. (%) of females	9 (45)
No. (%) <i>M. tuberculosis</i> or AFB <sup>a</sup> culture positive	9 (45)
No. (%) with household TB contact	16 (80)
No. (%) TST <sup>b</sup> positive	10/19 (53)
Mean no. of days on anti-TB treatment (SD)	106.0 (62.7)
No. (%) HIV infected	5 (25)
Mean no. of days on cART <sup>c</sup> (SD)	168.6 (97.7)
<b>Clinical features</b>	
No. (%) with pulmonary TB	11 (45)
No. (%) with extrapulmonary TB	9 (45)
No. (%) with TB meningitis	8 (40)
No. (%) with abdominal TB	1 (5)
No. (%) with hepatomegaly at inclusion	11 (55)
<b>Nutritional status</b>	
No. (%) with mass <3rd percentile for age	10 (50)
Mean Z score for wt (SD)	-1.74 (1.84)
Mean BMI <sup>d</sup> (SD)	16.02 (2.62)

<sup>a</sup> AFB, acid-fast bacilli.  
<sup>b</sup> TST, tuberculin skin test.  
<sup>c</sup> cART, combination antiretroviral therapy.  
<sup>d</sup> BMI, body mass index.

forms of TB. Recent studies have demonstrated that children require higher mg/kg body weight doses of anti-TB drugs to achieve the same serum drug concentrations as in adults (6, 10, 34, 38, 39, 41, 45). Consequently, the World Health Organization (WHO) has recently issued revised dosage recommendations (44) for the treatment of children with first-line drugs based on a systematic review of the literature. The WHO now advises giving INH at 10 mg/kg (range, 10 to 15 mg/kg), RMP at 15 mg/kg (range, 10 to 20 mg/kg), and PZA at 35 mg/kg (range, 30 to 40 mg/kg). This compares to the previous recommendation of INH at 5 mg/kg (range, 4 to 6 mg/kg), RMP at 10 mg/kg (range, 8 to 12 mg/kg), and PZA at 25 mg/kg (range, 20 to 30 mg/kg). All doses are advised for once-daily administration.

The serum INH, RMP, and PZA concentrations achieved following previous and revised WHO dosing guidelines for young children have not been examined. The aim of the present study was to investigate the pharmacokinetics of the first-line anti-TB agents INH, RMP, and PZA at previous and revised WHO-recommended doses for children younger than 2 years of age. We also investigated the effects of important clinical covariates, including HIV coinfection, nutritional status, age, gender, and type of TB, and the effect of NAT2 acetylator status.

**MATERIALS AND METHODS**

**Study population and setting.** This was a prospective, single-center hospital-based, observational intensive sampling pharmacokinetic study. The study was conducted at the Brooklyn Hospital for Chest Diseases (BHCD), Cape Town, South Africa, from May to October 2010. Eligible children were <2 years of age and on anti-TB treatment with a regimen that included INH and PZA, with or without RMP (all licensed for use in South Africa). Children had to be medically stable, and written informed consent was obtained from a parent or legal guardian, including permission for HIV testing. The study was approved by the Health

TABLE 2. Mean maximum serum concentrations ( $C_{max}$ ), area under the curve (AUC), and mean time until maximum serum concentration is reached ( $t_{max}$ ) with 95% confidence interval for INH, PZA, and RMP in children <2 years of age

Parameter	INH (n = 20)			PZA (n = 20)			RMP (n = 11)		
	5 mg/kg	10 mg/kg	P value <sup>a</sup>	25 mg/kg	35 mg/kg	P value <sup>a</sup>	10 mg/kg	15 mg/kg	P value <sup>a</sup>
$C_{max}$ (µg/ml)	3.19 (2.39–3.99)	8.11 (6.68–9.53)	<0.001 <sup>b</sup>	29.95 (26.16–33.73)	47.11 (42.64–51.58)	<0.001 <sup>b</sup>	6.36 (4.45–8.27)	11.69 (8.71–14.67)	0.005 <sup>b</sup>
$t_{max}$ (h)	0.70 (0.72–1.17)	0.52(0.64–1.12)	0.640	1.44 (0.98–1.41)	1.45 (0.96–1.41)	0.961	1.49 (1.07–1.91)	1.54 (1.16–1.93)	0.865
AUC (µg · h/ml)	8.09 (5.76–10.42)	20.36 (15.76–24.97)	<0.001 <sup>b</sup>	118.01 (101.33–134.70)	175.23 (155.50–194.96)	<0.001 <sup>b</sup>	17.78 (12.81–22.76)	36.95 (27.64–46.26)	0.006 <sup>b</sup>

<sup>a</sup> From paired *t* test comparing previous and revised doses.  
<sup>b</sup> Significant on the 0.05 level.

Research Ethics Committee of the Faculty of Health Sciences, Stellenbosch University, Stellenbosch, South Africa.

**Diagnosis of TB.** The diagnosis of TB was based on a history of TB contact, a positive tuberculin skin test (TST), a chest radiograph (CR) compatible with the diagnosis of pulmonary TB, and/or clinical signs of extrapulmonary TB. Whenever possible, the diagnosis was confirmed by culture of *Mycobacterium tuberculosis* from sputum or another clinical specimen. Children were treated according to the mycobacterial drug susceptibility test pattern from the child or that obtained from the most likely adult source case.

**Drug administration.** Fixed dosage combinations formulated for pediatric use are routinely prescribed for anti-TB treatment, as recommended by the South African National Tuberculosis Programme. Rimcure (INH at 30 mg, RMP at 60 mg and PZA at 150 mg) was manufactured by Sandoz SA Pty. Ltd., Spartan, South Africa. The doses given were calculated according to body weight at both WHO recommendations. For an adequate dose of INH, tablets (or fractions of tablets, respectively) containing INH at 100 mg (Be-Tabs Isoniazid; Be-Tabs Pharmaceuticals Pty. Ltd., Roodepoort, South Africa) were added. Fractions of 500-mg PZA (Pyrazide; Sanofi Aventis, Johannesburg, South Africa) and 100-mg INH tablets were given to children not receiving RMP. All formulations used were approved by the South African Medicines Control Council. Tablets were routinely crushed and dissolved in 2 to 5 ml of water and orally administered. All children were supplemented with pyridoxine and multivitamin syrup. Antiretroviral therapy (ART) consisted of two nucleoside reverse transcriptase inhibitors (lamivudine and stavudine) and a protease inhibitor (lopinavir/ritonavir). This was boosted with extra ritonavir if the child was prescribed RMP. Trimethoprim-sulfamethoxazole was given to all HIV-infected children.

**Pharmacokinetic sampling.** The first pharmacokinetic assessment was performed at 2 weeks or more following the initiation of anti-TB treatment. Two days prior to the assessment, the child was prescribed PZA at the previous WHO dosage of 25 mg/kg, due to the long half-life of the drug. On the morning of the assessment, the child was fasted for 4 h prior to receiving medications. An intravenous catheter was inserted, and a baseline blood sample was taken. The child was given INH, PZA, and, if appropriate, RMP at the previous WHO dosages. Further sampling took place at 0.5, 1.5, 3, and 5 h after dosing. Blood samples were collected in EDTA-coated tubes and placed on ice immediately. Plasma was separated by centrifugation within 15 min and then deep-frozen until analysis. Children received breakfast only after the 1.5-h blood sample was taken. If the child was HIV infected, combination ART (cART) was given with breakfast, while for all children other treatment was given after the last blood sample was taken. Following assessment, children were prescribed TB medications at the revised WHO dosages. One week later, a second assessment of each child took place, using identical methods but using the revised WHO dosages.

**Laboratory sampling.** The serum INH and PZA concentrations of plasma extract was determined by high-performance liquid chromatography (HPLC) and UV detection. INH had to be derivatized with cinnamaldehyde to be detectable at 340 nm, while PZA could be measured directly at 269 nm. For both compounds, a solvent gradient of 50 mM phosphate buffer (solvent A) and a 1:4 acetonitrile-isopropanol mixture (solvent B) was used. The RMP concentration was determined by HPLC-tandem mass spectrometry technology with an  $m/z$  ratio of 823.3/95.2, a gradient of water and acetonitrile, both containing 0.1% formic acid, was used in this instance as previously described (36, 37). The lower limit of detection was 0.5  $\mu\text{g/ml}$  for both INH and PZA and 0.1  $\mu\text{g/ml}$  for RMP; the lower limit of quantification was determined at a concentration where the inter- and intraday variations were less than 5.0% ( $n = 10$ ) for each compound. For all three compounds, we found a linear calibration range of 8 spiked samples ( $R^2, >0.9950$ ).

Following centrifugation, the remaining blood cells were pooled and used for the extraction of DNA for *NAT2* genotyping. After preparation of genomic DNA by a salting-out procedure, the *NAT2* gene was analyzed by PCR and restriction fragment length polymorphism as previously described (22, 34). The *NAT2* gene was analyzed for single nucleotide polymorphisms defining the *NAT2*\*5, *NAT2*\*6, *NAT2*\*7, *NAT2*\*12, *NAT2*\*13, and *NAT2*\*14 alleles. According to the current NAT nomenclature, the wild-type allele is designated *NAT2*\*4, which together with the *NAT2*\*12 and *NAT2*\*13 alleles, defines the rapid-acetylator (F) status. Decreased or impaired *NAT2* enzyme activity is encoded by the mutant alleles *NAT2*\*5, *NAT2*\*6, *NAT2*\*7, and *NAT2*\*14, which define the slow-acetylator (S) status. Accordingly, individuals were classified as homozygous fast (FF), heterozygous intermediate (FS), or homozygous slow (SS) acetylators, depending upon the allele combinations observed.

**Pharmacokinetic parameters.** Study endpoints were the following standard parameters for each patient:  $C_{\text{max}}$  (the highest drug concentration measured),  $t_{\text{max}}$  (the time after drug administration taken to reach  $C_{\text{max}}$ ), and the area under

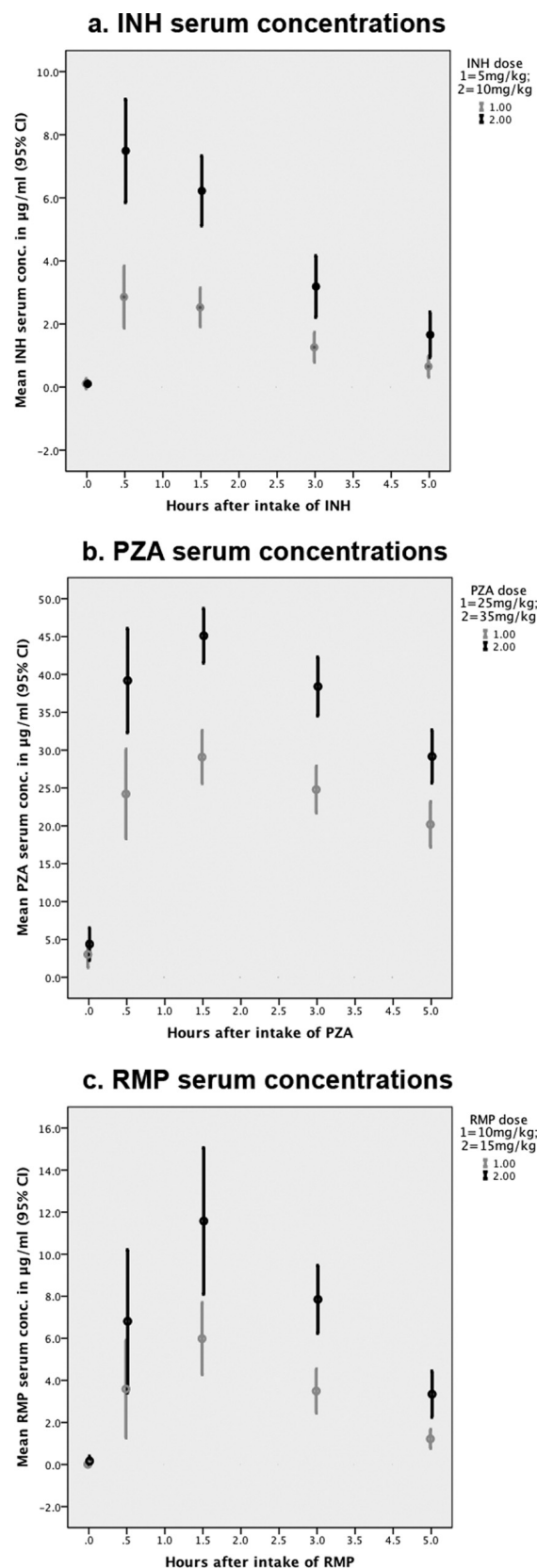


FIG. 1. Serum INH, PZA, and RMP concentrations following old and revised WHO dosage recommendations. Shown are means with 95% confidence intervals. Panels: a, INH; b, PZA; c, RMP.



the concentration-time curve (AUC) from 0 to 5 h of INH, PZA, and RMP. AUC was calculated according to the linear trapezoidal rule.

**Statistical methods.** Data were graphically checked for distribution and considered adequate to be analyzed using original (untransformed) values for all subsequent analyses. Data were summarized as means and 95% confidence intervals (95% CIs). Paired *t* tests were used to assess differences in pharmacokinetic parameters between the two doses. Independent *t* tests were used to assess the effect of HIV, type of TB, RMP coadministration, and gender on pharmacokinetic parameters. The effect of the *NAT2* genotype was evaluated using one-way analysis of variance (ANOVA). The effects of age and nutritional status (Z score for weight) was assessed by linear regression. The Z score for weight was calculated according to WHO growth charts (43). All tests were two sided, and the significance level was set at 5% without adjustment for multiple testing. Data were analyzed using SAS for Windows, version 9.2 (SAS Institute, Cary, NC).

**RESULTS**

**Patient characteristics.** Twenty children were studied (mean age, 1.09 years; standard deviation [SD], 0.49 years); 9 (45%) were female. Demographic, diagnostic, and clinical features are displayed in Table 1. At enrolment, all HIV-infected children (*n* = 5) were established on cART. Eleven children received a multidrug regimen including RMP, and nine children received treatment excluding RMP due to the presence of resistance. Eight children were treated for multidrug-resistant TB (i.e., resistance to INH and RMP with or without other drugs). INH at a dose of 20 mg/kg was continued in these children to overcome possible low-dose INH resistance.

**Differences between previous and revised WHO dosages.** Pharmacokinetic parameters for the two different dosages of INH, PZA, and RMP are shown in Table 2. Giving INH at a dose of 10 mg/kg resulted in a higher *C*<sub>max</sub> and a correspondingly higher AUC than giving INH at 5 mg/kg. No difference in *t*<sub>max</sub> was seen between the two INH doses. The same was true for the two doses of PZA and RMP: *C*<sub>max</sub> and AUC were significantly higher following the revised dose, while there was no difference regarding *t*<sub>max</sub> (Fig. 1). A high intraindividual variability was found: children with high serum drug concentrations at the lower INH, PZA, and RMP doses did not necessarily have comparatively high serum drug concentrations following the higher dose.

A low *C*<sub>max</sub> of INH (<3 µg/ml) was seen in half of the children (10 of 20) following an INH dose of 5 mg/kg, but in none after an INH dose of 10 mg/kg. For PZA, 15 of 20 children had a low *C*<sub>max</sub> (<35 µg/ml) following a dose of 25 mg/kg (2 children <20 µg/ml), while only one child had a concentration below this threshold following PZA at 35 mg/kg. Low (<8 µg/ml) RMP *C*<sub>max</sub>s were found in 6 of 11 children following a 10-mg/kg dose of RMP compared with three following a dose of 15 mg/kg. Very low (<4 µg/ml) RMP *C*<sub>max</sub>s were found in 3 of the 11 children following 10 mg/kg, whereas none had such low concentrations following dosing at 15 mg/kg.

**Influence of RMP on pharmacokinetics.** There were no differences in INH pharmacokinetics (with either 5 or 10 mg/kg) between children receiving RMP and those not receiving RMP (Table 3). Children receiving RMP achieved a significantly lower PZA *C*<sub>max</sub> and AUC following a PZA dose of 25 mg/kg than children not receiving RMP (Table 4). Following the higher PZA dose (35 mg/kg), the differences between children with and without RMP were diminished and not statistically

TABLE 3. Influence of HIV, type of TB, RMP, gender, age and nutritional status (weight for age Z score) on pharmacokinetic parameters of INH

Parameter	No.	<i>C</i> <sub>max</sub> (µg/ml)			<i>t</i> <sub>max</sub> (h)			AUC <sub>0-5</sub> (µg · h/ml)		
		5 mg/kg Mean (95% CI)	<i>P</i> value <sup>a</sup>	10 mg/kg Mean (95% CI)	<i>P</i> value <sup>a</sup>	5 mg/kg Mean (95% CI)	<i>P</i> value <sup>a</sup>	10 mg/kg Mean (95% CI)	<i>P</i> value <sup>a</sup>	
HIV status										
Negative	15	3.19 (2.18-4.19)	0.990	8.50 (6.69-10.32)	0.229	0.98 (0.71-1.24)	0.688	7.39 (4.98-9.80)	0.413	
Positive	5	3.20 (1.25-5.15)		6.91 (4.37-9.46)		0.86 (0.21-1.52)		10.21 (1.98-18.44)		
Type of TB										
Extrapulmonary	9	2.93 (1.89-3.97)	0.539	7.07 (5.17-8.98)	0.166	0.87 (0.50-1.23)	0.515	8.15 (3.86-12.44)	0.966	
Pulmonary	11	3.40 (2.06-4.75)		8.95 (6.72-11.18)		1.01 (0.68-1.34)		8.05 (4.88-11.22)		
RMP										
No RMP	9	3.31 (1.43-4.07)	0.791	8.89 (6.03-11.75)	0.337	0.91 (0.52-1.30)	0.738	7.24 (3.97-10.51)	0.491	
Included	11	3.09 (0.98-2.45)		7.46 (5.88-9.05)		0.98 (0.66-1.30)		8.79 (5.03-12.56)		
Gender										
Male	11	3.31 (2.05-4.56)	0.742	8.73 (6.87-10.60)	0.332	1.11 (0.79-1.44)	0.083	8.82 (5.13-12.51)	0.476	
Female	9	3.05 (1.82-4.28)		7.34 (4.76-9.92)		0.74 (0.42-1.07)		7.21 (3.81-10.60)		
Age	20		0.044 <sup>b</sup>		0.083		0.764		<0.001 <sup>b</sup>	
Nutritional status (Z score wt for age)	20		0.237		0.228		0.909		0.777	

<sup>a</sup> *P* values are from independent *t* test (HIV, TB, RMP, gender) or linear regression analysis (age, nutrition).  
<sup>b</sup> Significant at the 0.05 level.

TABLE 4. Influence of HIV, type of TB, RMP, gender, age and nutritional status (weight for age Z score) on pharmacokinetic parameters of PZA

Parameter	N	$C_{max}$ ( $\mu\text{g/ml}$ )				$t_{max}$ (h)				$AUC_{0-5}$ ( $\mu\text{g} \cdot \text{h/ml}$ )				
		25 mg/kg		35 mg/kg		25 mg/kg		35 mg/kg		25 mg/kg		35 mg/kg		
		Mean (95% CI)	P value <sup>a</sup>	Mean (95% CI)	P value <sup>a</sup>	Mean (95% CI)	P value <sup>a</sup>	Mean (95% CI)	P value <sup>a</sup>	Mean (95% CI)	P value <sup>a</sup>	Mean (95% CI)	P value <sup>a</sup>	
HIV status														
Negative	15	30.91 (26.15–25.67)	0.304	49.20 (43.62–54.77)	0.015 <sup>b</sup>	1.16 (0.91–1.42)	0.654	1.08 (0.79–1.37)	0.009 <sup>b</sup>	122.9 (101.6–144.3)	0.183	180.1 (154.0–206.3)	0.186	
Positive	5	27.05 (19.34–34.76)		40.84 (36.22–45.46)		1.28 (0.69–1.86)		1.50 (1.39–1.61)		103.2 (75.80–130.6)		160.5 (139.9–181.1)		
Type of TB														
Extrapulmonary	9	29.06 (22.29–35.83)	0.675	44.65 (35.77–53.53)	0.335	1.20 (0.84–1.57)	0.911	1.43 (0.75–1.53)	0.737	114.3 (84.97–143.6)	0.686	173.8 (140.6–207.0)	0.895	
Pulmonary	11	30.67 (25.43–35.91)		49.12 (44.06–54.19)		1.18 (0.87–1.49)		1.22 (0.89–1.55)		121.1 (97.53–144.6)		176.4 (147.2–205.6)		
RMP														
No RMP	9	34.07 (27.17–40.97)	0.049	50.70 (45.50–55.90)	0.117	1.12 (0.74–1.50)	0.541	1.25 (0.89–1.60)	0.616	138.2 (107.7–168.7)	0.029 <sup>b</sup>	182.7 (146.2–219.3)	0.498	
RMP included	11	26.58 (22.77–30.38)		44.17 (36.94–51.40)		1.25 (0.96–1.55)		1.13 (0.78–1.49)		101.5 (86.63–116.3)		169.1 (143.3–194.9)		
Gender														
Male	11	28.03 (24.31–31.75)	0.287	48.89 (42.84–54.95)	0.378	1.30 (1.03–1.57)	0.270	1.20 (0.87–1.52)	0.895	108.2 (92.19–124.1)	0.212	188.4 (164.8–212.1)	0.138	
Female	9	32.29 (24.38–40.19)		44.93 (37.03–52.83)		1.06 (0.67–1.46)		1.17 (0.76–1.57)		130.1 (95.55–164.6)		159.1 (123.6–194.6)		
Age	20		0.972		0.373		0.998		0.258		0.550		0.198	
Nutritional status (Z score wt for age)	20		0.529		0.676		0.787		0.693		0.679		0.416	

<sup>a</sup> P values are from independent t test (HIV, TB, RMP, gender) or linear regression analysis (age, nutrition).  
<sup>b</sup> Significant at the 0.05 level.

significantly different. The  $t_{max}$  of PZA was not influenced by RMP at either the previous or the revised dosage.

**Influence of patient characteristics on pharmacokinetics.**

Nutritional status, assessed by weight for age, body mass index, and weight-for-age Z score according to WHO growth charts, did not influence the pharmacokinetics of any of the drugs, irrespective of the dose (only data for Z scores are shown in Tables 3 to 5).

There were no differences in the pharmacokinetics of INH and RMP between HIV-infected and HIV-uninfected children (Tables 3 and 5). Following a PZA dose of 35 mg/kg, HIV-uninfected children achieved higher maximum serum PZA concentrations than did HIV-infected children after a shorter time ( $t_{max}$ , 1.08 versus 1.50 h) (Table 4). The AUC for HIV-uninfected children was also greater than that for HIV-infected children, but this difference was not significant. No difference in  $C_{max}$ ,  $t_{max}$ , or AUC was found in HIV-infected versus uninfected children following a PZA dose of 25 mg/kg.

Neither the type of TB (pulmonary versus extrapulmonary disease) nor gender affected the pharmacokinetics of INH, PZA, or RMP (Tables 3 to 5). Age had an impact on the pharmacokinetic parameters of INH, but not on those of PZA and RMP (Tables 3 to 5). At both INH dosages, younger age was associated with a higher maximum serum INH concentration (INH at 5 mg/kg,  $P = 0.04$ ; INH at 10 mg/kg,  $P = 0.22$ ) and a greater AUC (INH at 5 mg/kg,  $P = 0.004$ ; INH at 10 mg/kg,  $P = 0.007$ ). No difference was detected for  $t_{max}$ .

**The influence of acetylator status.** The *NAT2* genotype was distributed as follows: 8 children were FF acetylators (5 of 8 children <1 year old), 4 were FS acetylators (3 of 4 children <1 year old), and 8 were SS acetylators (4 of 8 children <1 year old). The maximum serum INH concentration and the AUC were greater for the SS acetylators than for the FF acetylators following INH dosing at both 5 mg/kg and 10 mg/kg (Table 6).

The impact of acetylator status is shown in Table 6. At both doses,  $C_{max}$  and AUC differ significantly between SS and FF acetylators. There were no differences found regarding  $t_{max}$  based on acetylator status.

At an INH dose of 5 mg/kg, 1 of 8 children with FF acetylator status, 1 of 4 with FS acetylator status, and 7 of 8 with SS acetylator status achieved  $C_{max}$ s above the recommended minimal therapeutic concentration for INH (3  $\mu\text{g/ml}$ ), while with INH at 10 mg/kg, all children achieved INH  $C_{max}$ s above the therapeutic target concentration.

**DISCUSSION**

We show that following administration of INH, PZA, and RMP at the revised WHO dosage recommendations, most children under 2 years of age had serum drug concentrations within the recommended therapeutic range. In contrast, at the previous WHO dosages, serum INH, PZA, and RMP concentrations were significantly lower and often below the recommended concentrations for anti-TB therapy. Serum INH concentrations in children are generally reported to be lower than in adults following the same mg/kg dose (3, 21, 34, 41). Although INH is the most extensively studied anti-TB drug, we are aware of only two reports of INH pharmacokinetics that included only children younger than 3 years (19, 30). In these

studies, INH was given at doses (15 mg/kg and 10 mg/kg, respectively) similar to those currently recommended by the WHO (10 to 15 mg/kg). The peak serum INH concentrations were comparable to those in our study and above the therapeutic minimum concentration of INH. In a previous study by our group, serum INH concentrations in children younger than 6 years of age receiving a dose of 4 to 6 mg/kg were significantly lower than in children prescribed a dose of 8 to 10 mg/kg (2.39 µg/ml versus 5.71 µg/ml) (21). The proportion of children with peak serum INH concentrations of <3 µg/ml following an INH dose of 5 mg/kg was even higher than in the present study (70% versus 50%).

In our study, coadministration with RMP had no influence on INH pharmacokinetics, while some inconsistent influence on PZA pharmacokinetics was found. Serum INH, PZA, and RMP concentrations were not associated with nutritional status, type of TB, or gender. The HIV status of the child did not affect INH and RMP pharmacokinetics, while the findings for PZA differed, dependent on the dosage given. Younger children achieved higher serum INH concentrations than older children, but for PZA and RMP, age did not influence the pharmacokinetic profile (within this 2-year age range). Children with a NAT2 fast-acetylator status had significantly lower serum INH concentrations than children with slow-acetylator status.

Rey et al. demonstrated a marked difference between slow and fast INH acetylators, with slow acetylators achieving higher serum INH levels. In further studies, a trimodal INH elimination was demonstrated (18, 26). Several studies have confirmed that fast- or intermediate-acetylator status is associated with lower serum INH concentrations than slow-acetylator status (21, 34). The distribution of acetylator phenotypes is population specific. This might explain the comparatively higher serum INH concentrations among Indian children, with mean serum INH concentrations of 4.75 µg/ml following an INH dose of 5 mg/kg and 10.1 µg/ml following a dose of 10 mg/kg (31). Routine determination of the acetylator status for clinical care is not feasible in resource-limited settings; however, with an INH dosage of 10 mg/kg, appropriate serum INH concentrations can be ensured in most children, irrespective of their acetylator status. Higher dosages might not be required in pediatric populations with predominantly slow acetylators and would unnecessarily expose patients to a higher risk of side effects.

Age-dependent elimination of INH has been demonstrated, with younger children eliminating INH more rapidly than older children and adults (21, 34, 46). This has been attributed mainly to a relatively larger liver size in proportion to total body weight in young children (3, 34). In our study, however, where all children were less than 2 years old, serum INH levels were higher in the younger children despite more children <1 year of age with the fast- and intermediate-acetylator phenotypes. In this very young age group, maturation of the NAT2 enzyme system might play an important role in INH metabolism. There is some evidence that maturation of acetylation occurs within the first 2 years of life and the cumulative percentage of fast acetylators increases with age, although the final acetylator status is genetically determined (25, 46). The effect of age in our study population might therefore be confounded by maturation of the NAT2 phenotype.

TABLE 5. Influence of HIV, type of TB, RMP, gender, age and nutritional status (weight for age Z score) on pharmacokinetic parameters of RMP

Parameter	N	$C_{max}$ (µg/ml)				$t_{max}$ (h)				$AUC_{0-5}$ (µg · h/ml)			
		10 mg/kg Mean (95% CI)	$P$ value <sup>a</sup>	15 mg/kg Mean (95% CI)	$P$ value <sup>a</sup>	10 mg/kg Mean (95% CI)	$P$ value <sup>a</sup>	15 mg/kg Mean (95% CI)	$P$ value <sup>a</sup>	10 mg/kg Mean (95% CI)	$P$ value <sup>a</sup>	15 mg/kg Mean (95% CI)	$P$ value <sup>a</sup>
HIV status													
Negative	7	6.85 (4.43-9.27)	0.520	12.52 (7.65-27.39)	0.350	1.612 (0.97-2.25)	0.390	1.56 (0.88-1.38)	0.901	18.41 (12.80-24.02)	0.774	36.12 (22.67-49.57)	0.810
Positive	4	5.49 (0.06-10.92)		10.24 (6.46-14.01)		1.283 (0.48-2.08)		1.52 (1.38-1.66)		16.68 (0.24-33.12)		38.40 (15.21-61.59)	
Type of TB													
Extrapulmonary	8	5.77 (3.23-8.31)	0.185	11.49 (7.19-15.80)	0.753	1.62 (1.09-2.15)	0.271	1.54 (0.97-2.11)	0.998	17.16 (10.10-24.23)	0.576	37.29 (23.97-50.62)	0.870
Pulmonary	3	7.93 (3.66-12.20)		12.21 (6.77-17.66)		1.14 (0.17-2.46)		1.34 (1.32-1.77)		19.44 (8.70-30.19)		36.03 (15.40-56.67)	
Gender													
Male	6	6.20 (3.81-8.60)	0.866	12.20 (9.09-15.31)	0.724	1.50 (1.43-1.57)	0.961	1.31 (0.87-1.75)	0.176	18.19 (11.28-25.09)	0.860	37.51 (28.27-46.75)	0.901
Female	5	6.53 (1.96-11.12)		11.09 (3.50-18.67)		1.48 (0.26-2.70)		1.82 (1.01-2.63)		17.30 (6.03-28.57)		36.28 (12.01-60.55)	
Age	11		0.240		0.232		0.351		0.153		0.415		0.527
Nutritional status (Z score wt for age)	11		0.344		0.774		0.437		0.217		0.832		0.270

<sup>a</sup>  $P$  values are from independent  $t$  test (HIV, TB, RMP, gender) or linear regression analysis (age, nutrition).

TABLE 6. Mean maximum serum INH concentrations and AUCs after administration of 5 and 10 mg/kg according to acetylator status

NAT2 status	No.	$C_{max}$				AUC <sub>0-5</sub>			
		5 mg/kg		10 mg/kg		5 mg/kg		10 mg/kg	
		Mean (95% CI)	<i>P</i> value <sup>a</sup>	Mean (95% CI)	<i>P</i> value <sup>a</sup>	Mean (95% CI)	<i>P</i> value <sup>a</sup>	Mean (95% CI)	<i>P</i> value <sup>a</sup>
Fast	8	2.16 (0.10–3.32)	0.139	6.69 (4.58–8.79)	0.650	4.28 (1.70–6.87)	0.196	12.89 (8.66–17.12)	0.416
Intermediate	4	3.64 (2.00–5.29)	0.031 <sup>b</sup>	7.48 (4.51–10.53)	0.039 <sup>b</sup>	7.14 (3.48–10.80)	<0.001 <sup>b</sup>	15.79 (9.80–21.77)	<0.001 <sup>b</sup>
Slow	8	4.00 (2.83–5.16)	0.714	9.84 (7.74–11.94)	0.189	12.38 (9.80–14.97)	0.024	30.12 (25.89–34.35)	<0.001 <sup>b</sup>

<sup>a</sup> From one-way ANOVA.<sup>b</sup> Significant at the 0.05 level.

There are no published studies of the pharmacokinetics of PZA specifically investigating children younger than 2 years of age. In two studies of older children, PZA was used at 35 mg/kg, the same dosage currently recommended by the WHO; in one study ( $n = 10$ ), the ages of the children studied ranged from 6 to 12 years and in the second ( $n = 27$ ), the median age was 5.7 years (12, 31, 44). The mean  $C_{max}$  in these studies was 40 to 50  $\mu\text{g/ml}$ , similar to that observed in our study. In a study of serum PZA concentrations across different age groups, PZA pharmacokinetics did not differ significantly with age (39). In our study, no influence of age on PZA pharmacokinetics was found. There is evidence that serum PZA concentrations increase linearly with increasing dose (45). In our study, serum PZA concentrations following a dose of 25 mg/kg were significantly lower than those following a 35-mg/kg dose. This is in contrast to a small study of PZA pharmacokinetics in children (>5 years of age) where serum PZA concentrations of 42.4 (SD, 3.3)  $\mu\text{g/ml}$  were reported following a dose of 25 mg/kg (13).

Of note is that the lower recommended limit of serum PZA concentrations (20  $\mu\text{g/ml}$ ) was achieved in 18 children following the previous WHO dosage recommendations and in all 20 following the higher dosages. However, mean serum PZA concentrations above 35  $\mu\text{g/ml}$  were only achieved following higher doses.

Most of the published pharmacokinetic studies of RMP in children were performed after a single dose of RMP and not after anti-TB therapy with RMP had been established. Due to self-induction of RMP metabolism, these findings might show higher serum drug concentrations than actually present during established anti-TB therapy. In a study with children 2 months to 5 years of age, low maximum serum drug levels (5.2  $\mu\text{g/ml}$ ) were found after a dose of 15 mg/kg (9). A more recent study of children (mean age, 3.8 years) established on RMP therapy assessed RMP parameters following a dose of 10 mg/kg (35). Serum RMP concentrations and AUCs were comparable to those in our younger study population. In both studies, the mean peak serum drug concentrations were low (<8  $\mu\text{g/ml}$ ). Again, our data confirm that the revised dosages recommended by WHO are necessary to achieve satisfactory serum RMP concentrations in young children. Although children have been documented to achieve lower serum RMP concentrations than adults in several studies, we could not find any influence of age within our young study population (1, 16, 35, 40).

Because RMP is a potent inducer of the cytochrome P450 system, concomitant treatment can result in a decreased half-life of a number of compounds (20, 42). Serum INH concen-

trations in our study were not affected by RMP, as has been previously described (24, 33). Our findings indicate that when PZA is given at a dose of 25 mg/kg, the concomitant use of RMP reduces the  $C_{max}$  of PZA. However, following a PZA dose of 35 mg/kg, RMP did not influence the PZA concentration. The clinical significance and mechanism of this finding are not clear. In previous studies, concurrent RMP did not influence the pharmacokinetics of PZA (2, 32). However, Bouhlal et al. reported significantly lower serum PZA concentrations in adults when the drug was given in combination with INH and RMP rather than as monotherapy (7).

HIV-infected patients have been reported to achieve lower serum anti-TB drug concentrations (8, 12, 14). This has been attributed to malabsorption caused by drug-drug interactions, gastrointestinal infections, or wasting, rather than the HIV infection itself. The different anti-TB drugs seem to be affected in various ways. Graham et al. demonstrated decreased serum PZA levels in Malawian HIV-infected children, while serum ethambutol concentrations were not lower in HIV-infected children than in non-HIV-infected children (12). In our study, HIV-infected children had lower serum concentrations of all of the first-line drugs tested, but only for PZA did this difference reach statistical significance. As only five children in our study were HIV infected, we may, however, not have been sufficiently powered to demonstrate such differences. The effect of HIV coinfection on the pharmacokinetics of anti-TB drugs in children requires further investigation.

**Conclusions.** Our data document low serum INH, PZA, and RMP concentrations following the previous WHO dose recommendations for children younger than 2 years of age. In contrast, administration of the revised WHO dose recommendations of INH at 10 mg/kg, RMP at 15 mg/kg, and PZA at 35 mg/kg results in satisfactory serum drug concentrations. Our data provide supportive evidence for the implementation of the revised WHO guidelines for first-line anti-TB therapy in young children.

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## Chapter 4

### Reviews on the use of second-line anti-tuberculosis drugs in children with tuberculosis: thioamides and fluoroquinolones

#### 4.1. Thioamides

##### **Abstract literature review thioamides (accepted for publication)**

Ethionamide (ETH) and prothionamide (PTH), both thioamides, have proven efficacy in clinical studies and form important components for multidrug-resistant tuberculosis treatment regimens and for treatment of tuberculous meningitis in adults and children. ETH and PTH are pro-drugs that, following enzymatic activation by mycobacterial EthA inhibit InhA, a target shared with isoniazid (INH), and subsequently inhibit mycolic acid synthesis of *Mycobacterium tuberculosis*. Co-resistance to INH and ETH is conferred by mutations in the mycobacterial *inhA* promoter region; mutations in the *ethA* gene often underlie ETH and PTH monoresistance. An oral daily dose of ETH or PTH of 15-20mg/kg with a maximum daily dose of 1,000mg is recommended in children to achieve adult-equivalent serum concentrations shown to be efficacious in adults, although information on optimal pharmacodynamic targets is still lacking. Gastrointestinal disturbances, and hypothyroidism during long-term therapy, are frequent adverse effects observed in adults and children, but are rarely life-threatening and seldom necessitate cessation of ETH therapy. More thorough investigation of the therapeutic effects and toxicity of ETH and PTH is needed in childhood TB while child-friendly formulations are needed to appropriately dose children.

## Introduction

The thioamides, ethionamide (ETH) and its propyl-analog prothionamide (PTH), are among the most frequently used oral second-line agents for the management of paediatric tuberculosis (TB). Although ETH is more widely available, ETH and PTH are considered interchangeable in TB chemotherapy regimens. ETH and PTH are recommended for the treatment of multidrug-resistant tuberculosis (MDR-TB; i.e. resistance to both isoniazid [INH] and rifampicin [RMP]), but also to treat drug-susceptible tuberculous meningitis (TBM) and miliary TB in some settings, due to good cerebrospinal fluid (CSF) penetration (170, 244, 245). Thioamides also exhibit activity against *Mycobacterium leprae* (246).

There is limited data on the efficacy, pharmacokinetics and safety of thioamides in children with TB, where treatment options especially for MDR-TB, are limited. We aimed to review the existing evidence for ETH and PTH use in the TB treatment in children to inform treatment recommendations for ETH and PTH, and highlight knowledge gaps for future research in children.

## Methods

A scoping review (247) was performed to assess the clinical evidence for thioamide use in children with TB. We searched PubMed without date or language restrictions (only English-, French-, Spanish- and German-language references were reviewed), using the following search terms: tuberculosis, thioamides, ethionamide, prothionamide, children/child, efficacy, therapy, treatment, resistance, pharmacokinetics, pharmacodynamics, safety and toxicity. The initial broad search using the terms “tuberculosis, children, ethionamide” retrieved 100 search results, using “tuberculosis, children, prothionamide (prothionamide)” 8 (9) results and “tuberculosis, children, thioamide” only 2 results. Narrowing the search by adding the following search terms to the initial broad ETH search resulted in the following subsets of articles: “efficacy” yielded 3 results, “therapy or treatment” yielded 84 results, “resistance” yielded 33 results, “pharmacokinetics” 3 results, “pharmacodynamics” 63 results, “safety” 2 results and “toxicity” 6 results. Abstracts were reviewed and full text articles retrieved for studies with relevant information. The reference lists of identified articles were searched for additional relevant reports. Adult data were reviewed if paediatric data

were lacking for each separate section, and were also included for comparison for pharmacokinetic reference points related to efficacy.

### **Mode of action**

ETH and PTH are structurally similar to INH, and like INH, cause actively growing bacilli to lose acid-fastness (248). Both INH and ETH (and PTH) are pro-drugs that, following activation, inhibit mycolic acid synthesis (249, 250). ETH is activated by the monooxygenase EthA, leading to the sulfoxide metabolite that has similar activity to the parent drug (251-253). It has been postulated that ETH and its sulfoxide are further transformed by EthA to a metabolite that accumulates intracellularly and acts as the final toxic compound (254). Activated ETH and PTH form adducts with nicotinamide adenine dinucleotide (NAD), which are inhibitors of the InhA enzyme in *Mycobacterium tuberculosis* (255-257). Inhibition of InhA results in inhibition of mycolic acid biosynthesis and cell lysis (258).

### **Mechanism of resistance and clinical application**

At an early stage of development it was noted that ETH was effective against strains of *M. tuberculosis* that were highly resistant to INH, but was less effective against strains that were “only slightly INH-resistant” (248, 259). More recent studies have shown that INH resistance is usually caused by mutations in either the *katG* gene or the *inhA* structural gene or the *inhA* promoter region (260). *KatG* encodes for catalase peroxidase, the enzyme which converts the pro-drug INH into its active form, and *katG* mutations are usually associated with high-level INH resistance. As *katG* is uninvolved in ETH activation, the efficacy of ETH is not affected by *katG* mutations. ETH shares the same final target with INH, and mutations in the *inhA* promoter region result in co-resistance to INH and ETH (256). However, INH resistance due to *inhA* promoter region mutations usually manifests as low-level resistance, and can often be overcome by giving INH at a higher dose (15-20 mg/kg), this being the rationale for the empirical use of high-dose INH combined with ETH in MDR-TB treatment, also in children (78, 79).

Resistance to ETH is further associated with mutations in the *ethA* gene encoding the activation of the drug, thus preventing drug activation (257).

*EthA* transcription is repressed by *ethR*, thus over-expression of *ethR* causes ETH resistance (261, 262). Less frequent mutations associated with ETH resistance are mutations in *mshA* and *ndh* genes. *MshA* encodes a glycosyltransferase, an enzyme involved in mycothiol biosynthesis. Mycothiol promotes ETH activation via EthA. Therefore mycothiol biosynthesis might be essential for ETH susceptibility in *M. tuberculosis* (263). Mutations in *ndh* gene result in increased intracellular NADH concentration, competitively inhibiting the binding of INH-NAD and ETH-NAD, therefore leading to co-resistance of INH and ETH (264).

For phenotypic drug susceptibility testing (DST) critical concentrations against *M. tuberculosis* using BACTEC MGIT 960 system of ETH 5.0µg/ml and PTH 2.5µg/ml and using BACTEC 460 of ETH 2.5µg/ml and PTH 1.25µg/ml have been proposed (265). For Middlebrook 7H11 agar, the critical concentration is 10µg/ml (266). Using this ETH concentration of 10µg/ml, isolates with *inhA* mutations were not detected by phenotypic testing in a surveillance study in children with MDR-TB, indicating that this critical concentration may be too high (10). Furthermore, ETH DST is notoriously unreliable, partly because the change in minimal inhibitory concentrations (MICs) associated with resistance is small, and partly because the drug is thermolabile. Therefore, molecular testing of ETH resistance might be specifically helpful in future, although not available in routine care settings (267, 268). The correlation between mutations conferring ETH resistance and the MIC warrants further studies. In a study from the European region on 137 drug resistant *M. tuberculosis* isolates, mutations in *inhA*- or *ethA*-gene (or both) could be identified in all 57 (42%) ETH-resistant isolates, but the level of resistance in phenotypic testing varied (269).

Regional differences in ETH resistance exist, but there is a global trend towards increasing resistance to thioamides, with as many as 50-75% of MDR-TB isolates having ETH resistance in some populations (10, 270-274). Therefore the use of thioamides might be precluded in some populations. In case of ETH resistance caused by *inhA* mutations, the use of ETH boosters may be an option for ETH treatment in the future (275, 276). These boosters enhance expression of EthA by inhibiting binding of the EthA regulator EthR, and have thereby shown to enhance the activity of ETH in *M. tuberculosis* in *in vitro* and in murine studies, but have yet to be studied in humans (277).

Furthermore, optimal serum drug concentrations should be achieved in order to minimize the risk of inadequate killing and the emergence of resistance.

### **Efficacy against *M. tuberculosis***

#### *Efficacy in in vitro studies*

*In vitro*, more than 99% of strains were inhibited at ETH concentrations between 0.3-1.25µg/ml (MIC) in 7H12 broth, while at higher concentrations ETH showed bactericidal activity. At ETH concentrations of 2.5-5.0µg/ml in 7H12 broth >99% of bacilli were killed (minimal bactericidal concentration, MBC) (72). Further studies confirmed bactericidal activity of ETH and PTH against *M. tuberculosis*, with PTH MICs often reported as half that of ETH (265, 278, 279).

#### *Efficacy in animal studies*

In an early report on studies in guinea pigs, increasing the daily ETH dose from 10mg/kg to 20mg/kg, led to anti-TB activity of ETH close to that of INH at 5mg/kg (280). In murine studies, ETH and PTH at dosages of 25mg/kg were very effective in preventing resistance to INH without further increasing INH bactericidal activity (281). In the mouse model, ETH alone had activity against *M. tuberculosis* less than that of INH at an equivalent dose (282). Following 4 weeks of therapy (5 days/week) the log colony forming units (CFUs)/lung for control, ETH 25mg/kg, ETH 50 mg/kg, ETH 75 mg/kg and INH 25 mg/kg were: 8.01, 6.67, 6.79, 6.58, and 5.59 respectively (282). In a further murine model, ETH at a dose of 125mg/kg showed activity against a clinical MDR-strain susceptible to ETH (MIC 2.0µg/ml), while ETH at a dose of 50mg/kg had no benefit (283).

#### *Efficacy in adult studies*

Shortly after the discovery of ETH activity in *M. tuberculosis in vitro* and in animal studies, it was evaluated in a number of two- and three-drug regimens in adult patients with TB. ETH showed favorable activity in the 'reversal of infectiousness' and in



prevention of drug resistance in companion drugs in a study utilizing a dosage of 1 g daily given in 4 doses of 250 mg or two doses of 500 mg in adults (284). If given as ETH monotherapy, loss of susceptibility against ETH has been reported (284). In an early study, investigating the efficacy of PTH in pulmonary TB, serial counts on CFU of *M. tuberculosis* in sputum culture were performed. Patients were either treated with PTH monotherapy at a dose of 500mg or 1,000mg, or with combination therapy of INH (10mg/kg) and PTH (1.0g). Following 14 days of PTH monotherapy at both dosages, the number of CFU in sputum were reduced to about a third, while in the INH/PTH group, a reduction of CFU of >80% was found (285).

In a study of 7 adult patients with sputum acid-fast bacilli (AFB) smear-positive TB, ETH, accompanied by streptomycin (SM) was given in a daily dose of 1,000mg divided in 4 doses. The full dose was achieved by increasing the dose over a week and was well tolerated (286). At 5 months, all 7 patients were culture-negative and ETH appeared to prevent the development of SM resistance (286). Another study on ETH (1,000mg/d in 4 doses) in combination with INH in previously untreated TB patients showed culture conversion at 6-months of 97% and at 12-months of 100% in those patients completing therapy (287). Because the rate of intolerance (mainly gastrointestinal) of ETH was high, studies investigating an oral dose of 750 (divided in three doses) and 500mg daily (divided in two doses) followed (288, 289). Efficacy of combination therapy with INH and ETH even at a dose of ETH 500mg was high (sputum culture conversion 100% after 7 months), without development of resistance. Following a dose of ETH 1,000mg, 750mg and 500mg, treatment with ETH had to be stopped due to adverse events in 31%, 18% and 8%, respectively (287, 289).

In a direct randomized comparison in 276 patients, the response to 6 months of treatment with ETH 500mg twice daily and SM did not differ significantly from that of INH and SM (also given for 6 months) (290). Bacteriological conversion at 4 months occurred in 80% of the INH/SM group and in 74% of the ETH/SM group. With regard to the emergence of resistance to SM, there was no difference between INH and ETH in efficacy (290). Several other trials, mainly in adult patients polyresistant to INH, *para*-aminosalicylic acid (PAS) and SM, showed some activity of ETH in combination with one or two other drugs (mostly cycloserine and pyrazinamide [PZA]) (291-293).

In a study on PTH given as either mono-therapy at 500mg or 1,000g daily or in combination with INH (PTH 1,000mg +INH 10mg/kg) for 90 days, sputum cultures became negative in 71%, 70.5% and 74%, respectively (285). Although culture negativity after 90 days was similar following therapy with PTH 1,000mg and 500mg, it occurred slower with PTH 500mg, with a high bacterial load in those patients remaining culture-positive (285).

In a comparative study, PTH and ETH both at a dose of 500mg daily (divided in 2 doses), were equally effective in combination therapy with INH and SM. Sputum cultures after six months treatment were negative in 98% of patients treated with ETH and 96% with PTH, respectively (294).

Nonetheless, gastrointestinal intolerance is a significant problem and has prevented ETH and PTH from playing a major role in first-line regimens. With the growing burden of MDR-TB, interest in these agents has been rekindled. In a meta-analysis of 9,153 patients with MDR-TB, treatment success was associated with the use of ETH/PTH in a multi-drug regimen (beside other factors) (295).

#### *Efficacy in paediatric studies*

Information on the value of ETH and PTH in childhood TB is limited. In an early anecdotal report on 225 children, it was stated that in 30% the treatment outcome results were better in children treated with ETH 15-25mg/kg in combination with INH than in those treated with standard therapy at that time (INH plus PAS or SM); ETH was tolerated well (296). In a study on 34 children with bacteriologically confirmed TB, culture conversion was achieved within 2 months of treatment with ETH, mainly accompanied by INH and radiographic improvement found in 98% (297). In one study ETH was given rectally at 12-35mg/kg (mean 15mg/kg) together with oral INH in 30 children; tolerability was good, and the combination of rectal ETH and oral INH was found at least as good as INH plus SM (298).

Two studies on 95 and 187 children with TBM, respectively, report using ETH at a dose of 20mg/kg as part of an intensified short-course regimen together with INH, RMP and PZA for 6 months (299, 300). The rationale for using ETH as a fourth drug in TBM



treatment is that after meningeal inflammation has subsided, RMP only poorly penetrates into the CSF and ethambutol or SM almost not at all. It is therefore likely that only INH, ETH and PZA reach their MIC in the CSF, which can be problematic in areas with high rates of INH resistance (170). The majority of these children were safely treated with this intensified short-course treatment with a very low relapse rate and low mortality (299, 300). In the more recent study, the overall mortality was 3.8%, and even in children with severe disease (stage III) mortality of 7.8% (5/64 children) was low (300). Good treatment outcome was seen in 100% of children with stage I disease, 97% with stage II and 47% with stage III disease, respectively. Therefore more than half of the children with stage III disease had severe developmental and neurological sequelae. Of note, using this treatment regimen for TBM, INH mono-resistance was not associated with poorer treatment outcome (300, 301).

A systematic review of children treated for MDR-TB reported a pooled treatment success of 81.67%; ETH formed part of the treatment regimen in all studies included, (66), therefore the individual role of ETH could not be ascertained. In a retrospective study on children with MDR-TB, ETH, given for a median duration of 13 (10.5-18) months, was part of the treatment regimen in 132 of 137 children (98.5%) who completed treatment (69). The overall treatment success was high (92%) (69).

In summary, ETH and PTH have significant activity against *M. tuberculosis* and prevent drug resistance in companion drugs in adult studies. *In vitro* studies show a bactericidal effect at higher doses, but due to intolerance doses of ETH/PTH of 500mg or divided doses have been used in adult studies. A dose of ETH 500mg seems sufficient to prevent development of drug resistance in the companion drugs (INH, SM). There is some evidence that ETH/PTH at higher doses might add to the overall efficacy of anti-TB therapy, but robust evidence on exact dosing is lacking. In children, ETH at doses between 15-20mg/kg is associated with good outcome as part of a multi-drug regimen against MDR-TB, or disseminated forms of drug-susceptible TB.

## **Pharmacokinetics**

### *Adult pharmacokinetic studies*

In adults, absorption of ETH and PTH from the intestinal tract is almost complete and

food and antacids appear to have little effect on this process (302, 303). Absorption can however be erratic, possibly due to altered gastrointestinal motility induced by the thioamides (160).

Protein binding is approximately 30% (262, 304). ETH is distributed throughout the body with a reported volume of distribution of 80 liters. In adults, peak serum concentrations ( $C_{max}$ ) of ETH occur at approximately 2 hours post-dose and have been reported to be between 1.9 to 2.5  $\mu\text{g}/\text{ml}$  following an oral dose of 500 mg (303, 305, 306). After a dose of 250 mg,  $C_{max}$  ranges from 0.9-1.1  $\mu\text{g}/\text{ml}$  and is similar between adult patients with and without acquired immunodeficiency syndrome (AIDS) (307). ETH concentrations close to serum concentrations are reached in the lungs, tuberculous lesions (308), and in the CSF (170, 244, 245). Considerably higher concentrations have been found in pulmonary epithelial lining fluid than in serum or lung alveolar cells (307). Significant variation in serum concentrations occurs, both in individual patients and between patients (309, 310). Bioavailability of ETH in TB patients has been reported to be lower compared to healthy volunteers consistent with a higher clearance and volume of distribution of ETH in TB patients. Table 1 summarizes the available data on ETH pharmacokinetics in adults and children.

In patients receiving PTH at a mean dose of 6mg/kg as part of MDR-TB treatment, a mean  $C_{max}$  of 2.2  $\mu\text{g}/\text{ml}$  occurred after approximately 3.6 hours (311). This differs from an earlier report in healthy volunteers, where following a dose of PTH 250mg a  $C_{max}$  of 1.0-1.5 and a time to  $C_{max}$  ( $T_{max}$ ) of about 2 hours have been reported (312). In the same study,  $C_{max}$  for ETH was about 1.8 times higher than for PTH, with the same ratios being observed for the sulfoxide metabolites (312). The clinical implication of this finding is unclear.

The first step of thioamide metabolism in the liver is transformation to sulphoxide metabolites by flavin-containing monooxygenase (FMO) in much the same manner as *M. tuberculosis* bioactivation of this pro-drug by EthA (313). Human FMOs appear to carry out the first reaction to the sulfenic acid more readily than the bacterial enzyme, but are slow to S-oxygenate a second time (313). Following oral administration of ETH intestinal (primarily FMO1) and liver FMO (FMO3) probably play an important role in the pharmacokinetics of this pro-drug and certainly in its demonstrated hepatotoxicity (313). The sulphoxide metabolite is thought to be responsible for hepatotoxicity of ETH

(251). In human lungs, FMO2 is expressed in high levels. A genetic polymorphism exists, resulting in FMO2 being inactive in individuals of Caucasian and Asian descent, while in 27% of individuals of African descent the active form of FMO2 is expressed (313). The influence of FMO2 genetic polymorphism on activity and/or toxicity of ETH in the lung is not yet known. These monooxygenases have many properties in common with cytochrome P450 system (CYP 450) and often have overlapping substrate specificities (313). RMP, commonly used with ETH, is a potent inducer of hepatic and intestinal P450 enzymes and information on the influence of RMP co-administration on ETH pharmacokinetics is limited.

Only a very small proportion of thioamides or their sulphoxide metabolites is recovered in urine or faeces, and there is still much unknown about ETH/PTH elimination (101, 258, 312, 314).

In order to overcome gastrointestinal intolerance, the use of ETH suppositories has been evaluated. The area under the time concentration curve (AUC) following rectal ETH application was only 57.3% of oral application and the  $C_{max}$  33%, respectively (315), making this a less attractive dosing option.

#### *Paediatric pharmacokinetic studies*

There is limited information on the pharmacokinetics of ETH in children. Two children were included in the study by Zhu et al., and received a dose of ETH 250mg orally: a 12.3 year old ( $C_{max}$  of 0.48  $\mu\text{g/ml}$ , AUC of 1.00  $\mu\text{g-h/ml}$ ) and a 6.7 year old ( $C_{max}$  of 1.11  $\mu\text{g/ml}$ , AUC of 9.88  $\mu\text{g-h/ml}$ ) (305).

In the first paediatric-specific pharmacokinetic study of ETH in children, 31 children overall were included, aged 3 months-<2 years, 2-<6 years, and 6-12 years of age receiving the World Health Organization (WHO) recommended dosage of ETH (15-20 mg/kg/day) (310). Of the 31 children (median age 2.8 years), 12 (39%) children were HIV-infected and 16 (52%) had pulmonary TB. Children younger than 2 years of age had significantly lower ETH exposure ( $C_{max}$  and AUC) compared to older children receiving the same mg/kg dose. This may imply a need for a higher dosage in this age group. HIV co-infection was associated with reduced exposure (AUC), but not with delay in absorption or elimination. RMP co-administration, nutritional status or duration of

treatment did not affect the pharmacokinetics of ETH. Following a standard oral dose of ETH 15-20mg/kg, the mean  $C_{max}$  of ETH was above 2.5µg/ml in all age groups, although a low serum  $C_{max}$  (<2.5 µg/ml) was found in 7 children (5 were <2 years of age) following 4 months of therapy; inter- and intra-individual variation was substantial (310). Preliminary data from an ongoing study in the same study setting showed that following an ETH dose of 20mg/kg in 34 children, younger children had higher serum concentrations compared to older children and overall ETH serum concentrations were higher than found by Thee et al (316). This difference in findings from the same study setting could be attributed to higher dosing and a different pharmacokinetic sampling scheme used, different laboratory assays, as well as to a potential crushing of tablets in the younger age group, which may alter bioavailability. In the follow-up study, HIV-infected children were also exposed to lower serum concentrations than HIV-uninfected children (median  $AUC_{0-8}$  21.9 vs 28.1µgh/ml).

For treatment of TBM in children, an ETH doses of 20mg/kg rather than 15mg/kg is needed to reach ETH concentration in the CSF >2.5µg/ml in the majority of children (245).

In conclusion, an ETH dose of 15-20mg/kg in children seems appropriate to achieve serum concentrations found in adults following a standard oral dose of 500-750mg, although HIV-infected children seem to be at risk for lower exposure. There are no child-friendly formulations of ETH, which is highly unpalatable, and the influence of crushing/breaking tablets on its bioavailability has not been systematically assessed. ETH suppositories use is associated with reduced bioavailability in adults, but paediatric data are lacking. At present, we are not aware of any planned or completed pharmacokinetic studies of PTH in children.

### **Pharmacodynamics**

There is limited data on the pharmacodynamics of thioamides. For anti-TB therapy, a targeted ETH  $C_{max}$  for susceptible strains of *M. tuberculosis* have been proposed at 2.5µg/ml (317). In therapeutic drug monitoring, an ETH  $C_{max}$  between 1 and 5 µg/ml, following a dose of 250-500 mg, are recommended targets (21, 160). Broth-determined MICs of ETH for drug-susceptible *M. tuberculosis* strains were 0.60-2.5µg/ml (317). Therefore a  $C_{max}$  target of 2.5µg/ml has been proposed. *M. tuberculosis* strains with an

MIC <1.25µg/ml are classified as very susceptible and therefore an ETH  $C_{max}$  of 1.0µg/ml might be efficacious against these strains. ETH has shown bactericidal activity at higher concentration of 2-5µg/ml *in vitro* (72).

In a study establishing a population pharmacokinetic model for ETH pharmacodynamic indices, such as the ratio of ETH  $C_{max}$ / MIC or AUC/MIC, values were calculated using a MIC against drug-susceptible *M. tuberculosis* of 1.0 µg/ml (305). Only a dose of 750mg/day achieved concentrations above the MIC for 3.6 hours, while concentrations following doses of ETH 500mg once or twice daily or 250mg twice or three times daily did not reach the MIC value. Daily ETH doses of more than 500mg are therefore required to achieve an appropriate AUC/MIC in adults (305). Nevertheless, pharmacodynamic targets of ETH in anti-TB treatment have not been prospectively assessed and more research is needed given its importance in existing and potentially, in new drug regimens.

### **Adverse effects**

The main adverse effect of thioamides is gastrointestinal intolerance resulting in nausea, vomiting, metallic taste, anorexia, abdominal discomfort, diarrhoea, weight loss, and hepatotoxicity (see table 2.). Gastrointestinal adverse effects of the thioamides are dose-related. In early studies on adults with TB, gastrointestinal disturbances occurred in about half the patients taking ETH or PTH at an oral dose of 500 mg, while following a dose of 1,000 mg, almost all patients reported gastrointestinal intolerance (290, 318-320). Gastrointestinal intolerance is often transient or at least improves after two to four weeks of therapy (284). To improve tolerability, divided daily doses can be given and then gradually adjusted to a single daily dose (195, 245). The use of suppositories has been suggested (306, 320) but pharmacokinetic, long-term safety, and acceptability data are lacking for this dosing strategy. Symptoms of intolerance (although less) still occur with rectal administration in adults (321). In children tolerability following rectal ETH administration has been demonstrated to be very good for short-term use. In 30 children rectal ETH at a mean dose of 15mg/kg (12-35mg/kg) was administered. Only 3 children experienced mild gastrointestinal disturbances at the beginning of therapy, which subsided during continuation of therapy (298). Following intravenous administration, ETH as well as its sulphoxide are excreted in gastric juice, possibly

leading to the observed gastrointestinal intolerance of ETH occurring despite rectal or intravenous administration (322).

The effect of supplementation with vitamin B complex on the tolerability of ETH/PTH is controversial. While in some studies, vitamin B supplementation has been reported to increase tolerability substantially and reduce nausea and vomiting in adults and children (323), other have not found a positive effect (324).

Originally, PTH was developed as an alternative to ETH, with a focus on reducing adverse effects. There is evidence that PTH might be better tolerated than ETH (324-326), although gastrointestinal disturbances and psychotoxic reactions still occur with PTH (285). Following an oral daily dose of PTH or ETH 750 mg, anorexia, nausea, vomiting was seen in 17/53 (32%) patients receiving PTH and in 24/48 (50%) receiving ETH; in 3 (6%) versus 9 (19%) cases, these adverse effects were classified as severe. These differences between ETH and PTH were not statistically significant (326). However, in an African cohort directly comparing adverse effects of ETH, PTH and placebo, there were more adverse effects with ETH than with PTH, although these differences only attained statistical significance in male and not in female patients. It is of interest that in this African cohort high doses of up to 1.75g of ETH/PTH were tolerated fairly well and most adverse effects were reported as being mild (324).

While asymptomatic elevation of liver transaminases occurs frequently during treatment with ETH or PTH, severe hepatotoxicity is rare (195, 246, 327); significant hepatotoxicity has been described in only about 2% of patients treated with ETH or PTH (195, 328-330). Nevertheless, occurrence of icterus was reported in 4 of 29 patients receiving 1,000mg/day PTH monotherapy, of which 2 patients had a history of alcohol-associated liver cirrhosis (285). In a study on treatment for leprosy, hepatotoxicity was reported in 4.5% of 596 patients. These findings differed from previous trials, in that ETH and RMP had been used for anti-leprosy treatment separately and hepatotoxicity was not mentioned. Therefore, the combination of ETH and RMP has been suggested as the toxic component (331). In a very early study on 59 children receiving ETH 10-30mg/kg (in combination with either INH, PAS or SM), no rise in liver transaminases above 50U/l was found (323) and the level of liver enzyme elevation did not correlate with increased ETH dose or with clinical features of nausea/vomiting (323). Similar findings were observed in a study on 107 children treated with ETH 20mg/kg. In 7

children ETH had to be stopped due to intolerability, of which 6 were gastrointestinal (297). Two studies on children with TBM treated with high doses of INH, RMP, PZA, and ETH found mildly elevated liver enzymes in 20% of cases (195, 300). Grade 3 or 4 drug-induced hepatotoxicity occurred in 5.6% of children, while no child was clinically jaundiced (300). In all cases treatment was temporarily changed to liver-friendly regimens until normalization of liver enzymes, and the original regimen was restarted without recurrence of hepatotoxicity (300).

During long-term therapy hypothyroidism may occur (332, 333) and has been recently recognized as an often undiagnosed but frequent adverse effect during MDR-TB treatment in adults and children (334, 335). ETH, which is structurally similar to methimazole, seems to inhibit thyroid hormone synthesis by inhibition of organification (336). From *in vitro* studies, it appears that ETH also inhibits the uptake of iodine into thyroid cells (333). Hypothyroidism was reported in 73/213 (34.2%) of adults with MDR-TB in a retrospective cohort study from Botswana, in 5/7 (71.4%) in a British cohort, and in 11/52 (21%) in an adult Indian cohort (335, 337, 338).

Combination therapy with *para*-aminosalicylic acid (PAS), which also may inhibit thyroid function (339), increases the risk of hypothyroidism (334, 338, 340, 341). In a study of adults with MDR-TB where 96.2% were treated with both ETH or PTH and PAS, 129 (69%) had a TSH >10mIU/L (334). It was stated that hypothyroidism due to ETH can be managed with thyroxine supplementation and was fully reversible after cessation of ETH therapy in adults (338). In a retrospective study on 137 children with TB treatment including ETH, abnormal thyroid function tests were reported in 79 (58%) children; of those, at least 41% were likely due to ETH treatment (340). HIV infection and concomitant treatment with PAS were associated with a higher risk for hypothyroidism (340). Of 137 children prospectively evaluated on MDR-TB treatment, 135 (98.6%) received ETH and 27 (19.7%) received PAS (69). Based on elevated thyroid-stimulating hormone (TSH) and low free thyroxine (fT4) levels, thyroxine supplementation was provided in 32 (23.4%) children. It is unclear whether the development of hypothyroidism on ETH and/or PAS is of clinical relevance in children. However, given the potential impact of hypothyroidism on neurodevelopment in young children, thyroid function tests should routinely be done in children on long-term ETH with or without concomitant PAS therapy, and thyroxine supplementation should be given if hypothyroidism is evident.



A further adverse effect associated with the use of thioamides is central nervous system toxicity. A high incidence (25-30%) of neuropsychotoxic effects has been described in adult patients receiving 1,000mg ETH or PTH (318, 319); however, these early studies do not describe how toxicity was assessed nor were adverse effects further specified or their severity graded. Nevertheless, there are several case reports of severe central nervous system toxicity including psychosis, seizures, behavioral disorders or pellagra-like encephalopathy (342-345). Nervous system toxicity may be responsive to niacin or pyridoxine (345). These have not been reported in children.

Other rare adverse effects of thioamides reported include gynaecomastia (346, 347), pellagroid dermatitis (348) and hypoglycaemia (349). The management of patients with diabetes mellitus may be more complicated in patients receiving ETH (350).

There are limited data on the use of ETH during breastfeeding and pregnancy (351, 352). In the mouse model, ETH has shown to reduce fertility and increase teratogenicity and mortality of newborn mice (353). There are single case reports on its possible teratogenic effect in humans, but this has never been studied systematically. In an early German national survey, no teratogenic effects were seen in 9 infants born to mothers receiving ETH during pregnancy (352). In a large study on 1082 delivering women with pulmonary TB of whom 22 women received ETH before or during pregnancy, developmental anomalies were described in 7 out of 23 (30.4%) children born to mothers receiving ETH compared to 35 out of 1082 (3.2%) children born to mothers on anti-TB therapy not including ETH. In 2 children with developmental anomalies (congenital heart disease, spina bifida) ETH was only given to the mothers in the month prior to labour and in two other children, the mothers had received ETH one year prior to pregnancy (354). It is thus questionable if these abnormalities can be attributed to ETH therapy, but the use of ETH is usually restricted during pregnancy.

## **Research agenda**

Although the efficacy of ETH/PTH against *M. tuberculosis* has been shown in *in vitro* studies, as well as in studies in animals and humans, pharmacokinetic and pharmacodynamic targets that correlate well with efficacy and prevention of resistance development in companion drugs are not known for drug-susceptible and drug-resistant TB in either adults or children.



Increased numbers of genetic mutations conferring ETH resistance in *M. tuberculosis* have been identified and are detected in patients with modern molecular testing. The correlation of molecular resistance testing with MIC needs further study.

In order to identify the optimal treatment for TBM, short intensified treatment regimens including ETH should be compared to standard WHO treatment recommendations in randomized controlled trials (355). A trial including high-dose RMP and levofloxacin is underway in India and Malawi (TBM-Kids study, PI K. Dooley).

Because tolerability is a major issue in the use of ETH/PTH with the currently available formulations, pharmacokinetic/pharmacodynamic and tolerability studies of new and child-friendly formulations (e.g. dispersible tablets or suppositories) are needed. For child-friendly formulations, issues of acceptability and palatability must be considered in addition to bioavailability and toxicity. In India, 125mg ETH tablet in addition to the standard 250mg tablets is available and a prototype of a scored dispersible ETH tablet is in development (356). This is a positive step forward, however substantial additional effort and resources will be needed to make this formulation widely available to children in the field.

Genetic polymorphism in enzymes involved in ETH metabolism (e.g. FM02) has been identified but their impact on bioavailability and tolerability of ETH is not known and should also be evaluated in future.

## **Conclusions**

ETH and PTH have substantial anti-TB activity proven in clinical studies and form important components of MDR-TB treatment regimens and in the treatment of disseminated forms of TB in adults and children. For children, an oral daily dose of ETH or PTH of 15-20mg/kg is recommended to achieve serum concentrations shown to be efficacious in adult studies, although information on the optimal pharmacodynamic targets is still lacking and recommendations are based on a limited amount of paediatric pharmacokinetic data. Gastrointestinal disturbances and hypothyroidism during long-term therapy are frequent adverse effects, but are rarely life-threatening and seldom necessitate cessation of ETH/PTH therapy. Child-friendly formulations of ETH/PTH are urgently needed; these need to be informed by rigorous pharmacokinetic and safety studies.

**Table 2. Ethionamide pharmacokinetic measures in adults and children**

Study	Method	Disease	HIV status	Age (years)	Number	ETH dosage	T <sub>max</sub> (h)	C <sub>max</sub> (µg/ml)	t <sub>1/2</sub> (h)	AUC
<b>Adults</b>										
Eule 1965 (306)	Microbiolog.	TB/healthy	NA	Adults	28	10mg/kg	Not reported	5.54	Not reported	Not reported
Jenner 1984 (312)	HPLC	Healthy	NA	Adults	9	250mg	2.0	2.0	2.1	Not reported
Auclair et al. 2001(303)	HPLC	Healthy	NA	36 (+/- 8)	12	500mg fasting	1.7 (0.75-3.0)	2.3 (0.99-6.1)	Not reported	10.0 (5.4-17)
						With orange juice	1.9 (0.50-3.0)	2.5 (0.47-5.2)	Not reported	9.6 (2.7-16)
						With food	2.6 (0.75-4.0)	2.3 (0.76-4.2)	Not reported	10.0 (4.1-18)
						With antacids	2.3 (0.75-4.0)	2.2 (0.68-6.2)	Not reported	10.4 (3.1-19)
Zhu M, et al. 2002(305)	HPLC	TB	NA	36.2 (12.2-57.6)	5	500mg (250-500mg)	2.00 (1.25-2.22)	1.35 (0.48-5.63)	1.63 (0.23-0.77)	2.80 (1.00-8.96)
		TB	NA	45.5 (6.7-79.0)	55	500mg (250-1,000mg)	Not reported	Not reported	1.94 (0.39-2.76)	3.95 (1.47-21.2)
		Healthy	NA		12	500mg	1.50	1.97	1.04	8.00

							(0.75-3.00)	(0.99-6.10)	(0.39-2.76)	(5.90-13.3)
Wyeth Inc. 2005(350)	Healthy	NA	Adults			250mg Film-coated tbl.	1.02 (0.55)	2.16 (0.61)	Not reported	7.67 (1.69)
						250mg Sugar-coated tbl.	1.49 (0.87)	1.48 (0.64)	Not reported	6.59 (1.76)
ETH review <i>Tuberculosis</i> 2008 (357)	--	--	NA	--	--	250mg Film-coated tbl.	Not reported	2,16	1,92	7,67

### Children

Thee et al. 2011 (310)	HPLC	TB	2	<2	5	15-20mg/kg	0.97 (0.9-1.0)	3.79 (1.59)	Not reported	7.84 (3.74)
			2	<2	5	15-20mg/kg (+RMP)	0.98 (0.9-2.1)	3.91 (1.61)	Not reported	8.75 (3.87)
			2	2-6	6	15-20mg/kg	1.11 (0.9-2.1)	4.43 (1.23)	Not reported	11.51 (6.90)
			2	2-6	5	15-20mg/kg (+RMP)	1.00 (1.0-1.1)	3.58 (1.36)	Not reported	8.72 (3.90)
			3	6-12	5	15-20mg/kg	2.00 (1.0-3.0)	3.62 (1.30)	Not reported	13.54 (4.96)
			1	6-12	5	15-20mg/kg	1.97	5.44	Not	15.04

					(+RMP)	(1.0-2.1)	(1.24)	reported	(5.50)
Hesseling 2012 (316)	HPLC	TB	<2	10	20mg/kg	1.80 (0.42)	7.66 (6.01-9.38)	Not reported	28.64 (24.58-33.41)
			2-5	11	20mg/kg	2.09 (0.70)	5.10 (4.37-7.48)	Not reported	25.36 (20.66- 39.06)
			6-15	13	20mg/kg	3.15 (1.14)	4.97 (4.35-6.27)	Not reported	26.07 (23.85- 32.62)

ETH: ethionamide, C<sub>max</sub>: maximum serum concentration, T<sub>max</sub>: time until C<sub>max</sub>, t<sub>1/2</sub>: half-life; AUC: area under the concentration time curve;  
HPLC: high performance liquid chromatography, RMP: rifampicin, NA: not applicable, tbl: tablet

**Table 3 Adverse effects associated with thioamide use in adults and children**

Author	Disease	Number	Drug	Dose	Concomitant drugs	Adverse events
ADULTS						
(318)	adults with chronic advanced TB	84 group A 14/20, group B 14/18, group C 10/15	ETH	group A: 500mg single dose or 250mg bd; group B 750mg or divided in 2 doses, group C 1,000mg single dose or 500mg bd	EMB, INH, PZA, CS	Group A 56.6%, group B 70%, group C 100%, mainly GI-intolerance, other: neuropsychotoxic reactions, skin-reactions, alopecia, goitre, hepatotoxicity; toxicity increased in patients receiving PZA
(319)	adults with chronic advanced TB	114; group A 20/25, group B 20/20, group C 15/20	PTH	group A: 500mg single dose or 250mg bd; group B 750mg or divided in 2 doses, group C 1,000mg single dose or 500mg bd	EMB, INH	70/114 (61.4%) patients with adverse effects, GI-intolerance occurred in 34.1%, 42.1% and 80% and neuropsychotoxic reactions in 4.8%, 15.8% and 25% of patients receiving 500mg, 750mg or 1,000mg PTH, respectively
(320)	adults with INH-resistant TB	82	ETH	1,000-1,250mg	KM, CA, PZA, EMB, CM	GI-intolerance in almost all patients in the beginning, disappearing after 2-3weeks of therapy classified as "important" in 18/82 (26%) patients, 1 hepatotoxic reaction; in 18/82 patients rectal administration of ETH
(290)	adults with TB	112	ETH	500mg bd	INH, SM	GI-intolerance leading to treatment interruption in 28/112 (25%) patients, hepatotoxicity in 11/112 (9.8%), 1 being jaundiced; neuropsychotoxic reactions 2/112 (2%)

						In 87/297 (29.3%) doses of ETH, 45/164 (27.4%) doses of PTH;
(324)	adults with TB		ETH/PTH	500-1,750mg	INH, SM	GI-intolerance 22%/23%, giddiness 10%/13%, headache 9%/4% following ETH/PTH, respectively, toxicity increased with dose, no effect of vit B supplementation
(326)	adults with TB	101 (53 PTH, 48 ETH)	ETH/PTH	375mg bd	INH, SM	GI-intolerance 17/53 (32%) and 24/48 (50%) for PTH and ETH, respectively, severe in 3 (6%) and 9 (19%); hepatotoxicity in 5/53 (9%) and 5/48 (5%) patients receiving PTH and ETH, respectively
(284)	adults with TB	29	PTH	1,000mg		Jaundice in 4/29 (.%)patients, of which 2 patients had alcohol-associated liver cirrhosis
(329)	adult with TB	1	ETH	500mg bd	SM	Jaundice
(330)	adults with TB	44	PTH	500-1,000mg	not stated	Elevated liver transaminases in 10/44 (22.7%) patients
(333)	NTM infection	1	ETH	1,000mg	RMP, EMB	Hypothyroidism with goitre
(332)	MDR-TB	1	ETH	250mg bd	not stated	Severe hypothyroidism
(338)	MDR-TB	52	ETH	250mg bd	not stated	11/53 /21.1%) with hypothyroidism, 3 with goitre, reversible after cessation of therapy, 1 patient not adherent to levothyroxine therapy died of myxoedema coma

(337)	MDR-TB	7	PTH	not stated	PAS+other	5/7 (71.4%) with hypothyroidism
(335)	MDR-TB	213	ETH	not stated	not stated	73/213 (34.3%) with TSH level >10U/l
(334)	MDR-TB	186	ETH/PTH (97.3% of patients)	not stated	PAS, PZA, CS, fluoroquinolone, 2nd- line injectable	129/186 (69%) TSH >10U/l; 100 (54%) had TSH >20U/l
(341)	MDR-TB	1	ETH	500mg+250mg/d	CS, OFX, PAS	TSH 28U/l, low thyroxine and low fT4-serum level
(343)	TB	1	ETH	750mg	INH, SM	severe psychologic changes after taking ETH for 1 year
(344)	TB	1	ETH	500mg	PAS, SM, INH	psychotic reaction, patient died after jumping from the window
(342)	TB	1	ETH	Not stated	SM, INH, TAZ	Psychotic reaction
(345)	TB	3	ETH	750mg	INH, CS, EMB, PAS	drug-induced encephalopathy with signs of myelopathy in two patients with symptoms similar to pellagra, rapid recovery with nicotinamide and other B group vitamin supplementation
(346)	MDR-TB	1	ETH	750mg	KM, OFX, EMB, PZA, PAS	painful gynaecomastia, subsiding after cessation of ETH
(347)	MDR-TB	1	ETH	250mg bd	Not stated	gynaecomastia, subsiding after cessation of treatment

(348)	MDR-TB/TBM	2	ETH	250mg bd	Not stated	pellagra-like skin lesions healing completely after administration of nicotinamide therapy
(349)	TB	1	ETH	750mg	INH, SM	patient died of severe hypoglycemia
<b>CHILDREN</b>						
(358)	TB	30	ETH	15 (12-35) mg/kg	INH+ vitamin B	3/30 children mild GI-intolerance subsiding with continuation of therapy, in 5/30 children transient leucopenia (3-4,000/ $\mu$ l), ETH administered rectally
(195)	TBM	56	ETH	15mg/kg	INH, RMP, PZA	Jaundice in 1/56 child, elevation of liver enzymes in 75-85% of children
(327)	TB	1	ETH	500mg	INH, SM, PAS	Patient died of acute hepatic failure
(296)	TB	59	ETH	10-30mg/kg	INH, SM or PAS + vitamin B	No rise in liver transaminases >50 IU/l
(297)	TB	107	ETH	20mg/kg	INH, SM or PAS	26/107 (24%) experienced adverse events, in 7 children leading to treatment interruption, mainly (6/7) because of GI-intolerance
(300)	TBM	184	ETH	20mg/kg	INH, RMP, PZA	8/143 (5%) children with drug induced hepatotoxicity



						In 79/137 (58%) children abnormal thyroid function tests;
(340)	TB/TBM	137	ETH	20mg/kg	INH, PAS, TZ, AMK, EMB	HIV infection and concomitant treatment with PAS associated with higher risk for hypothyroidism;
						thyroxine supplementation in 32 children

TB tuberculosis; MDR multidrug resistant; TBM tuberculous meningitis; ETH ethionamide; PTH prothionamide; INH isoniazid; RMP rifampicin; SM streptomycin; PZA pyrazinamide; EMB ethambutol; PAS *para*-amino salicylic acid; TZ terizidone; AMK amikacin; CS cycloserine; CM capreomycin; KM kanamycin; TAZ thioacetazone; OFX ofloxacin

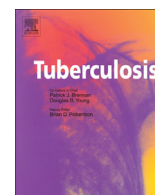
## 4.2. Fluoroquinolones

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## REVIEW

## Fluoroquinolones for the treatment of tuberculosis in children

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## SUMMARY

The fluoroquinolones are key components of current multidrug-resistant tuberculosis (MDR-TB) treatment regimens and are being evaluated in shortened treatment regimens as well as in the prevention of drug-resistant TB. The objective of this review was to identify existing evidence for the use of the fluoroquinolones ofloxacin, levofloxacin and moxifloxacin in the treatment of TB in children.

Existing data from *in vitro*, animal and human studies consistently demonstrate the efficacy of the fluoroquinolones against *Mycobacterium tuberculosis*, with superiority of levofloxacin and moxifloxacin compared to ofloxacin. *In vitro* and murine studies demonstrated the potential of moxifloxacin to shorten drug-susceptible TB treatment, but in multiple randomized controlled trials shortened fluoroquinolone-containing regimens have not been non-inferior compared to standard therapy. Resistance occurs frequently via mutations in the *gyrA* gene, and emerges rapidly depending on the fluoroquinolone concentration, with newer more potent fluoroquinolones less likely to develop resistance. Emerging data from paediatric studies underlines the importance of fluoroquinolones in the treatment of MDR-TB in children. There is a paucity of pharmacokinetic data especially in children <5 years of age and HIV-infected children; existing studies show substantially lower serum concentrations in children compared to adults at currently recommended doses, probably due to faster elimination. This has implications for optimizing paediatric treatment and for the development of resistance. Fluoroquinolone use has been restricted in children due to concerns about drug-induced arthropathy. The available data does not demonstrate any serious arthropathy or other severe toxicity in children. Although there is limited paediatric safety data for the prolonged treatment of MDR-TB, extended administration of fluoroquinolones in adults with MDR-TB does not show serious adverse effects and there is no evidence suggesting less tolerability of fluoroquinolones in children. Additional study of moxifloxacin and levofloxacin for TB treatment and prevention in children is an urgent priority.

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## 1. Introduction

The World Health Organization (WHO) estimated that in 2013 there were 550,000 new cases of TB in children <15 years of age [267]. Multidrug-resistant TB (MDR-TB; i.e. resistance to both rifampicin [RIF] and isoniazid [INH]) is an emerging epidemic. Model-based estimates suggest 32,000 children had MDR-TB in 2010 [112,206]. As many as 900,000 children were exposed to MDR-TB in 2012. These figures likely underestimate the true burden of childhood TB due to diagnostic challenges and poor recording and

reporting of TB in children [112,213]. New diagnostic tools, such as the Xpert MTB/RIF may increase the number of MDR-TB cases detected in adults and children, increasing the number of children needing MDR-TB treatment.

Fluoroquinolones are key components of current MDR-TB treatment regimens and are being evaluated as components of future shortened regimens for the treatment of drug-susceptible TB (DS-TB) [28,86,117,199]. They may also play an important role in the prevention of drug-resistant TB. Despite their increasingly prominent role in TB treatment, there is limited data on efficacy, pharmacokinetics (PK) and safety of the fluoroquinolones in children with TB. The objective of this review was to identify existing evidence for the use of the fluoroquinolones ofloxacin (Ofx), levofloxacin (Lfx) and moxifloxacin (Mfx) in the treatment of TB in children, to guide current treatment and highlight knowledge gaps for future research.

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## 2. Methods

We performed a scoping review [251] to broadly assess the evidence for fluoroquinolone use in children with TB. We searched PubMed without date or language restrictions (although only English-, French-, Spanish- and German-language references were reviewed), using the following search terms: tuberculosis, fluoroquinolone, ofloxacin, levofloxacin, moxifloxacin, children/child, efficacy, therapy, treatment, resistance, pharmacokinetics, pharmacodynamics, safety and toxicity. Abstracts were reviewed and full text articles retrieved for studies identified with relevant information. We reviewed the reference lists of identified articles for additional relevant reports and other sources known to the authors. *In vitro* and animal data were included to provide information on efficacy of fluoroquinolones and resistance. Adult data were reviewed if paediatric data were lacking, and also for comparison purposes.

## 3. Efficacy of fluoroquinolones in TB

The fluoroquinolone target in *Mycobacterium tuberculosis* is DNA gyrase (topoisomerase II) [150]. Inhibition of DNA gyrase disrupts bacterial DNA synthesis causing rapid cell death [107]. In adult TB patients, efficacy of anti-TB treatment can be monitored by sputum smear or culture conversion. In children, who frequently have paucibacillary disease, monitoring of response to treatment is challenging and is based on symptomatic improvement, weight gain, or regression of radiographic finding [233] in the absence of bacteriology. Given the lack of paediatric data on fluoroquinolone efficacy, we looked closely at evidence from *in vitro* and animal data as well as data from adult TB patients. As the principles of anti-TB treatment do not differ between adults and children, children are expected to respond as well or better, given similar drug exposures as adults [64].

### 3.1. *In vitro* activity of fluoroquinolones against *M. tuberculosis*

The good *in vitro* efficacy of the fluoroquinolones Ofx, Lfx and Mfx against *M. tuberculosis* has been extensively documented (Table 1). Several studies also demonstrate *in vitro* synergy of the fluoroquinolones given in combination with first-line and some second-line anti-TB drugs [21,102,142,184,186,187].

Fluoroquinolones have shown *in vitro* activity against intracellular and dormant *M. tuberculosis*, and therefore exhibit a sterilizing potential [52,75,108,130,155,202,220,232,243,244,250], although for Ofx data is conflicting [102]. The fluoroquinolones accumulate in macrophages and granulocytes with intracellular drug concentrations exceeding extracellular concentrations at least four-to five-fold (Mfx > Lfx > Ofx) [82,155,172,182,264]. Mfx had superior activity compared to Ofx and Lfx in three different *in vitro* models of RIF-tolerant persistent *M. tuberculosis*, predicting that Mfx may be able to shorten anti-TB treatment [108]. In contrast, Lfx was more active over 21 days than Mfx against *M. tuberculosis* in the dormant phase of an acid model [52,81].

Together, the *in vitro* data demonstrate that Ofx, Lfx and Mfx have bactericidal activity against metabolizing bacilli, with a relative potency against *M. tuberculosis* of Mfx > Lfx > Ofx; Lfx and Mfx show the greatest sterilizing potential.

### 3.2. Activity of fluoroquinolones in murine studies

In murine studies, Ofx has moderate activity against *M. tuberculosis* compared to newer fluoroquinolones [114,115,131,140,245], while Lfx and Mfx have dose-dependent bactericidal activity comparable or even superior to that of INH [9,113,114,123,134,153,215,220,275]. High

dose Lfx and Mfx showed similar activity after 2 months of treatment, but after 4 months, Lfx was inferior to Mfx in murine tuberculosis [3].

The potential of Mfx to shorten treatment suggested by *in vitro* studies was confirmed in mice. Substitution of Mfx for INH reduced the time needed to eradicate *M. tuberculosis* from the lungs of mice by up to 2 months [166], and relapse-free cure was established after 4 months of therapy with RIF, Mfx and pyrazinamide (PZA), compared to 6 months with standard treatment [167]. These studies have informed treatment-shortening trials in humans. However, recently completed trials using gatifloxacin (Oflotub) or Mfx (e.g. ReMox, RIFAQUIN) for treatment shortening could not confirm the potential to shorten TB treatment in humans [86,117,152].

Fluoroquinolones have also been studied in combination with second-line anti-TB drugs.

In drug-susceptible murine TB, Mfx in combination with different second-line drugs used for 9 months had bactericidal activity comparable to 6 months of the standard regimen [257]. Against MDR-strains in mice, the efficacy of Lfx and Mfx in combination with second-line agents were similar after 2 months of therapy, while relapse rates after 7 months of treatment were lower in the Mfx group [72].

Few studies have investigated the efficacy of fluoroquinolones in combination with newer anti-TB agents in the mouse model. A regimen consisting of two weeks daily RIF, INH, PZA and Mfx followed by a 5.5-months once weekly therapy with rifapentine, INH and Mfx was only slightly inferior to the 6-month standard regimen [139]. Mfx in combination with rifapentine also provided excellent sterilizing activity and shortened the time needed to prevent relapses [139,195,196]. Further evidence exists for the new anti-TB agents bedaquiline and PA-824 in combination with Mfx and PZA to shorten treatment with similar relapse rates compared to therapy with first-line agents in murine studies [10,165]. Recently, novel regimens to treat latent TB infection (LTBI) were identified in a murine model [132]. The combination of PA-824 and Lfx has been identified for the evaluation in future clinical trials of MDR prevention [132].

Murine studies confirmed the superior activity of Lfx and Mfx compared to Ofx against *M. tuberculosis* as demonstrated by *in vitro* studies. Evidence from the murine model further highlights the role of Mfx in possible shortening of TB treatment in combination with existing and novel anti-TB agents.

### 3.3. Activity in human TB

#### 3.3.1. Early bactericidal activity (EBA)

The EBA of an anti-TB agent is defined as the fall in log<sub>10</sub> colony forming units (cfu) of *M. tuberculosis* per ml sputum per day during the first days of treatment [60,63,118,218]. Table 2 summarizes EBA studies of Ofx, Lfx, and Mfx.

While the EBA of Ofx at 800 mg/day is lower than that of INH [32,218], Lfx at a daily dose of 1000 mg and Mfx at a dose of 400 mg show an EBA comparable to that of INH [87,92,119,176]. The mean EBA of the novel three-drug regimen consisting of PA-824/Mfx/PZA was comparable to that of a standard regimen (INH/RIF/PZA/ethambutol) [59].

#### 3.3.2. Drug-susceptible TB (DS-TB)

Early studies in TB patients utilized Ofx, as it was the most widely available fluoroquinolone until recently. In one of the first studies of fluoroquinolones in 13 TB patients, Ofx monotherapy resulted in reduced sputum cfu counts, with sputum culture conversion in five confirming the bactericidal activity of Ofx [247]. However Ofx resistance developed in all those not converting [246]. Two further studies provide additional evidence for the efficacy of

**Table 1**  
Minimum inhibitory concentrations (MIC) in µg/ml for ofloxacin, levofloxacin and moxifloxacin against *Mycobacterium tuberculosis*.

Source	<i>M. tb</i> strain	Middlebrook 7H11	Middlebrook 7H10	Lowenstein-Jensen medium	Bactec 460	Other
<b>Ofloxacin</b>						
Fenlon et al. 1986 [74]	Clinical isolates		1.0, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )			
Texier-Maugein, et al. 1987 [238]	Clinical isolates					Youmans broth; 1.0, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )
Gorzynski EA et al. 1989 [91]	Clinical isolates		1.3, 2.4 (MIC <sub>50</sub> , MIC <sub>90</sub> )			
Van Caekenberghe 1990 [254]	H37Rv, clinical isolates		0.5, 0.5 (MIC <sub>50</sub> , MIC <sub>90</sub> )			
Rastogi N, et al. 1991 [183]	H37Rv, clinical isolates	0.5, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )			0.5, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )	
Truffot-Pernot C, et al. 1991 [245]	H37Rv, Clinical isolates, DR and DS			1.0, 2.0 (DS), 2.0, 2.0 (DR) (MIC <sub>50</sub> , MIC <sub>90</sub> )		
Ji B, et al. 1991 [115]	H37Rv, Clinical isolates, DS	1.0, 2.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Tomioka H et al. 1993 [242]		0.78, 0.78 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Mor N, et al. 1994 [155]	H37Rv, Vertullo strain (MDR strain), Clinical isolates					7H12 broth; 0.5–2.0 (range MIC <sub>90</sub> )
Ji B, et al. 1995 [114]	Clinical isolates, DS	1.0, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Saito H, et al. 1995 [200]		0.78, 0.78 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Rastogi N, et al. 1996 [184]	Clinical isolates DS and DR				0.75–1.0 (range MIC <sub>90</sub> )	
Guillemin I, et al. 1998 [97]	H37Rv	1.0 (MIC <sub>90</sub> )				
Vacher S, et al. 1999 [250]		0.5, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Ruiz-Serrano MJ, et al. 2000 [197]	Clinical isolates, DS and DR		1.0, 2.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )			
Rodriguez JC, et al. 2001 [192]	Clinical isolates, DS	1.0, 2.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Hu Y, et al. 2003 [16]	H37Rv	0.71 (no growth)				
Singh M, et al. 2009 [216]	H37Rv, Clinical isolates, DS and DR			2.0 (MIC <sub>93</sub> )		
<b>Levofloxacin</b>						
Mor N, et al. 1994 [155]	H37Rv, Vertullo strain (MDR strain), Clinical isolates					7H12 broth; 0.25–1.0 (range MIC <sub>90</sub> )
Ji B, et al. 1995 [114]	Clinical isolates, DS	0.5, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Saito H, et al. 1995 [200]		0.39, 0.39 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Rastogi N, et al. 1996 [184]	Clinical isolates, DS and DR				DS 0.5; DR 0.5–0.75 (MIC <sub>90</sub> )	
Hoffner SE, et al. 1997 [105]	Clinical isolates, DS and DR	1–2 (range MIC <sub>90</sub> )				
Guillemin I, et al. 1998 [97]	H37Rv	0.5 (MIC <sub>90</sub> )				
Gillespie SH, Billington O. 1999 [85]	Clinical isolates			0.25, 0.5 (MIC <sub>50</sub> , MIC <sub>90</sub> )		
Ruiz-Serrano MJ, et al. 2000 [197]	Clinical isolates, DS and DR		0.5, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )			
Tomioka H, et al. 2000 [244]	Clinical isolates, DS	0.39, 0.39 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Rodriguez JC, et al. 2001 [192]	Clinical isolates, DR	3.13, 6.25 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Rodriguez JC, et al. 2001 [192]	Clinical isolates, DS	0.5–1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Rodriguez JC, et al. 2002 [193]	Clinical isolates, DS and DR	0.25, 0.5 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Hu Y, et al. 2003 [108]	H37Rv	0.35 (no growth)				
Lu T, Drlica K. 2003 [142]	TN6515					0.65 (MIC <sub>90</sub> )
Aubry A, et al. 2004 [12]	H37Rv	0.5 (MIC <sub>90</sub> )				
Singh M, et al. 2009 [216]	H37Rv, Clinical isolates, DS and DR			1.0 (MIC <sub>90</sub> )		
Tan CK, et al. 2009 [235]	Clinical isolates, DS			0.5, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )		
Guerrini V, et al. 2013 [96]	H37vR	0.25 (no growth)				

(continued on next page)

Table 1 (continued)

Source	<i>M. tb</i> strain	Middlebrook 7H11	Middlebrook 7H10	Lowenstein-Jensen medium	Bactec 460	Other
Moxifloxacin						
Woodcock JM, et al. 1997 [265]	Clinical isolates, DS and DR		0.12–0.5 (range MIC <sub>90</sub> )			
Ji, et al. 1998 [113]	H37Rv, clinical isolates, DS and DR	0.5, 0.5 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Gillespie SH, Billington O. 1999 [85]	Clinical isolates			0.12, 0.5 (MIC <sub>50</sub> , MIC <sub>90</sub> )		
Rodriguez JC, et al. 2001 [192]	Clinical isolates DS	0.5, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Rodriguez JC, et al. 2002 [193]	Clinical isolates, DS and DR	0.25, 0.5 (MIC <sub>50</sub> , MIC <sub>90</sub> )				
Lu T, Drlica K. 2003 [142]	TN6515					0.29 (MIC <sub>90</sub> )
Alvarez-Freites EJ, et al. 2002 [9]	Clinical isolates		0.062, 0.125 (MIC <sub>50</sub> , MIC <sub>90</sub> )			
Hu Y, et al. 2003 [16]	H37Rv	0.18 (no growth)				
Aubry a, et al. 2004 [12]	H37Rv	0.5 (MIC <sub>99</sub> )				
Krüüner A, et al. 2005 [129]	Clinical isolates DS				0.125 (no growth)	
Kam KM, et al. 2006 [121]	Clinical isolates, DR/Ofx-S Clinical isolates, DR/Ofx-R					0.5, 0.5 (MIC <sub>50</sub> , MIC <sub>90</sub> ) 1.0, 2.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )
Tan CK, et al. 2009 [235]	Clinical isolates, DS		0.25, 1.0 (MIC <sub>50</sub> , MIC <sub>90</sub> )			
Pucci MJ, et al. 2010 [181]	Erdmann strain Clinical isolates DS Clinical isolates MDR/XDR Clinical isolates, FQN resistant	0.06 (no growth) 0.06 (no growth) 0.06/2.0 (no growth) >8 (no growth)				
Drusano GL, et al. 2011 [67]	H37Ra		0.25 (MIC <sub>99</sub> )			

DS = drug susceptible, DR = drug resistant, MDR = multi-drug resistant.

MIC<sub>50</sub>/MIC<sub>90</sub>/MIC<sub>99</sub>: minimum drug concentration that inhibits 50%/90%/99% of bacterial isolates.

Ofx in DS-TB, without being superior to a standard first-line therapy [127,248]. Adding Lfx to first-line therapy had no impact on 2-month culture conversion compared to standard therapy [68].

There is extensive evidence for the clinical efficacy of Mfx in anti-TB treatment [22,28,33,35,49,65,174,180,198,199,252,260].

Three studies comparing Mfx to EMB added to INH, RIF, and PZA demonstrated not only Mfx efficacy in the intensive phase of treatment, but also the potential to shorten treatment [28,50,199]. In a further phase II trial comparing Mfx substituted for INH during the intensive phase of standard TB therapy, the Mfx group had a small, statistically non-significant increase in 2-month sputum culture conversion [65].

These four studies provided sufficient evidence for phase III trials of later generation fluoroquinolone-containing regimens for treatment shortening in adults with DS-TB.

A randomized controlled trial (RCT) from India using either Mfx or gatifloxacin in combination with INH plus RIF, and PZA in the first two months in a 4-month intermittent regimen (thrice weekly) in newly diagnosed smear-positive TB patients compared with an intermittent 6-month regimen of INH and RIF with EMB and PZA in the first two months was terminated prematurely due to the high risk of TB recurrence in the fluoroquinolone arms [15,111]. If Mfx was given as part of a five-drug daily treatment (Mfx/INH/RIF/PZA/EMB) in treatment-naïve patients with pulmonary TB, higher sputum culture conversion was achieved at 2 months compared to a thrice-weekly four-drug regimen (INH/RIF/PZA/EMB) [255].

The RIFAQUIN trial evaluated the potential of rifapentine in combination with Mfx to shorten anti-TB treatment to 4 months or to reduce the frequency of dosing during a 4-month continuation

phase by giving rifapentine/Mfx once weekly. While the 4-month regimen was inferior to standard therapy, the 6-month regimen with once weekly rifapentine/Mfx continuation phase was not and appeared more convenient than the current standard of care [117]. The REMox TB trial, comparing two 4-months Mfx-containing treatment regimens to 6-month standard first-line treatment, showed a more rapid initial decline in bacterial load, but again, non-inferiority of 4 months therapy compared to standard 6-month therapy could not be shown [86].

Taken together, a higher sputum culture conversion at two months has been demonstrated in Mfx-containing regimens, but current evidence does not support shortening treatment-duration from 6 to 4-months in DS-TB.

### 3.3.3. MDR-TB

Several studies have demonstrated that the use of Ofx compared to no fluoroquinolone is associated with higher treatment success in adult MDR-TB patients and with lower rates of relapse or death ([37]; [106,271,273]). In two retrospective analyses on patients with MDR-TB, Lfx had superior efficacy compared to Ofx [272] and high-dose Lfx (1000 mg) appeared to further improve treatment success [189,237].

Reports of Mfx efficacy in DR-TB are mainly from observational studies. Two individual patient data (IPD) meta-analyses and another systematic review reported improved treatment success with the use of a later-generation fluoroquinolone compared to no or earlier generation fluoroquinolone, and identified fluoroquinolone resistance as a risk factor for unfavourable outcome [4,71,120]. However, another systematic review and meta-analysis of outcomes of MDR-TB therapy found that the proportion of

**Table 2**  
The early bactericidal activity (EBA) of fluoroquinolones.

Study	Design	Population	Treatment	Outcome
Chambers 1998 [32]	Prospective, randomised, 2 study sites	31 adults with sputum smear pos. TB	1) Amox./clavul. 1,000/250 mg; 2) Oxf 600 mg; 3) INH 300 mg	EBA <sub>0–2</sub> /EBA <sub>2–7</sub> 1) amox./clavul. 0.34 (SD 0.03)/0.02 (SD 0.04) 2) Oxf 0.32 (SD 0.05)/0.16 (0.02); 3) INH 0.21 (SD 0.04)/0.60 (SD 0.30);
Sirgel 2000 [218]	Prospective, randomised, 4 study sites:	Total 233 patients with sputum smear pos. DS-TB	1) INH 300 mg 2) INH 18.5 mg 3) RIF 600 mg 4) Oxf 800 mg 5) no drug	EBA <sub>0–2</sub> /EBA <sub>3–5</sub> 1) INH 300 mg 0.37–1.01/0.00–0.08; 2) INH 18.5 mg –0.01–0.33/0.10–0.12 3) RIF 600 mg 0.17–0.63/0.24–0.30 4) Oxf 800 mg 0.01–0.39/0.17–0.29 5) no drug –0.02–0.04/–0.06–0.09
Gosling 2003 [92]	Prospective, randomised, 1 centre	43 patients with newly diagnosed sputum smear pos. TB	1) INH 300 mg 2) RIF 600 mg 3) Mfx 400 mg	Mean EBA <sub>0–2</sub> 1) INH 300 mg 0.77 (SD 0.37) 2) RIF 600 mg 0.28 (SD 0.21) 3) Mfx 400 mg 0.53 (SD 0.31)
Pletz 2004 [176]	Prospective, randomised, 1 centre	17 patients with sputum smear-pos. TB	1) Mfx 400 mg 2) INH 6–8 mg/kg	Mean EBA <sub>0–5</sub> 1) Mfx 400 mg 0.21 2) INH 6 mg/kg 0.27
Gillespie 2005 [87]	Prospective, 1 centre	7 patients with sputum smear pos. TB	Mfx 400 mg plus INH 300 mg	Mean EBA <sub>0–2</sub> 0.60 (95% CI 0.23–0.97)
Johnson et al. 2006 [119]	Prospective, randomised, 1 centre	40 patients with newly diagnosed sputum smear pos. TB	1) Mfx 400 mg 0.21 2) Lfx 1000 mg 3) gatifloxacin 400 mg 4) Mfx 400 mg	Mean EBA <sub>0–2</sub> /EBA <sub>2–7</sub> 1) INH 300 mg 0.67 (SD 0.17)/0.08 (SD 0.09), 2) Lfx 1000 mg 0.45 (SD 0.35)/0.18 (SD 0.13) 3) gatifloxacin 400 mg 0.35 (SD 0.27)/0.17 (SD 0.13) 4) Mfx 400 mg 0.33 (SD 0.39)/0.17 (SD 0.09)

INH = isoniazid; RIF = rifampicin, Oxf = ofloxacin, Lfx = levofloxacin, Mfx = moxifloxacin, amox = amoxicillin, clavul. = clavulanic acid.  
EBA = early bactericidal activity defined as fall in log<sub>10</sub> colony forming units (cfu)/ml sputum/day.

patients treated with a fluoroquinolone was not predictive of treatment success [168]. The authors stated that this might be due to reporting insufficiency rather than the absence of a true association.

No difference in early treatment outcome was shown comparing Lfx versus Mfx in MDR-TB treatment [116,126]. These findings are consistent with data from the murine model; however, whether Mfx is superior to Lfx measuring long-term outcome (and as found in mouse studies), still needs evaluation [199].

For the treatment outcome of XDR-TB, the use of later-generation fluoroquinolones significantly improves treatment outcomes, even in the presence of drug-susceptibility testing (DST) demonstrating resistance to a representative fluoroquinolone [110]. Several trials of MDR-TB prevention using Lfx are currently planned in adults and children.

### 3.3.4. Paediatric TB

There are no prospective RCTs on efficacy of fluoroquinolones in children with TB, but there are limited data available on their use in MDR-TB treatment. In a systematic review of children treated for MDR-TB, fluoroquinolones were an important component of the treatment regimen in all studies included, with a pooled treatment success of 81.67% [70]. In a retrospective study including 149 children with MDR-TB, Oxf was part of the regimen in 132 (96.4%) of those treated, with a median duration of Oxf therapy of 13 (interquartile range [IQR] 10.5–18) months and an overall treatment success (cure or clinical cure [212]) in 137 children (92%) [211]. Similar findings were reported from a paediatric cohort (n = 45) from Georgia; most children were treated with a 6-drug regimen including an injectable agent and Lfx; treatment success was 77% [83]. Several other smaller case series were recently published. Six children with MDR-TB received either Lfx (n = 5) or Mfx (n = 1) as part of their regimen; four were cured, one had pulmonary

sequelae and one child was lost to follow-up [35]. Mfx and linezolid were administered in 2 paediatric MDR-TB cases from Italy and cure was achieved in both [174]. Another series from Italy showed clinical and radiological cure in all 9 children treated with a regimen including Mfx for MDR and DS-TB [78]. Two further case reports show efficacy of fluoroquinolones in anti-TB therapy in children where first-line agents could not be used [69,103].

Existing evidence from paediatric studies underlines the importance of fluoroquinolones in the treatment of paediatric MDR-TB, although data supporting the potential in treatment-shortening is lacking.

## 4. Fluoroquinolone resistance

Mycobacterial resistance to the fluoroquinolones occurs frequently via missense mutations in *gyrA* gene, in a highly conserved region called the Quinolone Resistance Determining Region (QRDR) [5,88,234]. The MTBDR<sub>s</sub> test (Hain Lifescience, Nehren, Germany), a line probe assay for rapid detection of resistance to fluoroquinolones and other second-line TB drugs, detects the most common mutations in the *gyrA* gene, with a sensitivity for fluoroquinolone resistance of 76%–91% [26,104,128]. Other mutations, especially in the *gyrB* subunits, decrease cell wall permeability, active drug efflux, or drug inactivation, and may account for fluoroquinolone resistance not detected using current tools [88,217]. Different mutations result in different levels of resistance [36,121,125,269]; a single *gyrA* mutation increases the minimal inhibitory concentration (MIC) 4- to 16-fold leading to clinically significant Oxf resistance (MIC > 2 µg/ml), while at least a double gyrase mutation is required for high-level resistance [125]. There is broad cross-resistance among the fluoroquinolones, although differing degrees of resistance from one drug to another [5,36,57,58,157].



**Table 3**  
Pharmacokinetic studies of ofloxacin, levofloxacin, moxifloxacin in adults with TB, and children with TB or other conditions.

Study	Disease	HIV	age	N	dosage	Tmax [h]	t1/2 [h]	Cmax [µg/ml]	AUC <sub>0-24</sub> [µg·h/ml]	AUC <sub>0-inf</sub> [µg·h/ml]	AUC other [µg·h/ml]
<b>Ofloxacin</b>											
<b>Adults</b>											
Zhu M, et al. 2002 [280]	TB	3	42 (22–57)	11	800 mg orally (600–1200 mg)	1.03 <sup>a</sup> (0.5–6)	7.34 <sup>a</sup> (3.53–28.3)	10.5 <sup>a</sup> (8.0–14.3)	103 (48–755)		
Chulavatnatol S, et al. 2003 [47]	DR-TB	0	43 (2.5–79)	63	600 mg orally	1.68 <sup>b</sup> (1.21)	5.7 <sup>a</sup> (0.79–173)	8.52 <sup>a</sup> (1.83–19.9)	68.7 <sup>a</sup> (0.78–374)		
Chigutsa E, et al. 2012 [44]	MDR-TB Cape Town	16	34 (20–63)	11	10 mg/kg orally	1.68 <sup>b</sup> (1.21)	8.03 <sup>b</sup> (3.37)	9.61 <sup>b</sup> (2.17)	70.57 <sup>b</sup> (26.4)		
Mueller, et al. 1994 [156]	MDR-TB Durban	13	34 (20–63)	38	800 mg (with meal)	3	7.8	8.8			
Yuk, et al. 1991 [276]	Healthy			27	800 mg (empty stomach)	1.2	7.8	10.4			
Bethell DB, et al. 1996 [20]	Healthy		25	13	400 mg (crushed)	0.81 <sup>b</sup> (0.69)	5.25 <sup>b</sup> (1.01)	5.47 <sup>b</sup> (1.18)		40.42 <sup>b</sup> (11.01)	
	Typhoid fever		10.4 (5–14)	20	400 mg orally	1.74 <sup>b</sup> (0.57)	5.48 <sup>b</sup> (0.81)	3.14 <sup>b</sup> (0.53)		28.36 <sup>b</sup> (4.32)	
Garcia-Prats AJ, et al. 2013 [80]	MDR-TB		0–2	17	7.5 mg/kg iv.	1.39 <sup>a</sup> (0.77–2.05)	4.45 <sup>b</sup> (3.79–5.10)	9.21 <sup>b</sup> (8.22–10.2)			AUC <sub>0-12</sub> 31.15 <sup>b</sup> (26.1–36.2)
Thee S, et al. 2014 [240]	MDR-TB	4	0–8	17	7.5 mg/kg orally	1.33 <sup>b</sup> (0.48)	3.26 <sup>b</sup> (2.31–3.75)	5.73 <sup>b</sup> (4.87–6.59)			AUC <sub>0-12</sub> 26.5 <sup>b</sup> (25.0–28.0)
				24	20 mg/kg orally	1.74 <sup>b</sup> (0.72)	3.21 <sup>a</sup> (2.50–3.27)	10.43 <sup>b</sup> (1.96)			AUC <sub>0-8</sub> 45.85 <sup>b</sup> (8.83)
			2–5	39	20 mg/kg orally	2.5 <sup>b</sup> (1.10)	3.34 <sup>a</sup> (3.02–3.88)	8.52 <sup>b</sup> (2.37)			AUC <sub>0-8</sub> 43.75 <sup>b</sup> (11.96)
			6–15	22	20 mg/kg orally	1.59 <sup>b</sup> (0.73)	3.54 <sup>a</sup> (3.39–4.72)	8.18 <sup>b</sup> (2.01)			AUC <sub>0-8</sub> 43.08 <sup>b</sup> (8.89)
			0–8	23	20 mg/kg orally	1.59 <sup>b</sup> (0.73)	3.18 <sup>a</sup> (2.83–3.44)	9.75 <sup>a</sup> (7.51–10.90)			AUC <sub>0-8</sub> 43.84 <sup>a</sup> (36.7–54.5)
			44 (30–54)	10	1000 mg orally	1.0 <sup>a</sup> (1.0–4.0)	7.37 <sup>a</sup> (4.14–16.3)	15.55 <sup>a</sup> (8.55–42.99)	131 <sup>a</sup> (52–702)		
					750 mg orally		7.7–8.9	7.0–12.0			71.4–110
<b>Levofloxacin</b>											
<b>Adults</b>											
Petoquin CA, et al. 2008 [173]	TB	0	0.5–2	6	7.0 mg/kg iv.	1.4 <sup>b</sup> (0.4)	4.1 <sup>b</sup> (1.3)	5.19 <sup>b</sup> (1.26)		21.5 <sup>b</sup> (6.1)	
Tuberculosis Drug Review 2008 [160]	Bacterial infections		0.5–2	8	7.0 mg/kg orally (Lfx suspension)	1.4 <sup>b</sup> (0.4)	5.0 <sup>b</sup> (1.3)	4.21 <sup>b</sup> (1.49)		25.8 <sup>b</sup> (9.2)	
Chien S, et al. 2005 [38]	Bacterial infections		2–<5	7	7.0 mg/kg iv.	1.6 <sup>b</sup> (0.5)	4.0 <sup>b</sup> (0.8)	6.02 <sup>b</sup> (1.07)		22.7 <sup>b</sup> (4.7)	
			2–<5	8	7.0 mg/kg orally (Lfx suspension)	1.3 <sup>b</sup> (0.4)	4.6 <sup>b</sup> (1.3)	4.56 <sup>b</sup> (0.83)		25.9 <sup>b</sup> (4.8)	
			5–<10	10	7.0 mg/kg iv.	1.3 <sup>b</sup> (0.4)	4.8 <sup>b</sup> (0.8)	7.30 <sup>b</sup> (3.85)		29.2 <sup>b</sup> (6.4)	
			5–<10	8	7.0 mg/kg orally (Lfx suspension)	1.9 <sup>b</sup> (0.9)	5.3 <sup>b</sup> (1.6)	4.64 <sup>b</sup> (0.39)		29.0 <sup>b</sup> (10.0)	
			10–<12	7	7.0 mg/kg iv.	1.9 <sup>b</sup> (0.9)	5.4 <sup>b</sup> (0.8)	6.12 <sup>b</sup> (1.19)		39.8 <sup>b</sup> (11.3)	
			10–<12	8	7.0 mg/kg orally (Lfx suspension)	1.6 <sup>b</sup> (1.0)	5.5 <sup>b</sup> (0.7)	3.99 <sup>b</sup> (0.87)		37.3 <sup>b</sup> (9.8)	
			12–<16	10	7.0 mg/kg iv.	1.6 <sup>b</sup> (1.0)	6.0 <sup>b</sup> (2.1)	6.34 <sup>b</sup> (1.58)		40.5 <sup>b</sup> (7.6)	
			12–<16	8	7.0 mg/kg orally (Lfx suspension)	1.6 <sup>b</sup> (1.0)	5.8 <sup>b</sup> (1.4)	4.76 <sup>b</sup> (0.86)		41.1 <sup>b</sup> (6.8)	

Mase SR, et al. 2012 [148]	MDR-TB+LTBI	4	1–14 (median 8)	33	5–20 mg/kg median 8 mg/kg (oral gel)	1 (1–2)	3.70 <sup>a</sup> (2.4–141.3)	6.39 <sup>a</sup> (1.97–17.03)	39.81 <sup>a</sup> (15.3–103.4)	
Thee S, et al. 2014 [240]	MDR-TB	4	0–8	23	15 mg/kg orally	1.45 <sup>b</sup> (0.51)	3.16 <sup>a</sup> (2.68–3.51)	6.79 <sup>a</sup> (4.69–8.06)		32.92 <sup>a</sup> (25.4–40.9) AUC <sub>0–8</sub> 30.47 <sup>b</sup> (24.4–36.4)
<b>Moxifloxacin</b>										
<b>Adults</b>										
Nijland HMJ, et al. 2007 [159]	TB		30 (20–55)	19	400 mg orally (with 450 mg RIF)	2.5 <sup>a</sup> (0.5–6.0)	7.1 <sup>a</sup> (5.0–9.6)	3.2 <sup>a</sup> (2.5–4.5)	33.3 <sup>a</sup> (25.1–55.5)	
Peloquin CA, et al. 2008 [173]	TB		35 (18–46)	9	400 mg orally	1.0 <sup>a</sup> (0.5–3.0)	9.9 <sup>a</sup> (7.4–14.0)	4.7 <sup>a</sup> (3.4–6.0)	48.2 <sup>a</sup> (37.2–60.5)	
Pranger AD, et al. 2011 [180]	TB		adults	16	400 mg orally	1.0 <sup>a</sup> (1.0–2.0)	6.53 <sup>a</sup> (4.25–10.6)	6.13 <sup>a</sup> (4.47–9.00)	60 <sup>a</sup> (24–140)	
Zvada SP, et al. 2012 [281]	TB		40 (21–51)	27	400 mg orally	1 <sup>a</sup> (1–2)	8 <sup>a</sup> (6–10)	2.5 <sup>a</sup> (2.0–2.9)	24.9 <sup>a</sup> (20.7–35.2)	
Manika K, et al. 2012 [146]	MDR/XDR-TB	0/7	40.1 (15.7)	7	400 mg orally	2.36 <sup>b</sup> (0.56)		4.59 <sup>b</sup> (2.06)	37.96 <sup>b</sup> (16.5)	
Ruslami R, et al. 2013 [198]	TB-meningitis		18–60	19	400 mg orally	2 <sup>a</sup> (1–6)		3.9 <sup>b</sup> (3.2–4.8)	28.6 <sup>b</sup> (24.2–33.8)	AUC <sub>0–6</sub> 15.1 <sup>b</sup> (12.8–17.7)
Magis-Escurra C, et al. 2014 [143]	TB		18–64	16	800 mg orally	2 <sup>a</sup> (1–6)		7.4 <sup>b</sup> (5.6–9.6)	60.4 <sup>b</sup> (45.4–80.3)	AUC <sub>0–6</sub> 28.6 <sup>b</sup> (24.2–33.8)
Thee S, et al. 2014 [239]	MDR-TB	6/23	42 <sup>a</sup> (27)	41	7.0 <sup>a</sup> mg/kg orally	2.0 <sup>a</sup> (1.0–4.0)	8.3 <sup>b</sup> (5.4–13.1)	3.3 <sup>b</sup> (2.4–5.8)	33.6 <sup>b</sup> (15.2–84.2)	
			8–15	23	10 mg/kg orally	2.0 <sup>a</sup> (1.0–8.0)	4.14 <sup>a</sup> (3.45–6.11)	3.08 <sup>a</sup> (2.85–3.82)	23.31 <sup>a</sup> (19.2–42.3)	21.51 <sup>a</sup> (17.7–30.4) AUC <sub>0–8</sub> 17.24 <sup>a</sup> (14.5–22.0)

Abbreviations: C<sub>max</sub> = maximum serum concentration; T<sub>max</sub>: time (h) until C<sub>max</sub>; t<sub>1/2</sub> = half-life; AUC = area under the concentration-time curve; TB = tuberculosis; DR = drug-resistant, MDR = multi-drug resistant; XDR = extensive-drug resistant, RIF = rifampicin.

<sup>a</sup> Median.

<sup>b</sup> Mean.



*In vitro*, the development of *M. tuberculosis* resistance depends on the fluoroquinolone concentration used. Low fluoroquinolone concentration produces many low-level resistance mutants [279], none containing alterations in the QRDR. As selection pressure increases, a variety of *gyrA* variants become prevalent. High fluoroquinolone concentrations produce only a few mutation types, and eventually a concentration is reached at which no mutant is recovered. The minimum concentration allowing no mutant recovery when  $>10^{10}$  bacilli are applied to drug-containing agar was defined as the mutant prevention concentration (MPC) [278], and may be an important pharmacodynamic consideration. The MPC for Mfx *in vitro* is 4–8 µg/ml, a target that is not achieved with Mfx at current recommended doses, but possibly with new fluoroquinolones with more potent activity [89,239]. To date, combination therapy is the most effective strategy to prevent emergence of resistance during TB treatment.

Fluoroquinolone resistance in clinical isolates of *M. tuberculosis* is a growing problem, with 4–30% of MDR isolates having fluoroquinolone resistance depending on the region/country [266]. Of great concern is the finding that resistance may emerge during treatment of patients infected with initially fluoroquinolone-susceptible *M. tuberculosis* strains [29,61,185,247]. The widespread use of fluoroquinolones in various lower respiratory tract and other infections is considered to be partially responsible for increasing resistance among *M. tuberculosis* strains [88]. Previous treatment with a fluoroquinolone is a risk factor for fluoroquinolone resistance [259,274], and *M. tuberculosis* bacilli acquire fluoroquinolone resistance within a few weeks ([106,185,231,273]). Later-generation fluoroquinolones like Mfx are more potent and appear to be less likely to develop resistance [88]. Fluoroquinolone resistance might further be due to activation of efflux pumps that seem to be induced in RIF-resistant *M. tuberculosis* strains exposed to RIF [141]. Of particular clinical importance in MDR-TB, Mfx retains significant activity in many Ofx-resistant isolates, with Mfx minimal inhibitory concentrations (MICs) 4–8-fold lower than those of Ofx [36,121,149,177,219,255]. There are, however, mutations that only confer resistance to Mfx, but not to Ofx or Lfx [145,258]. Nevertheless, improved treatment outcomes in patients with XDR-TB have been observed if treated with a later-generation fluoroquinolone (Mfx, Lfx or gatifloxacin) [110,199,260].

An improved understanding of the fluoroquinolone resistance mutations in *M. tuberculosis* could facilitate more rapid molecular and clinical diagnosis and the initiation of appropriate treatment, resulting in improved treatment outcomes and reduced transmission.

## 5. Pharmacokinetics of fluoroquinolones

Given the challenges with assessing TB drug efficacy in children, an understanding of the pharmacokinetics of TB drugs in children is critical. As a general approach, the recommended dose of anti-TB drugs in children should lead to a pharmacokinetic profile that approximates the adult exposures associated with efficacy and safety. As such, we have reviewed published studies of the pharmacokinetics of Ofx, Lfx, and Mfx in adults with TB, and in children with TB or other conditions.

### 5.1. General information

The fluoroquinolones are easily and rapidly absorbed after oral administration [109,138,173]. The maximum serum concentration ( $C_{max}$ ), as well as the area under the time–concentration curve (AUC) of the fluoroquinolones increase linearly with dose [109,138].

Bioavailability of Ofx, Lfx and Mfx is  $> 85$ –99% following oral administration [18,41,137,276]. Protein-binding might be

concentration-dependent for Ofx and Lfx [214], while for Mfx it is consistent at about 48% over a range of plasma concentrations [226]. Ofx and Lfx have a half-life of approximately 5 h in adults and are eliminated mainly unchanged by the kidney (70–90%) [41,137]. Mfx has a relatively long half-life in adults of about 6–12 h [161,230]. About half of Mfx undergoes phase II biotransformation in the liver [227], while 45% is excreted unchanged in urine and faeces [161].

Fluoroquinolones are distributed widely with good tissue penetration (including intrapulmonary tissue, bones and soft tissue), with tissue concentrations exceeding serum concentrations [43,51,76,191]. Fluoroquinolones penetrate well into the cerebrospinal fluid (CSF), with published CSF-concentrations of 60% of serum concentrations for Ofx,  $>70$ % for Lfx and  $>80$ % for Mfx [6,62,241].

Lfx accumulates only marginally following once-daily administration and steady state is reached within 48 h [76]. For Mfx, steady state is reached within 3 days after the first dose and the serum concentrations at steady state are approximately 30% higher than after the first dose [230]. Food delays absorption of fluoroquinolones, but has no effect on the AUC [228,229,256]. Absorption is decreased by co-administration of sucralfate, antacids, vitamins and minerals containing divalent and trivalent cations (e.g. zinc, iron) and these agents should not be taken within 2 h of fluoroquinolone administration [137,138,170,178,229]. Calcium may also reduce absorption, although the effect appears less than with other cations, and administration of Lfx and Mfx with dairy has only a minimal impact on drug concentrations [228]. These interactions are of particular importance for children who often take crushed adult tablets mixed in a multivitamin syrup, milk, or foods [17]. The pharmacokinetics of fluoroquinolones are not appreciably affected by gender or race [39,76]. HIV infection is associated with reduced absorption of some anti-TB agents [94,99], although the influence of HIV on the pharmacokinetics of fluoroquinolones has not been systematically studied. Available data indicate that Lfx pharmacokinetics in HIV-infected patients are comparable to that observed in healthy subjects [90,175]. Information on drug–drug interactions between fluoroquinolones and antiretroviral agents is still limited. Co-administration of ciprofloxacin (Cfx) and didanosine might reduce Cfx bioavailability due to the antacid-containing didanosine formulation rather than a true drug interaction [53,124]. No influence on Lfx pharmacokinetics by concomitant zidovudine use has been found [40]. In two studies in adults, oral solutions or crushing of tablets did not result in altered drug exposure to Ofx compared to published data [144,156]. Plasma drug concentrations of Mfx are lowered by 30% with RIF co-administration due to induction of hepatic enzymes involving phase II biotransformation [159,262]. The impact of this interaction on the outcome of TB treatment still needs to be evaluated.

Table 3 summarizes pharmacokinetic data on Ofx, Lfx and Mfx in adult patients with TB as well as the available paediatric data in TB and other conditions.

### 5.2. Ofloxacin pharmacokinetics in children

In a randomized cross-over study Ofx 7.5 mg/kg bodyweight was given either orally or intravenously to 17 children with typhoid fever (mean age 10.4 years, range 5–14 years) [20]. The time to maximum concentration ( $T_{max}$ ), half-life ( $t_{1/2}$ ) and volume of distribution were similar to those obtained from adults, while the systemic clearance was more rapid [20]. Following an oral dose of Ofx 20 mg/kg in children  $<15$  years of age  $C_{max}$  approximated published adult data following a standard oral dose of Ofx 800 mg, while AUC was less than half the adult values [79,240]. This was attributed to faster elimination in children. There was no difference

seen by HIV- or nutritional status or if Ofx was given for TB disease or for preventive therapy [79,80]. Crushing tablets also did not lower drug exposure compared to administration of whole Ofx tablets [240].

### 5.3. Levofloxacin pharmacokinetics in children

There are two published pharmacokinetic studies of Lfx in children with MDR-TB and one study in children with other bacterial infections [38,147,240]. Lfx pharmacokinetics was studied in more than 80 children with bacterial infections after a single dose of Lfx 7 mg/kg, given intravenously or orally. Elimination occurred faster in children <5 years of age compared to older children. The authors concluded that in order to approximate exposures in adults after a 500 mg Lfx dose, children >5 years need a daily dose of 10 mg/kg, whereas children 6 months to <5 years should receive 10 mg/kg 12 hourly [38]. Applying pharmacometric techniques to this data [38] in the context of treatment for post-exposure inhalational anthrax, Lfx doses of 8 mg/kg twice daily for children <50 kg and 500 mg once daily for children >50 kg best approximated Lfx exposure in adults after a 500 mg dose [135].

Mase et al. studied the pharmacokinetics of Lfx in 33 children (median age 8 years) with MDR-TB or MDR-TB exposure receiving a median Lfx dose of 8 mg/kg [147]. In the only other study, the pharmacokinetics of Lfx was investigated in 22 children <8 years of age (median age 3 years) receiving Lfx 15 mg/kg either for MDR-TB disease or preventive treatment [240]. Although children in the Mase study received a lower dose than children in the study by Thee et al. the  $C_{max}$  as well as the  $AUC_{0-\infty}$  were similar in the two studies [147,240]. In the study by Mase et al. Lfx was given as oral gel of verified potency, while in the Thee study, Lfx tablets, either whole or crushed, were administered [147,240] and no association was found between age and Lfx, exposure, although weight predicted drug exposure [240].

### 5.4. Moxifloxacin pharmacokinetics in children

We only identified two studies on Mfx pharmacokinetics in children. One is a single case study in a premature infant with *Mycoplasma hominis* meningitis, with limited generalizability [261]. The other study included 23 children (age 7–15 years) with MDR-TB receiving Mfx at a daily dose of 10 mg/kg, showing that children were exposed to lower serum levels than adults receiving a comparable dose, and that HIV infection is associated with lower Mfx serum concentrations [239].

In summary, the available information on fluoroquinolone pharmacokinetics in children show substantially lower serum concentrations compared to adults, using noncompartmental analysis. There is a paucity of data especially in children <5 years of age and HIV-infected children and child friendly drug formulations are lacking, making the study of drugs like Mfx in younger children challenging. Pharmacokinetic modelling and meta-analyses of larger combined datasets might be useful tools to compare paediatric versus adult data, and assess covariate effects.

## 6. Pharmacodynamics

Pharmacodynamic (PD) indices are used as surrogate markers for clinical efficacy. An  $AUC_{0-24}/MIC$  ratio of >100–125 or  $C_{max}/MIC$  of 8–10 is associated with clinical and microbiological success in Gram-negative bacteria and presumably also in *M. tuberculosis* [88,268]. In non-mycobacterial respiratory infections  $AUC/MIC$  is the best PD index for fluoroquinolones, and data in mice support the applicability to TB [54,158,208,214]. In an EBA study, an  $AUC/MIC$  >100 of Lfx and Mfx showed similar activity against

*M. tuberculosis* as INH [119]. There are no human studies prospectively establishing the target  $AUC/MIC$  ratios for the fluoroquinolones against *M. tuberculosis* [158,214].

A Mfx dose of 800 mg in adults has been identified in *in vitro* studies and in mathematical modelling as the dose most likely to achieve the proposed drug targets [98,214,282]. In adult MDR-TB patients, an oral Mfx dose of 400 mg resulted in less favourable free  $AUC/MIC$  ratios than a Lfx dose of 1,000 mg [173]. Existing evidence from *in vitro* and animal studies shows that either higher doses or the use of newer fluoroquinolones with a lower MIC should be considered to achieve proposed  $AUC/MIC$  targets [11,44,54,214,280].

PD indices in the treatment of TB need to be prospectively assessed. For paediatric TB there is no information on fluoroquinolone drug targets available. An improved understanding of the relative importance of  $C_{max}/MIC$  ratio versus  $AUC/MIC$  ratio is particularly important for children, who may more easily approximate the adult  $C_{max}$  but have much lower AUCs due to more rapid drug elimination.

## 7. Safety

The fluoroquinolones are generally well-tolerated compounds. Adverse effects were described as mild to moderate in severity and necessitated discontinuation of treatment in 1–2% in adults [160,161,171,203]. The most common adverse effects reported in adults were gastrointestinal disturbances (0.9–4.7%), neurotoxicity (0.9–4.7%), hypoglycemia, anaemia, conjunctivitis, and rash [45,127,154]. Further rare adverse effects include phototoxicity, tendonitis, prolongation of QT interval and ophthalmological and nephrologic complications [45,221].

The use of fluoroquinolones in children has been limited because of their potential to induce arthropathy in juvenile animals (see below) [46,77,236]. However, the majority of adverse effects reported with fluoroquinolones use in children are mild, require little or no intervention and are reversible with cessation of the drug [93].

### 7.1. Gastrointestinal adverse effects

In a report on >1,700 children receiving Cfx for compassionate use, adverse effects involving the digestive system were the most common, occurring in 5% [100]. In a cohort of 186 children receiving ofloxacin, ethambutol, and high-dose isoniazid as part of MDR-TB preventive therapy, loss of appetite and nausea were the most common grade 2 or higher adverse events (12 children [6.2%]), followed by itchy skin (9 children [4.7%]), disturbance of sleep or mood (7 children [3.6%]), and skin rash (7 children [3.6%]) [209]. Grade 3 events were only reported in 6 children (3.2%), and no grade 4 events occurred [209]. No dose-related increase in gastrointestinal adverse effects was seen for Lfx in adult dose-ranging studies [42].

### 7.2. Central nervous system (CNS) reactions

Central nervous system reactions are mostly mild (e.g. dizziness, headache, tiredness, sleeplessness, restlessness), are dose dependent [23], typically start within a few days after initiation of fluoroquinolone treatment and stop with cessation of the fluoroquinolone [45,225,249]. Nevertheless, in adults, there are reports on peripheral neuropathy and possibly Guillain-Barré syndrome associated with fluoroquinolone use [8]. Few case reports on benign raised intracranial pressure associated with fluoroquinolone use have been published [133,263]. In a cohort of children receiving an Ofx-containing MDR-TB preventive treatment

regimen, 9 (4.6%), 4 (2.1%) and 3 (1.5%) of 193 children experienced Grade 1, 2 and 3 mood/sleep disturbances, respectively, the latter probably related to inadvertent overdosing of Ofx [210]. Some cases of hallucinations following treatment with Ofx have also been reported [34,209]. In several previous reports on management of children with MDR-TB, neurological adverse effects occasionally occurred, but were attributed to cycloserine [66,73,207]. Subtle neurological adverse effects (e.g. sleep disturbances) have to be specifically ascertained and might be underreported.

### 7.3. Chondrotoxicity

Fluoroquinolones at high doses (e.g. Ofx 600 mg/kg) cause irreversible joint cartilage defects in studies in juvenile rats [77], while in dogs, representing the most sensitive species, cartilage lesions are inducible at a rather low dose of Ofx 10–50 mg/kg/d [223]. The underlying mechanism may be chelation of magnesium in joints leading to subsequent reactions, including radical formation [101]. Data from juvenile rats indicate that chondrotoxicity of Ofx not only increased with increasing single oral doses, but also when the drug is given for several days [222]. Although chondrotoxicity is considered a class effect, considerable differences seem to exist. Absorption, tissue penetration and AUC of fluoroquinolones have to be taken into account to assess the risks associated with individual fluoroquinolones [224]. Tendinopathies are another described toxic effect with a higher risk with concomitant corticoid therapy [188,222]. Although multiple observational studies have demonstrated no unequivocal documentation of fluoroquinolone-induced arthropathy in children or adolescents, their use in children remains debated and the available paediatric data limited [19,24,27,100,205]. Schaad reviewed published reports which included monitoring for fluoroquinolone-induced cartilage toxicity and found no unequivocal documentation of fluoroquinolone-induced arthropathy in children [204]. Clinical observations temporally related to quinolone use are reversible and are coincidental rather than representing adverse effects [204]. Multiple further reviews of fluoroquinolone safety in children concluded that there may be some association between fluoroquinolones and reversible arthralgia in children, but there is no evidence for severe or irreversible arthropathy and no evidence for sustained injury on bone or joint growth [1,7,24,48,100,194,203]. In a comprehensive review on quinolone arthropathy in children versus animals, Burkhardt et al. identified 10 published case reports of suspected quinolone arthropathy, of which 7 were associated with treatment with pefloxacin, 2 with Cfx and 1 with nalidixic acid [27]. With the exception of one case, complete clinical recovery was observed. In 31 further reports from multi-patient studies in >7000 children, arthralgia following the use of either Cfx, nalidixic acid, norfloxacin or Ofx did not occur beyond the level of severity expected as a result of the underlying disease [27]. The authors concluded that quinolone arthropathy is not convincingly correlated with use of these compounds in children and estimated that the occurrence of chondrotoxicity, as seen in juvenile animals, would not be greater than 0.04% [27]. In a prospective trial comparing safety of fluoroquinolone use in children (n = 276) to that of other antibiotics in children (n = 249) with different underlying conditions, adverse events (including musculoskeletal events) occurred more frequently in the fluoroquinolone group than in the control group but were all transient [31]. No relationship between dose and duration of therapy was seen. Prescribed fluoroquinolones were Cfx (87%), Ofx (9%) and pefloxacin (4%). No adverse event was associated with Ofx use [31].

Noel et al. assessed the safety profile of Lfx in 2523 children based on observations for 1 year after therapy [162]. The incidence of pre-defined musculoskeletal disorders (arthralgia, arthritis, tendinopathy,

gait abnormality) was statistically greater in Lfx-treated children and occurred in about 1 of 400 children treated with Lfx. Arthralgia in weight-bearing joints was the major complaint. Lfx was given in a dose of 10 mg/kg twice daily to a maximum of 500 mg/day for 7–14 days. Reported incidence of musculoskeletal adverse effects increased over time, even after exposure to Lfx, but disorders were typically transient and resolved spontaneously without sequelae. The authors noted that the lack of blinding may have biased these results, particularly as the difference was mainly related to subjective parental reports of arthralgia [162]. After 5 years of follow up on 1340 children receiving Lfx, no long-term effects on the musculoskeletal system were seen [24]. In a retrospective study of more than 6000 children, the incidence of tendon or joint disorders associated with the use of Ofx, Lfx or Cfx was <1% and comparable with the reference group receiving azithromycin [270]. Data on the risk of arthropathy in children on long-term fluoroquinolone therapy (as for anti-TB treatment) is limited. Seddon et al. reported joint, muscle or bone pain only as grade 1 or 2 in 13 of 137 (9%) children receiving Ofx 15–20 mg/kg as part of a multi-drug preventive therapy for MDR-TB (once-daily dosing for 6 months) [209].

### 7.4. Cardiovascular reactions

The fluoroquinolones prolong the QT interval by blocking voltage-gated potassium channels expressed by the human ether-a-go-go-related gene, HERG [201], potentially leading to the development of torsades de pointes, which may degenerate into ventricular fibrillation and, potentially, sudden death. Studies *in vitro* and with healthy volunteers show that Mfx has the highest potency for QT prolongation compared with Lfx and Ofx [56,122,163,164], although even for Mfx the amplitude of QT-prolongations is small, and the risk of torsades de pointes is expected to be minimal at the current recommended doses [25,56]. However, fluoroquinolones should not be used in patients with predisposing factors for torsades de pointes including electrolyte disturbances and bradycardia or during coadministration of proarrhythmic drugs [25,56].

An additional consideration for patients on MDR-TB treatment is the frequent drug-induced hypothyroidism related to *para*-aminosalicylic acid or ethionamide. Hypothyroidism and subclinical hypothyroidism are associated with prolongation of the QT interval [13,169]. Care should be taken if other drugs with potential to prolong the QT interval (e.g. delamanid, bedaquiline, clarithromycin or clofazimine) are used in combination with fluoroquinolones [30,84,179,277].

Our search did not identify any report on QT prolongation, arrhythmia or sudden death associated with fluoroquinolone use in children. Garazzino et al. reported no QT prolongation in 9 children with MDR TB treated with Mfx [78]. In two further studies on children with MDR-TB treated with Lfx (n = 23) or Mfx (n = 23) no QT-prolongation >450 ms was seen [239,240].

### 7.5. Other adverse effects

Mild, usually transient, elevations in hepatic enzymes have been associated with fluoroquinolone therapy, while the incidence of acute liver injury is very low (<1 per 100,000 users) [14,55,239]. There are two reports on higher frequencies of hepatitis, if Ofx or Lfx are given in combination with pyrazinamide [2,190]. Acute liver failure reporting rates using U.S. Food and Drug Administration (FDA) data per 10 million prescriptions have been given as 2.1 for Lfx, compared with 6.6 for Mfx [253].

Disordered glucose regulation, either hypo- or hyperglycemia, can occur with any of the fluoroquinolones, with marked differences between individual fluoroquinolones [136]. For Lfx and Mfx



the risk is reported to be low [95,151]. We did not identify any report on hypoglycaemia or acute liver failure in children receiving fluoroquinolones.

## 8. Conclusions

Existing data from *in vitro*, animal and human studies consistently demonstrate the efficacy of Ofx, Lfx and Mfx against *M. tuberculosis*. The later-generation fluoroquinolones Lfx and Mfx have shown a higher potency than Ofx. Mfx is currently considered the most potent against *M. tuberculosis*, but Lfx at higher doses (1000 mg/d or 20 mg/kg) may be equivalent. If available, Mfx and Lfx should be the fluoroquinolones of choice in the treatment of MDR-TB. Despite a lack of RCTs of the fluoroquinolones in the treatment of MDR-TB in adults or children, their strong bactericidal and sterilizing activity, favourable pharmacokinetics and toxicity profile have made them the most important component of existing MDR-TB treatment regimens. The current evidence on Mfx containing regimen does not yet support their use in the shortening of TB treatment for DS-TB.

Existing pharmacokinetic data suggests that fluoroquinolones should be given at higher doses in children compared to adults. Further studies are needed to evaluate these higher doses and their safety in long-term anti-TB treatment in children, including in children below 2 years of age and HIV-infected children. Data on drug–drug interactions between fluoroquinolones and antiretroviral agents is largely absent in children. Existing formulations make appropriate dosing of the fluoroquinolones in the paediatric population challenging. It is common practice to split or crush tablets to administer to children without available information on subsequent bioavailability and therefore efficacy. Widely available, child-friendly formulations especially of Mfx and Lfx, for children with MDR-TB, are urgently needed.

The use of fluoroquinolones has been traditionally restricted in children due to safety concerns, especially drug-induced arthropathy. The available data does not demonstrate any serious arthropathy or other severe toxicity in children. Although there is a paucity of paediatric data for the treatment of MDR-TB, extended administration of fluoroquinolones in adults with MDR-TB does not show serious adverse effects and there is no evidence suggesting less tolerability of fluoroquinolones in children.

Available data supports the use of newer fluoroquinolones as key components in the treatment of drug resistant TB in children, while their role in treatment shortening and preventive therapy in children needs further investigation.

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## Chapter 5

### **The pharmacokinetics of the second-line anti-tuberculosis drugs ethionamide, ofloxacin, levofloxacin and moxifloxacin in children with tuberculosis**

The second-line anti-tuberculosis agents ethionamide (ETH) and the fluoroquinolones ofloxacin (OFX), levofloxacin (LFX) and moxifloxacin (MFX) were selected for pharmacokinetic investigation in children. ETH, because it is widely used in multidrug-resistant tuberculosis (MDR-TB) as well as in drug-susceptible (DS)-TB; fluoroquinolones, because they form the backbone of MDR-TB chemoprevention and treatment of MDR-TB not only in current regimens, but will also be used in future regimens with novel compounds- both for DR-TB and DS-TB

#### **5.1. The pharmacokinetics of ethionamide in children with tuberculosis**

The aim of this first study investigating the pharmacokinetics of ethionamide (ETH) in children, was to document the pharmacokinetics ETH in children 3 months-<2 years, 2-<6 years, and 6-12 years of age receiving the current WHO recommended dosage of ETH (15-20 mg/kg/day) (310). For anti-tuberculosis therapy, a maximum serum concentration ( $C_{max}$ ) of 2.5 $\mu$ g/ml has been suggested (72). The hypothesis was that the current dosing recommendations for ETH would achieve serum concentrations in children with TB treated with a multiple drug regimen (with and without rifampicin [RMP]) comparable to those found in adults treated with a standard dose of ETH 500-750mg/day.

Two groups of children were studied: those receiving ETH with RMP, and those receiving ETH without accompanying RMP, in order to detect possible influences on the pharmacokinetics of ETH by the strong enzyme inducer RMP. In this explorative study a valid estimation of number of cases needed for statistical significance could not be made as there were neither data on ETH serum concentrations nor on ETH standard

deviations in children of different age groups. Sample size considerations were based on reasons of practicability and according to the frequency of treatment regimen use in children containing ETH.

Children younger than 13 years of age with TB routinely admitted to Brooklyn Hospital for Chest Diseases (BHCD) were included. Pharmacokinetic assessment of ETH was completed through an intensive sampling approach approximately 1 month after starting a routine anti-tuberculosis treatment regimen which included ETH, and again at 4 months of anti-tuberculosis treatment, in order to assess intra-individual differences and changes in the pharmacokinetics of ETH over time. Blood samples were drawn before and at 1, 2, 3, 4 and 6 hours after oral administration of ETH. The serum concentrations were determined by high performance liquid chromatography (HPLC).  $C_{max}$ , time until  $C_{max}$  ( $t_{max}$ ), and area under the curve (AUC) of ETH concentrations were primary outcome measures.

Thirty-one (n=31) children (median age 2.8 years) were included. Twelve children (39%) were HIV-infected, and 16 had pulmonary TB (52%). Observed mean (SD) ETH PK measures after 1 month of therapy with/without RMP in children <2years, 2-6years and 6-12years were as follows:  $C_{max}$  3.8 (1.6)/3.9 (1.6) $\mu\text{g/ml}$ , 4.4 (1.2)/3.6 (1.4) $\mu\text{g/ml}$ , and 3.6 (1.3)/5.4 (1.2) $\mu\text{g/ml}$  respectively;  $\text{AUC}_{0-6}$  7.6 (3.5)/8.4 (3.7) $\text{mg}\cdot\text{h/l}$ , 10.6 (5.6)/8.3 (3.5) $\text{mg}\cdot\text{h/l}$ , and 12.3 (4.3)/13.9 (4.9) $\text{mg}\cdot\text{h/l}$ , respectively. Younger children were exposed to lower ETH concentrations than older children at the same mg/kg body weight dose. Age correlated significantly with AUC after both 1 month ( $r=0.50$ ,  $p=0.001$ ) and 4 months ( $r=0.63$ ,  $p=0.001$ ) of therapy. HIV co-infection led to lower ETH serum concentrations (statistically significant). Neither co-medication with RMP nor the duration of ETH treatment influenced pharmacokinetic measures of ETH in children.

This study showed that with an ETH dose as currently recommended by WHO, the majority of children achieved sufficient serum concentrations. Children younger than 2 years of age and HIV-infected children were exposed to substantially lower ETH concentrations than older children or HIV-uninfected children. This may imply a need for a higher dosage in younger and HIV-infected children, which should be evaluated in future studies.

Pharmacokinetics of Ethionamide in Children<sup>∇</sup>S. Thee,<sup>1,2\*</sup> H. I. Seifart,<sup>3</sup> B. Rosenkranz,<sup>3</sup> A. C. Hesseling,<sup>2</sup>  
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**Ethionamide (ETH), a second-line antituberculosis drug, is frequently used in treating childhood tuberculosis. Data supporting ETH dose recommendations in children are limited. The aim of this study was to determine the pharmacokinetic parameters for ETH in children on antituberculosis treatment including ETH. ETH serum levels were prospectively assessed in 31 children in 3 age groups (0 to 2 years, 2 to 6 years, and 6 to 12 years). Within each age group, half received rifampin (RMP). Following an oral dose of ETH (15 to 20 mg/kg of body weight), blood samples were collected at 0, 1, 2, 3, 4, and 6 h following 1 and 4 months of ETH therapy. The maximum serum concentration ( $C_{max}$ ), time to  $C_{max}$  ( $T_{max}$ ), and area under the time-concentration curve from 0 to 6 h ( $AUC_{0-6}$ ) were calculated. Younger children were exposed to lower ETH concentrations than older children at the same mg/kg body weight dose. Age correlated significantly with the AUC after both 1 month ( $r = 0.50$ ,  $P = 0.001$ ) and 4 months ( $r = 0.63$ ,  $P = 0.001$ ) of therapy. There was no difference in the AUC or  $C_{max}$  between children receiving concomitant treatment with RMP and those who did not. Time on treatment did not influence the pharmacokinetic parameters of ETH following 1 and 4 months of therapy. HIV infection was associated with lower ETH exposure. In conclusion, ETH at an oral dose of 15 to 20 mg/kg results in sufficient serum concentrations compared to current adult recommended levels in the majority of children across all age groups. ETH levels were influenced by young age and HIV status but were not affected by concomitant RMP treatment and duration of therapy.**

The thioamides, ethionamide (ETH) and prothionamide, are among the most frequently used second-line drugs for the management of pediatric tuberculosis (TB) and are active against several species of mycobacteria. They are oral drugs recommended for treatment of multidrug-resistant tuberculosis (MDR-TB) and also used to treat drug-susceptible tuberculous meningitis (TBM) and miliary disease due to good cerebrospinal fluid penetration (8, 19). Absorption of ETH from the intestinal tract is almost complete, and food and antacids appear to have little effect on this process (2). Protein binding is approximately 30% (9). ETH is distributed with ease throughout the body (28). In adults, peak plasma concentrations of ETH occur at approximately 2 h postdosing (2, 10, 29). For clinical purposes, the suggested serum levels for susceptible strains of *Mycobacterium tuberculosis* vary in the literature, as follows: 2.5  $\mu\text{g/ml}$  or between 1 and 5  $\mu\text{g/ml}$  (13, 14, 22). There is evidence that ETH has a bactericidal effect in higher doses (15). Higher doses are often poorly tolerated, limiting the dose in adults to 500 to 1,000 mg per day. A very small proportion of the drug is excreted unchanged, and the first step in thioamide metabolism in the liver is transformation to the active sulfoxide metabolites by monooxygenases (17). These monooxygenases have many properties in common with the cytochrome P450 system (CYP) and often have overlapping

substrate specificities (17). Rifampin (RMP), commonly used with ETH, is a potent inducer of hepatic and intestinal cytochrome P450 enzymes and *p*-glycoprotein and activates the nuclear pregnane X receptor and the constitutive androstane receptor (20).

The optimal dose of ETH for children has not yet been established. While a total daily pediatric dose of 10 to 20 mg/kg of body weight has been suggested by the WHO and 15 to 20 mg/kg is the standard in South Africa, the resulting serum levels in children of different ages are largely unknown. Little is known regarding the accumulation of ETH during continuous therapy or whether drug interactions relevant to combination TB therapy in children lead to changes in ETH serum levels over time.

In drug-susceptible tuberculous meningitis or miliary disease, ETH is given in a combination regimen which includes RMP. The effects of coadministered drugs, such as RMP, on the concentrations of ETH are unknown.

We studied ETH serum levels in children of different age groups who were routinely on two different treatment regimens in a hospital setting at 1 and 4 months after initiation of routine antituberculosis treatment. Besides a single previous study of two children, this is to our knowledge the first evaluation of ETH pharmacokinetics in children (29).

## MATERIALS AND METHODS

This study was conducted at Brooklyn Hospital for Chest Diseases (BHCD), Cape Town, South Africa. From September 2009 through May 2010, children aged 3 months to 13 years admitted to BHCD were eligible for enrollment. Children were divided in 3 age groups (3 months to 2 years, 2 to 5 years, and 6

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TABLE 1. Demographic, clinical, and radiological features of children by age group and treatment regimens

Characteristic <sup>a</sup>	No. of children:		
	<2 yr (n = 10) without/with RMP	2 to 6 yr (n = 11) without/with RMP	6 to 12 yr (n = 10) without/with RMP
Total	5/5	6/5	5/5
Mean age (SD)	1.0 (0.7)/1.0 (0.6)	2.7 (1.2)/3.2 (1.3)	9.3 (2.1)/8.5 (1.7)
Female	3/3	4/1	5/1
Culture of <i>M. tuberculosis</i> or AFB positive	2/4	4/2	4/2
Household TB contact	4/4	2/3	4/1
HIV infected	2/2	2/2	3/1
Mantoux test done	5/4	4/3	2/2
Clinical features			
Pulmonary TB	5/2	5/0	4/0
Tuberculous meningitis	0/3	0/5	0/5
Hepatomegaly at inclusion	3/1	2/2	2/1
Nutritional statuses			
Mass < 3rd percentile	1/0	0/2	2/1
Mean wt, 1 month (SD)	8.3 (2.6)/8.7 (2.4)	13.1 (2.0)/12.8 (2.6)	26.1 (7.5)/23.7 (2.0)
Mean wt, 4 months (SD)	9.6 (2.6)/9.2 (2.7)	13.5 (2.1)/13.0 (2.7)	25.7 (7.3)/23.1 (2.0)
Mean MUAC, 1 month (SD)	13.6 (2.1)/14.1 (2.0)	16.0 (1.2)/15.6 (0.9)	17.9 (1.9)/17.6 (0.9)
Mean MUAC, 4 months (SD)	14.4 (2.1)/15.7 (2.2)	15.8 (1.1)/15.2 (1.2)	18.0 (2.0)/17.2 (0.6)
Mean BMI, 1 month (SD)	15.8 (3.0)/16.9 (1.7)	15.8 (1.1)/16.6 (2.0)	15.5 (2.1)/15.2 (1.2)
Mean BMI, 4 months (SD)	16.9 (2.7)/16.4 (2.6)	15.7 (0.9)/15.8 (1.6)	15.2 (2.0)/14.4 (0.6)
Radiological features <sup>b</sup>			
Hilar adenopathy	3/3	3/1	2/1
Airway compression	2/0	1/1	0/1
Alveolar opacification	4/1	4/2	2/2
Cavitation	1/0	0/1	2/0
Bronchopneumonic opacification	1/0	0/0	1/0
Pleural effusion	1/0	1/0	0/0

<sup>a</sup> SD = standard deviation; AFB = acid-fast bacilli; MUAC = mid-upper-arm circumference; BMI = body mass index (kg/m<sup>2</sup>).

<sup>b</sup> For one child with tuberculous meningitis, no chest X-ray was available.

to 13 years of age), with 10 children in each age stratum, of whom half routinely received RMP.

All parents or legal guardians gave written informed consent for their child's participation prior to enrollment. The study was approved by the Health Research Ethics Committee of the Faculty of Health Sciences of Stellenbosch University, South Africa, where ETH is licensed for use in children.

**Diagnosis of tuberculosis.** The diagnosis of TB was based on a chest radiograph compatible with a diagnosis of pulmonary TB and/or clinical signs of extrapulmonary TB (e.g., peripheral lymph nodes, meningitis, abdominal TB) with supporting special investigations, including mycobacterial culture. Middlebrook 7H9 broth base (mycobacterial growth indicator tubes [MGIT]; Becton Dickinson, Sparks, MD) culture medium was used for primary isolation. The presence of *M. tuberculosis* was confirmed by PCR amplification. Phenotypic drug susceptibility testing was done using the Bactec 460TB system (Becton Dickinson, Sparks, MD), according to international criteria. Strain susceptibility was judged by comparing growth of organisms in drug- versus nondrug-containing media. Resistance was defined as 1% or more bacterial growth in the drug-containing media (24). A history of household TB contact and a positive Mantoux tuberculin skin test (TST), along with suggestive symptoms, were considered supportive evidence. When no culture was obtained, children were treated empirically according to the drug susceptibility result of isolates from the likely source case.

**Treatment.** Children were included if ETH was part of their standard antituberculosis daily treatment and only if they could tolerate taking ETH as a single daily dose. South African Medicines Control Council-approved ETH 250-mg tablets (Sanofi Aventis, South Africa) were used.

All children received supplementary pyridoxine and multivitamin syrup. Trimethoprim-sulfamethoxazole was given to all HIV-infected children. Antiretroviral treatment consisted of two nucleoside reverse transcriptase inhibitors (lamivudine and stavudine) and either lopinavir-ritonavir (boosted with extra ritonavir if on RMP) in children younger than 3 years of age or efavirenz in children older than 3 years of age.

**Pharmacokinetic investigation.** A routine recommended ETH dose of 15 to 20 mg per kg of body weight was calculated for each child, and ETH tablets were measured accordingly. ETH was administered by study personnel in the morning after an overnight fast (minimum of 4 h of fasting in younger children). In children who were <2 years of age and if necessary in other children, ETH tablets were crushed and suspended in 2 to 5 ml of water. For one child, all drugs were given via a gastrostomy tube. Children had breakfast only after the 1-hour blood sample was taken. In case of HIV infection, antiretroviral therapy was given with breakfast, while other treatment was given after the last blood sample was taken.

Blood samples were collected at 0, 1, 2, 3, 4, and 6 h following dosing and following 1 and 4 months of antituberculosis therapy.

Blood samples were collected in EDTA-containing sampling vials, centrifuged, frozen at -80°C, and stored. To 100 µl of sample, 300 µl of methanol, containing 1.0 µg/ml thiacetazone (Sigma, St. Louis, MO), was added. The supernatant was transferred into autosampler vials for analysis using a 5-µl injection volume.

Specimens were analyzed using a binary high-performance liquid chromatographer (HPLC) (Agilent series 1100 HPLC; Agilent Technologies, Waldbronn, Germany) equipped with an Agilent Zorbax analytical column (150 mm by 2.1 mm inside diameter [i.d.], 3.5-µm particle size). The column temperature was maintained at 40°C at a flow rate of 300 µl/min. The mobile phase A was water containing 0.1% formic acid (FA) (Fluka Chemie GmbH, Buchs, Switzerland), while phase B was methanol (E. Merck, Darmstadt, Germany) containing 0.1% FA. All solvents were of HPLC grade. The concentration of ETH was determined using an API 2000 tandem mass spectrometer (MS/MS; Applied Biosystems, MDS Sciex, Foster City, CA) equipped with an atmospheric turbulon ionization chamber. A single transition range for ETH of *m/z* 167.13/107.00 was used, while for the internal standard, a transition of *m/z* 237.12/119.96 was used. All samples and spiked standards were analyzed in duplicate. The within-day variation between the duplicates was less than 2%. The overall analytical precision over the entire duration of the study was not more than 3% over the entire calibration range. The day-to-day variation of standards and patient samples was

in the range of 1.5 to 2.0%. The retention times of ETH and thiacetazone were 2.73 min and 7.0 min, respectively. The temperature of the nebulizer gas of the ionization chamber was set to be 400°C. Spiked calibrators in the range of 0.5 µg/ml to 15.0 µg/ml showed a linear relationship. Quality control samples were added to each sample batch.

**Pharmacokinetic parameters.**  $C_{max}$  is the observed maximum serum concentration for each individual, and  $T_{max}$  is the time at which  $C_{max}$  is recorded. The elimination rate constant ( $k_{el}$ ) was determined from the concentration versus time curve. The area under the time-concentration curve (AUC) from 0 to 6 hours ( $AUC_{0-6}$ ) was calculated according to the linear trapezoidal rule.  $AUC_{0-\infty}$  and clearance were also calculated.

**Statistical methods.** The data were summarized as means and standard deviations (SD). The Z-score for weight for age was calculated according to WHO growth charts (27).

For binominal data, differences between groups were determined using Fisher's exact test. The Spearman rank correlation coefficient described associations between continuous variables. A mixed-model repeated-measures analysis of variance (ANOVA) was performed to evaluate the influence of HIV infection and to assess the differences for the pharmacokinetic parameters between the two treatment groups and following 1 and 4 months of therapy.

**RESULTS**

A total of 31 children were enrolled; 15 children received a regimen that included ETH and RMP, and 16 received an ETH-containing regimen without RMP. For one 2-year-old child, RMP was added only after inclusion in the study and after completion of the first study day. For one child, the 4-month data point was missing because of failed venipuncture. All HIV-infected children were established on antiretroviral therapy (at least 2 to 4 weeks of therapy) at enrollment.

The demographics, diagnostic criteria, and clinical features of participants are shown in Table 1. A RMP-including treatment regimen is used in the treatment of TBM but usually not in the treatment of MDR-TB, explaining the different clinical features in the two study groups.

ETH pharmacokinetic parameters are displayed in Table 2. The variability of pharmacokinetic parameters at both time points was high (Table 2). Children were on daily treatment with ETH for a mean of 36 days (SD, 7.13 days) at the 1-month sampling point and 120 days (SD, 5.5 days) at the 4-month sampling point, respectively. The mean ETH doses administered at the respective sampling points were 17.8 mg/kg (SD, 2.4 mg/kg) and 17.6 mg/kg (SD, 1.9 mg/kg), respectively.

Low maximum serum levels ( $C_{max} < 2.5$  µg/ml) were found in 4 children (2 were <2 years of age) at the 1-month sampling and in 7 children (5 were <2 years of age) following 4 months of therapy. Only one child had a  $C_{max}$  below 2.5 µg/ml at both time points.  $C_{max}$  ranged between 2.5 µg/ml and 5.0 µg/ml in 15 children (48%) at 1 month and in 13 children (42%) following 4 months of therapy.

HIV coinfection was associated with reduced exposure to ETH at both time points (1 month/4 months,  $C_{max} P = 0.002/0.023$ ,  $AUC_{0-6} P = 0.523/0.047$ ; ANOVA); there was no HIV-associated delay in absorption or elimination (1 month/4 months,  $T_{max} P = 0.594/0.970$ , half-life [ $t_{1/2}$ ]  $P = 0.435/0.602$ ).

We found no association between body mass index, mid-upper-arm circumference, or weight-for-age Z scores and any ETH pharmacokinetic parameters (data not shown).

**Differences between age groups.** Mean ETH serum concentrations are shown by treatment regimens depicted in Fig. 1a and b, and the corresponding pharmacokinetic parameters appear in Table 2.

TABLE 2. Pharmacokinetic parameters of ethionamide in children in different age groups on multidrug regimens with and without rifampin

Pharmacokinetic parameter	Value for children <sup>a</sup> :											
	<2 yr				2 to 6 yr				6 to 12 yr			
	No. of children on ETH	ETH	No. of children on ETH+RMP	ETH+RMP	No. of children on ETH	ETH	No. of children on ETH+RMP	ETH+RMP	No. of children on ETH	ETH	No. of children on ETH+RMP	ETH+RMP
<b>1 month of therapy</b>												
$C_{max}$ (µg/ml)	5	3.79 (1.59)	5	3.91 (1.61)	6	4.43 (1.23)	5	3.58 (1.36)	5	3.62 (1.30)	5	5.44 (1.24)
$T_{max}$ (h)	5	0.97 (0.9-1.0)	5	0.98 (0.9-1.3)	6	1.11 (0.9-2.1)	5	1.00 (1.0-1.1)	5	2.00 (1.0-3.0)	5	1.97 (1.0-2.1)
$k_{el}$ (1/h)	5	0.94 (0.58)	5	0.68 (0.23)	6	0.57 (0.15)	5	0.71 (0.27)	5	0.51 (0.67)	5	0.53 (0.09)
$t_{1/2}$ (h)	5	0.92 (0.40)	5	1.11 (0.33)	6	1.30 (0.46)	5	1.08 (0.37)	5	1.38 (0.20)	5	1.32 (0.19)
$AUC_{0-6}$ (mg · h/liter)	5	7.60 (3.50)	5	8.41 (3.71)	6	10.61 (5.59)	5	8.31 (3.51)	5	12.33 (4.34)	5	13.94 (4.87)
$AUC_{0-\infty}$ (mg · h/liter)	5	7.84 (3.74)	5	8.75 (3.87)	6	11.51 (6.90)	5	8.72 (3.90)	5	13.54 (4.96)	5	15.04 (5.50)
Clearance (no. of liters/h)	5	22.01 (9.89)	5	19.5 (8.59)	6	24.29 (8.93)	5	31.89 (14.80)	5	34.49 (9.38)	5	30.61 (11.01)
<b>4 months of therapy</b>												
$C_{max}$ (µg/ml)	5	2.78 (1.15)	4	2.15 (1.19)	6	4.67 (1.02)	6	4.28 (1.98)	5	5.06 (2.83)	5	6.37 (2.37)
$T_{max}$ (h)	5	0.97 (0.9-1.0)	4	0.99 (1.0-2.0)	6	1.90 (1.0-3.2)	6	1.96 (1.0-3.0)	5	1.97 (1.0-3.0)	5	1.98 (1.0-3.0)
$k_{el}$ (1/h)	5	0.58 (0.12)	4	1.34 (1.41)	6	0.46 (0.15)	6	0.63 (0.25)	5	0.43 (0.09)	5	0.43 (0.14)
$t_{1/2}$ (h)	5	1.22 (0.24)	4	0.87 (0.45)	6	1.63 (0.56)	6	1.30 (0.61)	5	1.68 (0.33)	5	1.76 (0.52)
$AUC_{0-6}$ (mg · h/liter)	5	6.38 (2.38)	4	4.82 (2.71)	6	12.84 (1.52)	6	13.14 (7.69)	5	15.25 (8.22)	5	20.43 (9.74)
$AUC_{0-\infty}$ (mg · h/liter)	5	6.67 (2.33)	4	4.97 (2.82)	6	14.51 (2.82)	6	14.74 (8.88)	5	17.30 (8.67)	5	24.92 (13.64)
Clearance (no. of liters/h)	5	25.68 (8.56)	4	37.98 (19.25)	6	16.97 (3.29)	6	25.70 (21.70)	5	30.90 (14.41)	5	21.71 (12.76)

<sup>a</sup> Shown as mean values (SD), except for  $T_{max}$  shown as median values (range).

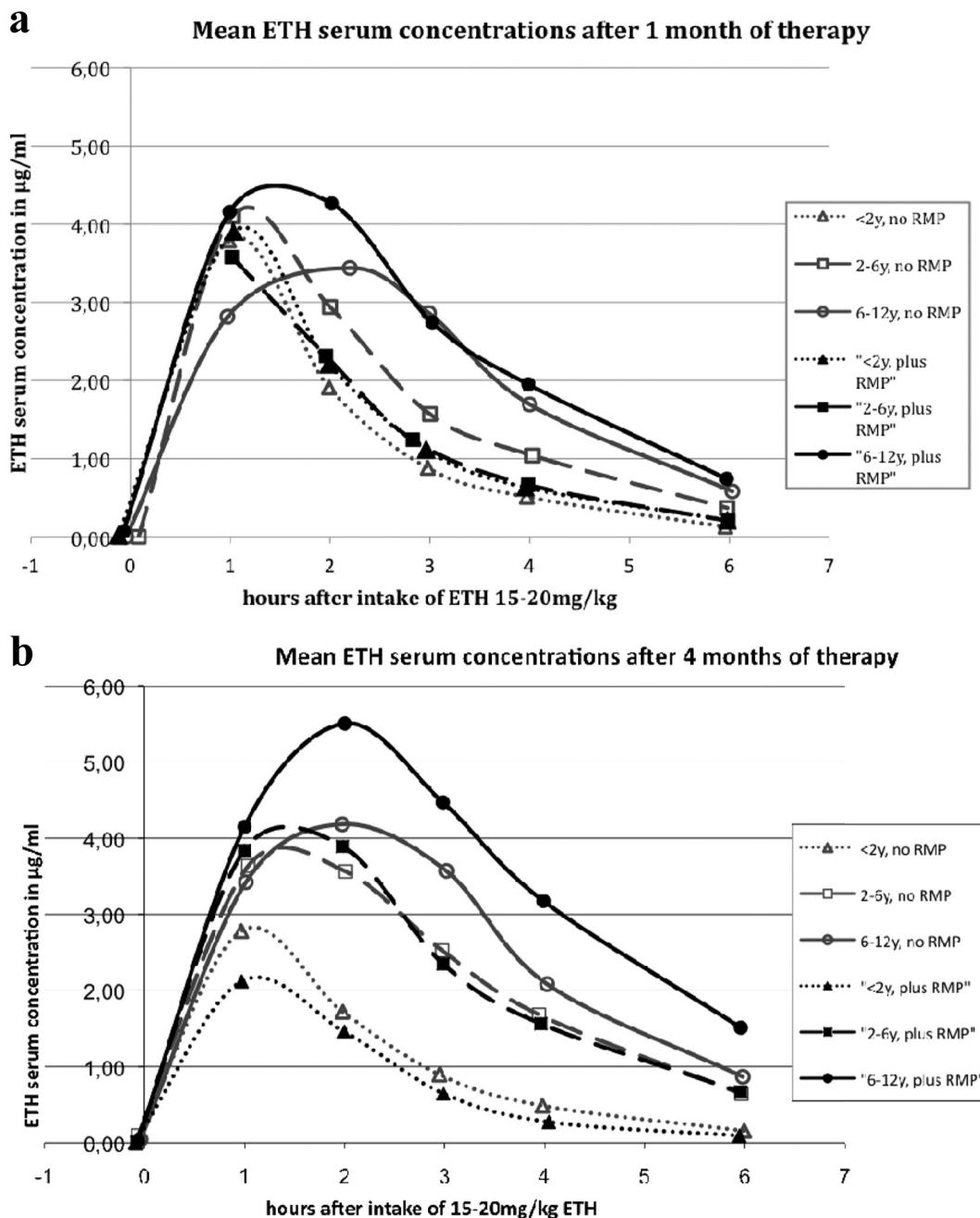


FIG. 1. (a and b) Mean ETH serum concentrations after oral intake of 15 to 20 mg/kg ETH in children of different age groups with or without concomitant treatment with rifampin (RMP). y, years.

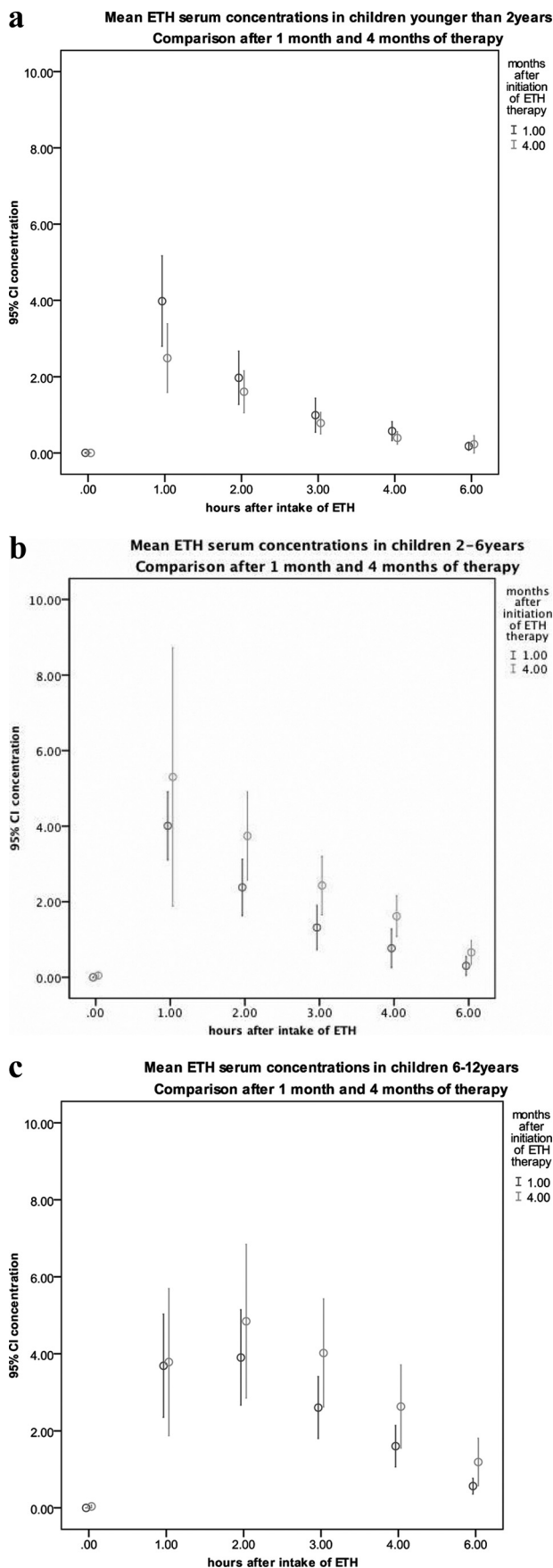
Children in both treatment groups ( $n = 31$ ) were therefore combined for analysis of pharmacokinetic parameters in different age groups.

After month 1 [referred to by subscripted "(1)"], younger children were exposed to lower ETH concentrations than older children.  $AUC_{0-6(1)}$  in children of <2 years of age was lower than that in children 6 to <12 years ( $P = 0.035$  and  $0.013$ , respectively) (Table 2). While the differences in  $C_{max(1)}$  between children of <2 years of age and older children were slight ( $P = 0.756$  and  $0.2922$ , respectively), absorption [ $T_{max(1)}$ ,  $P = 0.372$  and  $0.004$ , respectively] and elimination [ $t_{1/2(1)}$ ,  $P =$

$0.215$  and  $0.038$ , respectively;  $k_{el(1)}$ ,  $P = 0.197$  and  $0.036$ , respectively] occurred more rapidly in younger children.

At month 4 [referred to by subscripted "(4)"], differences between the age groups (children of <2 years of age and older children) became more pronounced [ $AUC_{0-6(4)}$ ,  $P = 0.027$  and  $0.001$ , respectively].  $C_{max(4)}$  in children younger than 2 years of age was significantly lower than that in older children ( $P = 0.034$  and  $0.002$ , respectively). Again, absorption [ $T_{max(4)}$ ,  $P = 0.033$  and  $0.08$ , respectively] and elimination [ $t_{1/2(4)}$ ,  $P = 0.168$  and  $0.051$ , respectively;  $k_{el(4)}$ ,  $P = 0.140$  and  $0.002$ , respectively] occurred more rapidly in younger children than in older children.





Age correlated significantly with  $T_{\max(1)}$  ( $r = 0.43$ ,  $P = 0.02$ ),  $AUC_{0-6(1)}$  ( $r = 0.50$ ,  $P = 0.001$ ), and  $clearance_{(1)}$  ( $r = 0.44$ ,  $P = 0.01$ ) at month 1, while the correlations with  $C_{\max(1)}$  ( $r = 0.18$ ,  $P = 0.32$ ),  $k_{el(1)}$  ( $r = -0.34$ ,  $P = 0.06$ ), and  $t_{1/2(1)}$  ( $r = 0.34$ ,  $P = 0.06$ ) were not statistically significant. At month 4, age correlated significantly with  $T_{\max(4)}$  ( $r = 0.47$ ,  $P = 0.01$ ),  $AUC_{0-6(4)}$  ( $r = 0.63$ ,  $P = 0.001$ ),  $C_{\max(4)}$  ( $r = 0.60$ ,  $P = 0.00$ ),  $k_{el(4)}$  ( $r = 0.43$ ,  $P = 0.02$ ), and  $t_{1/2(4)}$  ( $r = 0.43$ ,  $P = 0.02$ ) but was not correlated with ETH clearance (2) ( $r = -0.10$ ,  $P = 0.60$ ).

**Differences: RMP/no RMP.** No differences in ETH pharmacokinetic parameters were detected at either sampling point between children receiving a RMP-including regimen or not (1 month/4 months of therapy), as follows:  $C_{\max}$ ,  $P = 0.530/0.859$ ;  $T_{\max}$ ,  $P = 0.304/0.748$ ;  $AUC_{0-6}$ ,  $P = 0.835/0.627$ ;  $k_{el}$ ,  $P = 0.735/0.109$ ;  $t_{1/2}$ ,  $P = 0.891/0.102$ ; and clearance,  $P = 0.833/0.372$ .

Similar results were obtained in the RMP/no RMP analysis stratified by age (data not shown).

**Differences in ETH levels over time.** Differences in ETH serum levels at 1 and 4 months of therapy are shown in Fig. 2a to c by age group.

Taking all children together, there was no difference between the two time points for  $C_{\max}$  ( $P = 0.6934$ ),  $AUC_{0-6}$  ( $P = 0.131$ ),  $k_{el}$  ( $P = 0.887$ ), and clearance ( $P = 0.949$ ).  $T_{\max}$  was significantly lower at 1 month [mean  $T_{\max(1)}$  versus mean  $T_{\max(4)}$ , 78 min versus 98 min, respectively;  $P = 0.048$ ], as was  $t_{1/2}$  [ $t_{1/2(1)}$  versus  $t_{1/2(4)}$ , 71 min versus 83 min, respectively;  $P = 0.035$ ].

## DISCUSSION

With an ETH dose as currently recommended by WHO, the majority of children achieved sufficient serum levels. Children younger than 2 years of age were exposed to lower ETH concentrations than older children receiving the same mg/kg dose. This may imply a need for a higher dosage in this age group. HIV coinfection led to lower ETH serum levels.

Neither comedication with RMP nor the duration of ETH treatment influenced ETH pharmacokinetic parameters in children.

Our study documents high variability in ETH serum levels in children receiving a standard oral dose of ETH of 15 to 20 mg/kg body weight. In adults, significant intra- and interindividual variations in ETH serum concentrations have been reported (2, 19, 21), consistent with our findings. Although in the youngest children, the observed variability might be partly attributable to difficulties in taking medication, we observed similar variability in older children, where no such difficulties were experienced.

ETH peak serum concentrations and the resulting drug exposures were lower in younger children. In up to 50% of children (5 of 10) younger than 2 years of age, recommended

FIG. 2. (a to c) Mean ETH serum levels before and after intake of ETH after 1 and 4 months of antituberculosis therapy including ETH. Both children with and without RMP are combined in graphs ( $n = 31$ ). CI, confidence interval.

serum levels of ETH were not achieved. Lower levels of first-line antituberculosis drugs have previously been reported in children than in adults; these have been attributed to changes in the relative size of body compartments and body surface area (BSA) and changes in the ability to absorb, metabolize, and excrete drugs (1, 4, 7, 25, 26, 29). While the time until maximum serum concentrations were reached was similar ( $T_{max}$ , 1 to 2 h),  $C_{max}$  was considerably lower in children younger than 2 years than in older children or adults. After the first weeks of life, gastrointestinal absorption is less relevant for the bioavailability of a drug than the volume of distribution and first-pass metabolism (3). ETH is widely distributed into body tissues and fluids, resulting in a high volume of distribution in adults and children (28). After maturation of liver enzymes, the rate of metabolism is determined mainly by liver growth, which correlates well with BSA. Most hepatic enzymes are mature by the age of 1 year (3). Therefore, a comparatively larger liver size and correspondingly higher hepatic blood flow in young children may account for the high first-pass effect, resulting in reduced bioavailability of ETH. The same principles are the reason for the more rapid elimination of ETH in children than in adults. In studies of ETH pharmacokinetics in adults, a  $k_{el}$  of 0.4/h and a corresponding elimination half-life of 1.6 to 1.8 h has been reported (2, 29). In our study, the mean half-life in children below 2 years of age was approximately 1 h and increased with age. Because ETH metabolism is predominantly by hepatic sulfoxidation, ETH dosing according to BSA should therefore be considered to allow for age-related changes in liver size.

Comedication with RMP did not influence the pharmacokinetics of ETH in the present study. RMP is a strong inducer of several enzymes, including different monooxygenases (17). Reduced bioavailability of several drugs (e.g., antiretroviral drugs, oral contraceptives, and oral anticoagulants) when given in combination with RMP has been reported (5, 16), and reduced serum levels of antituberculosis drugs in the context of an RMP-containing treatment regimen has therefore been a concern. RMP does not influence the bioavailability of isoniazid (18). The monooxygenases responsible for ETH sulfoxidation are not part of the P450 system. We did not find any evidence that these monooxygenases are induced by RMP, which is, to our knowledge, the first study to have assessed this potential drug interaction.

Accumulation or self-induction of metabolism during continuous antituberculosis therapy might alter the pharmacokinetic parameters of a drug over time. For ETH, both the absorption and elimination seemed to be delayed after 4 months of therapy. The reason for this is unclear. Slowing down of the enzyme activity responsible for ETH metabolism over time might be a possible reason but has not yet been described. However, since children were exposed to the same amount of drug, measured by the AUC at the beginning of and during continuous therapy, the clinical relevance of this finding is probably minor.

Although not part of our primary study aims, we found that HIV-infected children were exposed to lower ETH concentrations than their HIV-uninfected counterparts. Reduced serum levels of several antituberculosis drugs in HIV-infected adults and children have been reported and have been attributed to

malabsorption caused by drug-drug interactions, diarrhea, and/or concurrent gastrointestinal infections (6, 11, 13, 23).

Despite the observed differences, the mean ETH serum concentrations in children of all age groups were above the recommended serum level of 2.5  $\mu\text{g/ml}$  following a standard oral dose of ETH of 15 to 20 mg/kg. In older children and adults, ETH causes dose-dependent serious gastrointestinal irritability and clinically, dosing is therefore driven by tolerance rather than efficacy. Lower mg/kg doses in older children might therefore increase patients' tolerability and compliance to ETH-inclusive treatment regimes without reducing the potential therapeutic efficacy. Despite adequate doses in most children, some possible concerns regarding lower ETH doses remain, as follows: a considerable proportion of children in our study were exposed to low ETH serum concentrations, and after 4 months of therapy, 7 of 31 children (5 aged <2 years) did not achieve the recommended ETH serum levels. A previous study of children with TBM compared ETH concentrations in the cerebrospinal fluid (CSF) after an oral dose of ETH at 15 mg/kg versus 20 mg/kg (8). After the 15-mg/kg dose, the recommended ETH level was achieved in the CSF in very few children (27%); therefore, a single daily dose of 20 mg/kg ETH was recommended for treatment of TBM (8). There is evidence that ETH can produce a bactericidal effect in concentrations of 2.5 to 5.0  $\mu\text{g/ml}$  *in vitro*, with a 99% killing of *M. tuberculosis* (15). *In vivo*, ETH is metabolized mainly to ETH-sulfoxide, which is less stable than ETH but also has antimycobacterial activity and might add to the therapeutic efficacy of ETH (12). At this stage, it is unknown whether higher *in vivo* ETH serum concentrations (e.g., above 2.5  $\mu\text{g/ml}$ ) increase the bactericidal activity or if lower ETH levels are sufficient in the presence of this active metabolite. A limitation of our study is that adverse effects were not studied systematically. Our findings in this relatively small study group also need to be validated in further studies.

In conclusion, ETH at the recommended oral dose of 15 to 20 mg/kg leads to mean serum levels at or above the recommended concentration in the majority of children of all ages. ETH levels are not influenced by concomitant treatment with RMP and do not change substantially during treatment. Nevertheless, the variability in ETH serum levels is high, and inadequate levels in some children were achieved. Younger children and HIV-infected children are at higher risk for subtherapeutic serum levels, and higher dosages can be considered if clinically tolerated. The recommended dose of 15 to 20 mg/kg seems appropriate in older children.

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## 5.2. The pharmacokinetics of ofloxacin, levofloxacin, and moxifloxacin in children with tuberculosis

In these observational, intensive sampling pharmacokinetic studies, the pharmacokinetic measures of the 3 most frequently used fluoroquinolones in anti-tuberculosis therapy, OFX, LFX and MFX, were investigated in children younger than 15 years of age with MDR-TB (359, 360). MFX, the most effective fluoroquinolone against *M. tuberculosis*, is currently only available in 400mg tablets, which are only breakable into half. Therefore appropriate dosing is only possible in children weighing more than 20kg (older than 8 years). Following the revision of the 2013 South African treatment guidelines, which had previously recommended OFX as the only available fluoroquinolones in the country (361), children older than 8 years would receive MFX and children younger than 8 years LFX instead of OFX. OFX is, however, still used extensively in other settings where the newer, more costly fluoroquinolones, are not readily available. Paediatric data on the pharmacokinetics of OFX will therefore remain highly relevant. I hypothesized that the current dosing recommendations for the fluoroquinolones OFX, LFX and MFX, would achieve similar serum concentrations compared to adult target values (see chapter 4).

In adults, standard daily doses of the fluoroquinolones are OFX 800mg, LFX 750mg and MFX 400mg. Following these doses a  $C_{max}$  of 8.5-10  $\mu\text{g}/\text{ml}$  for OFX, 7-12 $\mu\text{g}/\text{ml}$  for LFX, and 2.5-6 $\mu\text{g}/\text{ml}$  for MFX and an AUC of 70-100 $\mu\text{g}\cdot\text{h}/\text{ml}$  for OFX, 70-110  $\mu\text{g}\cdot\text{h}/\text{ml}$  for LFX and 25-60  $\mu\text{g}\cdot\text{h}/\text{ml}$  for MFX, have been reported (362-368). Proposed pharmacodynamic targets for fluoroquinolones against *M. tuberculosis* are  $\text{AUC}_{0-24}/\text{MIC} >100$  or  $C_{max}/\text{MIC}$  8-10 (71, 369-371). Efficacy studies of fluoroquinolones in Middlebrook 7H11 revealed the following MICs: 1.0 $\mu\text{g}/\text{ml}$  for Ofx (370, 372, 373), 0.5-1.0 $\mu\text{g}/\text{ml}$  for Lfx (370, 372-374) and 0.25-0.5 $\mu\text{g}/\text{ml}$  for Mfx (373-375). Following these assumptions, presumably efficacious AUCs for anti-TB therapy are: 100 $\mu\text{g}\cdot\text{h}/\text{ml}$  for Ofx, 50-100 $\mu\text{g}\cdot\text{h}/\text{ml}$  for Lfx and 25-50  $\mu\text{g}\cdot\text{h}/\text{ml}$  for Mfx. This is matching well the drug exposure achieved in adults following standard fluoroquinolone therapy (as shown above). Although not optimal, the major tools at hand to determine desired serum levels of an anti-TB drug in children are comparative clinical data from adults and their pharmacokinetic “optimal” target values.

Noncompartmental analysis (NCA) based on serum concentrations following extensive sampling schedule of 6 samples over 8 hours were used to derive standard pharmacokinetic measures for OFX, LFX and MFX. Co-variables of interest included age, HIV co-infection and nutritional status. These fluoroquinolone pharmacokinetic studies were sub-studies (principal investigator S. Thee) nested in a larger ongoing NIH-funded study investigating the pharmacokinetics and toxicity of second-line anti-tuberculosis drugs in HIV-infected and uninfected children with MDR-TB (N11/03/059: principal investigator A. Hesselring).

Eligibility criteria for these studies included: HIV-infected and -uninfected children aged 0-<15 years, routinely started on second-line treatment regimen for drug-resistant TB (DR-TB) disease or preventive therapy and which included a fluoroquinolones. Written informed consent and assent for all aspects of the study as age-appropriate was obtained. The serum concentrations of OFX, LFX and MFX were determined by LC-mass spectrometry methodology at the Division of Pharmacology of the University of Cape Town (University of Cape Town, by collaborators Professors Peter Smith and Helen McIlleron). The medians of  $C_{max}$ ,  $t_{max}$ , and AUC were compared by age, HIV status and study group using the Mann-Whitney U test.

### **Ofloxacin and Levofloxacin**

The pharmacokinetics of OFX and LFX were studied in 23 children (median age 3.14 years, interquartile range [IQR] 1.3-4.0 years). Four children were HIV-infected. Following an oral dose of OFX 20mg/kg and LFX 15mg/kg the median (IQR) pharmacokinetic measures for Ofx/Lfx were as follows:  $C_{max}$  9.7 (7.5-10.9)/6.8 (4.7-8.1)  $\mu\text{g/ml}$ ,  $t_{max}$  1.6 (0.73)/1.4 (0.5)h,  $AUC_{0-8}$  43.8 (36.7-54.5)/30.5 (24.4-36.4)  $\mu\text{g}\cdot\text{h/ml}$ .

This study documents substantially lower OFX and LFX drug exposure represented by the AUC in children compared to adult studies, and also compared to other reported paediatric studies. This may be related to differences in drug formulation or administration method, or the study population. Although elimination tended to be higher in younger children, we found no difference in drug exposure to OFX and LFX by age group. This may be attributable to confounding by HIV, as HIV infection is associated with reduced absorption of some anti-tuberculosis agents, and all the children in our

study >6 years of age were HIV-infected. Children in this study failed to achieve an  $AUC_{0-24}/MIC >100$ , even if a lower  $MIC_{90}$  was used. These data are concerning, and indicate the need to optimize the dosing of fluoroquinolones in children with TB. Nevertheless, our estimated pharmacodynamic indices favour LFX over OFX.



# Pharmacokinetics of Ofloxacin and Levofloxacin for Prevention and Treatment of Multidrug-Resistant Tuberculosis in Children

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**Limited data on fluoroquinolone pharmacokinetics and cardiac effects in children exist. Among 22 children receiving drug-resistant tuberculosis prophylaxis or treatment, serum concentrations following oral doses of levofloxacin (15 mg/kg of body weight) and ofloxacin (20 mg/kg) were lower than those expected from existing pediatric data, possibly due to differences in the formulations (crushed tablets). Drug exposures were lower than those in adults following standard doses and below the proposed pharmacodynamic targets, likely due to more rapid elimination in children. No QT prolongation was observed.**

Multidrug-resistant tuberculosis (MDR-TB) (i.e., resistance to rifampin and isoniazid) is an emerging epidemic with an estimated 63,000 pediatric cases per year (1, 2). Fluoroquinolones are essential in drug-resistant TB (DR-TB) treatment, but pharmacokinetic and safety data (including data on potential QT interval prolongation) for children are limited (3–6). South African DR-TB treatment guidelines were revised during 2012 to recommend levofloxacin (Lfx) and moxifloxacin (Mfx) instead of ofloxacin (Ofx) in children. Due to tablet sizes, children >8 years receive Mfx and children <8 years receive Lfx for prevention or treatment of DR-TB. We aimed to characterize the pharmacokinetics and cardiac effects of Ofx and Lfx in children <8 years of age.

A prospective crossover intensive pharmacokinetic sampling study was conducted at Brooklyn Chest Hospital and Tygerberg Children's Hospital in Cape Town, South Africa from May 2012 through March 2013. Children aged 3 months to 8 years routinely started on either Ofx or Lfx for the prevention or treatment of MDR-TB were eligible. Exclusion criteria were a hemoglobin level of <8 g/dl or a weight of <5 kg. Child contacts (<5 years or HIV infected) of infectious MDR-TB cases without TB disease received preventive therapy, including 20 mg/kg of body weight Ofx or 15 mg/kg Lfx daily for 6 months. Children with MDR-TB received MDR-TB treatment based on World Health Organization recommendations (7). South African Medicines Control Council-approved tablets of Ofx (Tarivid, 200-mg tablets; Sanofi-Aventis, Midrand, South Africa) and Lfx (250-mg tablets; Cipla, Cape Town, South Africa) were used.

Two pharmacokinetic samplings were done, alternately for Ofx and Lfx, both at steady state. Exact doses of Ofx of 20 mg/kg and of Lfx of 15 mg/kg were used, and the tablets were broken accordingly and weighed to the nearest 0.1 mg. After an overnight fast, tablets were given whole or crushed and suspended in water, and the dose taken was observed. Blood samples were collected predose and at 1, 2, 4, 6, and 8 h after dosing in EDTA-containing tubes and centrifuged, and plasma was separated and frozen within 30 min at –80°C. Lfx concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay developed in the Division of Clinical Pharmacology, University of Cape Town, South Africa. Plasma

TABLE 1 Demographic and clinical characteristics of children receiving ofloxacin and levofloxacin for prevention or treatment of DR-TB<sup>a</sup>

Characteristic	MDR disease group (n = 12) (n [%])	MDR PT <sup>b</sup> group (n = 11) (n [%])
Age at enrollment		
0–2 yr	4 (36.4)	5 (45.5)
2–6 yr	3 (27.3)	6 (54.6)
≥6 yr	4 (36.4)	0 (0.0)
Male	6 (54.6)	7 (63.6)
Previous TB episode or treatment	4 (36.4)	0 (0.0)
Certainty of TB diagnosis (n = 12)		
Bacteriological confirmation	5 (45.5)	
Probable TB	5 (45.5)	
Suspected TB	1 (9.1)	
TB disease type (n = 12)		
PTB <sup>c</sup> only	7 (63.6)	
EPTB <sup>d</sup> only	0 (0.0)	
PTB and EPTB	4 (36.4)	
Reason DR PT started (n = 11)		
DR contact only		8 (72.7)
DR contact and positive TST <sup>e</sup>		3 (27.3)
HIV infected at baseline	4 (36.4) <sup>f</sup>	0 (0.0)
Weight-for-age Z score < –2.0 (n = 23)	5 (45.5)	1 (9.1)
Height-for-age Z score < –2.0 (n = 22)	6 (54.6)	2 (18.2)
Weight-for-length Z score < –2.0 (n = 22)	1 (9.1)	0 (0.0)

<sup>a</sup> DR-TB, drug-resistant tuberculosis.

<sup>b</sup> PT, preventive therapy.

<sup>c</sup> PTB, pulmonary TB.

<sup>d</sup> EPTB, extrapulmonary TB.

<sup>e</sup> TST, tuberculin skin test.

<sup>f</sup> Note that all HIV-infected children were >6 years of age.

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**TABLE 2** Summary statistics for pharmacokinetic parameters of ofloxacin and levofloxacin in children receiving treatment or prophylaxis for multidrug-resistant tuberculosis ( $n = 22$ )<sup>a</sup>

Drug	$C_{\max}$ ( $\mu\text{g/ml}$ )	$T_{\max}$ (h)	$k_{\text{el}}$ (1/h)	$t_{1/2}$ <sup>b</sup> (h)	$\text{AUC}_{0-8}$ ( $\mu\text{g} \cdot \text{h/ml}$ )	$\text{AUC}_{(0-\infty)}$ ( $\mu\text{g} \cdot \text{h/ml}$ )
Ofloxacin	9.75 (7.51–10.90)	1.59 (0.73)	0.22 (0.20–0.25) <sup>c</sup>	3.18 (2.83–3.44) <sup>d</sup>	43.84 (36.73–54.46)	47.51 (40.26–58.50)
Levofloxacin	6.79 (4.69–8.06)	1.45 (0.51)	0.22 (0.20–0.26)	3.16 (2.68–3.51)	30.47 (24.41–36.39)	32.92 (25.44–40.88)

<sup>a</sup> All parameters are reported using medians (interquartile ranges [IQRs]), except for time to maximum concentration in serum ( $T_{\max}$ ), which is reported using means (SDs).

<sup>b</sup>  $t_{1/2}$ , half-life.

<sup>c</sup> One child was excluded from the analysis because a wrong dose of Lfx (10 mg/kg) was given.

<sup>d</sup> One child had a late peak in the concentration-time curve. For this reason, no half-life or elimination rate constant is reported for that child.

samples were extracted, and chromatographic separation was achieved on a Gemini-NX 5- $\mu\text{m}$  C18 (50-mm by 2-mm) analytical column. An AB Sciex API 3000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode. The calibration curve fitted a linear (weighted by 1/concentration) regression over the range of 0.0781 to 5.0  $\mu\text{g/ml}$ . Ofx plasma concentrations were determined using an LC-MS/MS method validated over the concentration range of 0.0781 to 20.0  $\mu\text{g/ml}$  (8).

Noncompartmental analysis was used to calculate pharmacokinetic measures. The area under the curve from 0 to 8 h ( $\text{AUC}_{0-8}$ ) and the AUC from 0 to 24 h ( $\text{AUC}_{0-24}$ ) were calculated according to the trapezoidal rule. Extrapolated Lfx and Ofx concentrations at 24 h were estimated assuming first-order elimination. The pharmacodynamic indices  $\text{AUC}_{0-24}/\text{MIC}$  and the maximum concentration of drug in serum ( $C_{\max}$ )/MIC ratios were calculated using published  $\text{MIC}_{90}$  estimates for Ofx of 1.0 and 2.0  $\mu\text{g/ml}$  and for Lfx of 0.5 and 1.0  $\mu\text{g/ml}$  (9–12). Stata 12.1 (StataCorp, College Station, TX) was used for analysis. A 12-lead electrocardiogram (ECG) was done at 3 h postdose on each pharmacokinetic assessment day, and corrected QT intervals (QTc) were calculated (ac-

ording to Fridericia [13]). A QTc of >450 ms was considered prolonged.

Parental written informed consent was obtained. The study was approved by the Stellenbosch University Human Research Ethics Committee.

Twenty-three children (median age, 3.14 years; interquartile range, 1.3 to 4.0 years) were enrolled and completed both pharmacokinetic assessments (Table 1). One child was excluded because of incorrect dosing of Lfx. Seven Ofx and 10 Lfx concentration measures sampled at time zero were listed as below the limit of quantitation and were taken as 0; there were no missing concentration measurements for Ofx or Lfx. Summary pharmacokinetic parameters are described in Table 2, with pharmacokinetic measures stratified by the clinical characteristics shown in Tables 3 (Ofx) and 4 (Lfx). Table 5 shows the estimated pharmacodynamic measures which favored Lfx. The mean QTc was 361 ms (standard deviation [SD], 37 ms) for Ofx and 369 ms (SD, 22 ms) for Lfx. No child in either group had a QTc of >450 ms.

This study documents lower drug exposure to Ofx and Lfx in children compared to the expected values based on existing pedi-

**TABLE 3** Pharmacokinetic measures of ofloxacin by study group, age, HIV status, and nutritional status ( $n = 22$ )

Clinical characteristic	Pharmacokinetic measure of ofloxacin											
	$C_{\max}$			$T_{\max}$			$\text{AUC}_{0-8}$			$t_{1/2}$ <sup>a</sup>		
	No.	Median (IQR) ( $\mu\text{g/ml}$ )	<i>P</i>	No.	Mean (SD) (h)	<i>P</i>	No.	Median (IQR) ( $\mu\text{g} \cdot \text{h/ml}$ )	<i>P</i>	No.	Median (IQR) (h)	<i>P</i>
<b>Study group</b>												
MDR disease	11	10.20 (7.51–10.90)		11	1.82 (0.87)		11	46.53 (38.88–54.98)		10	3.13 (2.89–3.44)	
MDR PT <sup>b</sup>	11	8.88 (7.05–12.70)	0.77 <sup>c</sup>	11	1.36 (0.51)	0.15 <sup>c</sup>	11	43.34 (29.75–50.89)	0.53 <sup>c</sup>	11	3.18 (2.64–3.61)	0.89 <sup>c</sup>
<b>Age group</b>												
0–2 yr	9	10.90 (10.20–12.70)		9	1.22 (0.44)		9	46.53 (43.05–54.46)		9	2.91 (2.61–3.26)	
2–6 yr	9	8.78 (5.39–9.82)		9	1.56 (0.53)		9	44.34 (28.99–48.76)		9	3.21 (3.00–3.61)	
$\geq 6$ yr <sup>d</sup>	4	7.69 (6.21–9.39)	0.02 <sup>e</sup>	4	2.5 (1.0)	0.01 <sup>e</sup>	4	39.01 (33.47–48.06)	0.54 <sup>c</sup>	3	3.44 (2.89–3.57)	0.29 <sup>e</sup>
<b>HIV status</b>												
HIV infected <sup>f</sup>	4	7.69 (6.21–9.39)		4	2.5 (1.0)		4	39.01 (33.47–48.06)		3	3.44 (2.89–3.57)	
HIV uninfected	18	9.86 (8.09–11.57)	0.25 <sup>c</sup>	18	1.39 (0.50)	0.11 <sup>c</sup>	18	44.67 (36.73–54.46)	0.55 <sup>c</sup>	18	3.16 (2.63–3.34)	0.48 <sup>c</sup>
<b>WAZ<sup>g</sup></b>												
$\geq -2.0$	18	10.05 (8.78–11.57)		18	1.44 (0.51)		18	45.77 (39.12–54.98)		18	3.20 (2.89–3.44)	
$< -2.0$	4	6.63 (5.15–7.98)	0.04 <sup>c</sup>	4	2.25 (1.26)	0.29 <sup>c</sup>	4	29.37 (28.52–34.45)	0.03 <sup>c</sup>	4	2.64 (2.56–3.57)	0.42 <sup>c</sup>

<sup>a</sup>  $t_{1/2}$ , half-life. For one child, no  $t_{1/2}$  could be reported due to a late peak serum concentration of Ofx.

<sup>b</sup> PT, preventive therapy.

<sup>c</sup> *P* value was calculated using the Mann-Whitney *U* test.

<sup>d</sup> One child was excluded from the analysis because a wrong dose of Lfx (10 mg/kg) was given.

<sup>e</sup> *P* value was calculated using the Kruskal-Wallis test.

<sup>f</sup> Note that all of the HIV-infected children were also >6 years old.

<sup>g</sup> WAZ, weight-for-age *Z* score using WHO reference standards (29).

TABLE 4 Pharmacokinetic measures of levofloxacin by study group, age, HIV status, and nutritional status ( $n = 22$ )

Clinical characteristic	Pharmacokinetic measure of levofloxacin											
	$C_{\max}$			$T_{\max}$			$AUC_{0-8}$			$t_{1/2}^a$		
	No.	Median (IQR) ( $\mu\text{g}/\text{ml}$ )	$P$	No.	Mean (SD) (h)	$P$	No.	Median (IQR) ( $\mu\text{g} \cdot \text{h}/\text{ml}$ )	$P$	No.	Median (IQR) (h)	$P$
Study group												
MDR disease	11 <sup>b</sup>	7.00 (4.69–8.06)		11 <sup>b</sup>	1.46 (0.52)		11 <sup>b</sup>	32.50 (24.41–38.83)		11 <sup>b</sup>	3.24 (3.01–3.99)	
MDR PT <sup>c</sup>	11	6.32 (4.63–8.17)	0.53 <sup>d</sup>	11	1.46 (0.52)	1.00 <sup>d</sup>	11	29.89 (21.07–32.75)	0.34 <sup>d</sup>	11	2.98 (2.64–3.51)	0.31 <sup>d</sup>
Age group												
0–2 yr	9	7.00 (6.32–8.06)		9	1.33 (0.50)		9	29.89 (24.50–36.39)		9	2.79 (2.62–3.14)	
2–6 yr	9	6.86 (4.69–7.51)		9	1.56 (0.53)		9	31.69 (23.81–33.11)		9	3.22 (2.98–4.24)	
$\geq 6$ yr <sup>e</sup>	4	4.98 (4.52–7.48)	0.40 <sup>f</sup>	4	1.50 (0.58)	0.66 <sup>f</sup>	4	27.49 (24.73–34.03)	0.91 <sup>f</sup>	4	3.37 (3.12–4.01)	0.18 <sup>f</sup>
HIV status												
HIV-infected <sup>c</sup>	4	4.98 (4.52–7.48)		4	1.5 (0.58)		4	27.49 (24.73–34.03)		4	3.37 (3.12–4.01)	
Not HIV-infected	18	6.88 (5.36–8.06)	0.39 <sup>c</sup>	18	1.44 (0.51)	0.85 <sup>c</sup>	18	31.38 (24.41–36.39)	0.67 <sup>c</sup>	18	3.09 (2.64–3.51)	0.23 <sup>c</sup>
WAZ <sup>g</sup>												
$\geq -2.0$	17	6.86 (4.69–8.06)		17	1.47 (0.52)		17	31.06 (23.81–36.39)		17	3.14 (2.78–3.51)	
$< -2.0$	5	6.71 (5.38–7.12)	0.91 <sup>c</sup>	5	1.40 (0.55)	0.79 <sup>c</sup>	5	29.24 (25.75–32.50)	0.91 <sup>c</sup>	5	3.23 (2.68–3.51)	0.97 <sup>c</sup>

<sup>a</sup>  $t_{1/2}$ , half-life.<sup>b</sup> One child was excluded from the analysis because a wrong dose of Lfx (10 mg/kg) was given.<sup>c</sup> PT, preventive therapy.<sup>d</sup>  $P$  value was calculated using the Mann-Whitney  $U$  test.<sup>e</sup> Note that all of the HIV-infected children were also  $>6$  years old.<sup>f</sup>  $P$  value was calculated using the Kruskal-Wallis test.<sup>g</sup> WAZ, weight-for-age  $Z$  score using WHO reference standards (29).

atric studies (4–6). This may be related to differences in drug formulations or administration methods. Tablets are the only oral formulation available in our setting; these need to be crushed for administration to young children. Although crushing might influence absorption, which is almost complete for the fluoroquinolones (4, 5, 14, 15), a study in adults comparing the pharmacokinetics of tablets and oral solutions of Ofx showed no differences (16). Although elimination tended to be higher in younger children, there were no differences for the Ofx and Lfx AUCs by age group. This may be related to confounding by HIV, as HIV infection is associated with reduced absorption of some anti-TB agents (17–19), and all the HIV-infected children in our study were  $>6$  years of age. A larger study sample would better characterize the pharmacokinetics of these drugs and provide insights into any differences in this population compared to those in previous reports.

We also documented lower Ofx and Lfx exposures in children compared to those in adults using the currently recommended doses (20–22). Children eliminated Ofx and Lfx more rapidly than adults, with a half-life of 3 to 4 h and an elimination rate constant ( $k_{el}$ ) of  $0.22 \text{ h}^{-1}$  in our study compared to half-lives of 4 to 8 h and  $k_{el}$  of 0.09 to  $0.13 \text{ h}^{-1}$  in adults (14, 20–22); this is likely due to the

well-described age-related changes in drug clearance in children compared to drug clearance in adults (23). The proposed pharmacodynamic targets for fluoroquinolones against *Mycobacterium tuberculosis* are an  $AUC_{0-24}/\text{MIC}$  of  $>100$  and  $C_{\max}/\text{MIC}$  of 8 to 10 (12, 24–26). Children in this study failed to achieve an  $AUC_{0-24}/\text{MIC}$  of  $>100$  even if a lower  $\text{MIC}_{90}$  was used. Nevertheless, our estimated pharmacodynamic indices favor Lfx over Ofx. The failure to approximate adult exposures or to achieve the pharmacodynamic targets has important implications for current and future dosing recommendations in children for these essential anti-TB drugs. With the current recommended dosing, successful MDR-TB treatment outcomes are reported in 80 to 90% of children (27, 28). However, to optimize treatment, higher or more frequent dosing of Ofx and Lfx in young children may be required to approximate adult exposures and meet the proposed pharmacodynamic targets.

Although the ECG timing may have been suboptimal in our study and additional research is needed, the lack of any QT prolongation is reassuring.

The limitations of this study are the modest sample size, the unavailability of individual MICs, and the administration of dif-

TABLE 5 Estimated pharmacodynamic parameters using published MICs of ofloxacin and levofloxacin for *Mycobacterium tuberculosis* in children

Pharmacokinetic parameter	Estimate for 20 mg/kg ofloxacin with MIC ( $\mu\text{g}/\text{ml}$ ) of:		Estimate for 15 mg/kg levofloxacin with MIC ( $\mu\text{g}/\text{ml}$ ) of:		$P^a$
	2.0	1.0	1.0	0.5	
$AUC_{0-24}/\text{MIC}$ ( $n = 21$ ) (mean [SD])	31.5 (10.3)	63.0 (20.5)	43.8 (13.3)	87.6 (26.7)	$<0.01$
$C_{\max}/\text{MIC}$ ( $n = 22$ ) (mean [SD])	4.5 (1.5)	9.6 (3.1)	6.5 (2.0)	13.1 (4.0)	$<0.01$

<sup>a</sup> Paired  $t$  test comparing pharmacodynamic parameters of Ofx and Lfx with an MIC of 2.0  $\mu\text{g}/\text{ml}$  for Ofx versus an MIC of 1.0  $\mu\text{g}/\text{ml}$  for Lfx and an MIC of 1.0 mg/ml for Ofx versus an MIC of 0.5  $\mu\text{g}/\text{ml}$  for Lfx.

ferent oral formulations of Ofx and Lfx. Additional studies on the safety and pharmacokinetics of Ofx and Lfx in children using higher doses to determine any impacts on treatment responses and outcomes should be considered.

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## Moxifloxacin

The pharmacokinetics of MFX were studied in 23 children (median age 11.1 years; IQR 9.2-12.0); 6/23 (26.1%) were HIV-infected. Following an oral Mfx dose of 10mg/kg a median (IQR)  $C_{max}$  of 3.1 (2.8-3.8) $\mu$ g/ml and a median  $AUC_{0-8}$  of 17.2 (14.5-22.0) $\mu$ g·h/ml were found. Three children, all HIV-infected, were underweight-for-age (UWA). HIV infection and UWA were both associated with lower MFX  $AUC_{0-8}$ , while  $C_{max}$  and  $t_{max}$  did not differ by nutritional status. There was a non-significant trend towards a lower  $C_{max}$  in HIV-infected children ( $p=0.08$ ).  $AUC_{0-8}$  was reduced by 6.85 $\mu$ g·h/ml (95% CI -11.15-2.56) in HIV-infected children. Crushing of tablets was associated with more rapid absorption of MFX, while  $C_{max}$  or AUC did not differ by administration method.

This first study investigating the pharmacokinetic and safety of MFX in children with TB demonstrated that exposure to MFX was substantially lower than that achieved with a standard dose of 400mg in adults, despite the relatively high mg/kg dose compared to adult dosing. In our study, MFX MIC of isolates from the children could not practically be determined and MFX MIC<sub>90</sub> of 0.5 $\mu$ g/ml was taken from published data from the same study setting (376). Children failed to achieve the proposed pharmacodynamic targets for MFX with a median  $AUC_{0-24}/MIC$  (IQR) of 46.6 (38.5-84.6) ( $n=12$ ) and a median  $C_{max}/MIC$  (IQR) of 6.2 (5.7-7.6) ( $n=23$ ).

With increasing numbers of paediatric MDR-TB especially in the context of HIV, anti-tuberculosis drug doses need be optimized to further improve outcome, shorten the duration of treatment and prevent the emergence of resistance. Since fluoroquinolones are the backbone of existing MDR-TB therapy and will remain fundamentally important in novel regimens that may be shorter and injectable-sparing, their optimal use in children with MDR-TB, and also DS-TB, is critically important. Failure to approximate adult exposures or to achieve pharmacodynamic targets has important implications for current and future dosing recommendations in children for these essential anti-tuberculosis drugs.

# Pharmacokinetics and Safety of Moxifloxacin in Children With Multidrug-Resistant Tuberculosis

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**Background.** Moxifloxacin is currently recommended at a dose of 7.5–10 mg/kg for children with multidrug-resistant (MDR) tuberculosis, but pharmacokinetic and long-term safety data of moxifloxacin in children with tuberculosis are lacking. An area under the curve (AUC) of 40–60  $\mu\text{g} \times \text{h/mL}$  following an oral moxifloxacin dose of 400 mg has been reported in adults.

**Methods.** In a prospective pharmacokinetic and safety study, children 7–15 years of age routinely receiving moxifloxacin 10 mg/kg daily as part of multidrug treatment for MDR tuberculosis in Cape Town, South Africa, for at least 2 weeks, underwent intensive pharmacokinetic sampling (predose and 1, 2, 4, 8, and either 6 or 11 hours) and were followed for safety. Assays were performed using liquid chromatography–tandem mass spectrometry, and pharmacokinetic measures calculated using noncompartmental analysis.

**Results.** Twenty-three children were included (median age, 11.1 years; interquartile range [IQR], 9.2–12.0 years); 6 of 23 (26.1%) were human immunodeficiency virus (HIV)-infected. The median maximum serum concentration ( $C_{\text{max}}$ ), area under the curve from 0–8 hours ( $\text{AUC}_{0-8}$ ), time until  $C_{\text{max}}$  ( $T_{\text{max}}$ ), and half-life for moxifloxacin were 3.08 (IQR, 2.85–3.82)  $\mu\text{g/mL}$ , 17.24 (IQR, 14.47–21.99)  $\mu\text{g} \times \text{h/mL}$ , 2.0 (IQR, 1.0–8.0) h, and 4.14 (IQR, 3.45–6.11), respectively. Three children, all HIV-infected, were underweight for age.  $\text{AUC}_{0-8}$  was reduced by 6.85  $\mu\text{g} \times \text{h/mL}$  (95% confidence interval, –11.15 to –2.56) in HIV-infected children.  $T_{\text{max}}$  was shorter with crushed vs whole tablets ( $P = .047$ ). Except in 1 child with hepatotoxicity, all adverse effects were mild and nonpersistent. Mean corrected QT interval was 403 (standard deviation, 30) ms, and no prolongation >450 ms occurred.

**Conclusions.** Children 7–15 years of age receiving moxifloxacin 10 mg/kg/day as part of MDR tuberculosis treatment have low serum concentrations compared with adults receiving 400 mg moxifloxacin daily. Higher moxifloxacin dosages may be required in children. Moxifloxacin was well tolerated in children treated for MDR tuberculosis.

**Keywords.** moxifloxacin pharmacokinetics; moxifloxacin toxicity; children; MDR tuberculosis.

The estimated global incidence of multidrug-resistant (MDR) tuberculosis (ie, resistance to at least isoniazid and rifampin) exceeded 450 000 cases in 2012 [1]. The true burden of tuberculosis in children is unknown, but

approximately 1 million children are estimated to have developed tuberculosis in 2010, of whom 32 000 had MDR tuberculosis [2]. New diagnostic tools, such as the Xpert MTB/RIF assay, will likely increase the number of MDR tuberculosis cases diagnosed in adults and therefore increase the number of children identified requiring MDR tuberculosis treatment [3]. MDR tuberculosis treatment should typically comprise 4–5 drugs to which the isolate of the child or the source case is susceptible, including an injectable drug for 6 months and a total duration of therapy of 18–24 months [4]. Fluoroquinolones have shown good in vitro and in vivo activity against *Mycobacterium tuberculosis* [5] and are an essential part of current MDR tuberculosis treatment

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regimens in adults and children [4, 6]. Moxifloxacin (MFX) has an early bactericidal activity close to that of isoniazid and is currently considered the most potent fluoroquinolone against *M. tuberculosis* [7, 8]. There is good evidence for its clinical efficacy at a standard dose (400 mg) in antituberculosis treatment in adults [9, 10].

The pharmacokinetics of MFX have been well characterized in adults with tuberculosis [10–15]. Bioavailability after oral intake of MFX is >90%, with little effect of food on absorption [16]. About half of the drug is metabolized in the liver, whereas 45% is excreted unchanged in urine and feces [17]. Following the standard oral dose, MFX pharmacokinetic measures in adults are as follows: maximum serum concentration ( $C_{max}$ ) 4–6  $\mu\text{g/mL}$ , elimination half-life ( $T_{1/2}$ ) up to 12 hours, and area under the concentration–time curve (AUC) of 40–60  $\mu\text{g} \times \text{h/mL}$  [8, 12–14, 18]. Coadministration of rifampin lowers MFX serum concentrations by up to 30% [11].

Although there are no prospective randomized trials of fluoroquinolones for the treatment of tuberculosis in children, high-quality observational data underscore their importance in successful MDR tuberculosis treatment in children [19, 20]. Pharmacokinetic data in children are limited to a single case study in a 1-month-old 1000-g former 28-week premature infant treated for *Mycoplasma hominis* meningitis [21]. Despite its routine use in children with MDR tuberculosis, there are limited data on MFX safety.

The South African treatment guidelines for MDR tuberculosis were revised during 2012 to recommend the use of levofloxacin and MFX both at a dose of 7.5–10 mg/kg instead of the less potent ofloxacin in children, consistent with World Health Organization (WHO) guidelines [4]. For MFX, appropriate dosing is not feasible in children weighing <20 kg (typically <8 years of age) due to the lack of a pediatric formulation. Only the 400-mg tablet formulated for adults is available. Hence, children weighing  $\geq 20$  kg routinely receive MFX and children weighing <20 kg receive levofloxacin (which is available in a 250-mg tablet), for the treatment of drug-resistant tuberculosis in most settings.

We characterize the pharmacokinetics and safety of MFX routinely given in combination with individualized optimized background regimen for the treatment of MDR tuberculosis in children >7 years of age and describe the effect of key clinical covariates.

## METHODS

### Study Design and Setting

This was a prospective intensive pharmacokinetic sampling study among children with MDR tuberculosis in Cape Town, South Africa.

### Study Population

From May 2012 through March 2014, human immunodeficiency virus (HIV)-infected and -uninfected children aged 7–15

years routinely started on MDR tuberculosis therapy including MFX were eligible. Children underwent pharmacokinetic sampling if on MDR tuberculosis treatment for 2–8 weeks. HIV-infected children also had to be on combination antiretroviral therapy (cART) for  $\geq 2$  weeks. Exclusion criteria were laboratory-documented anemia (hemoglobin <8 g/dL) or lack of informed consent/assent. Pharmacokinetic sampling in children with severe acute illness was deferred until patients were clinically stable.

### Diagnosis of Tuberculosis

The diagnosis was made according to consensus clinical research case definitions for pediatric tuberculosis [22]. Bacteriologic confirmation was attempted using sputum and other specimens and Middlebrook 7H9 broth-base (Mycobacterial Growth Indicator Tubes; Becton Dickinson, Sparks, Maryland) culture medium; a commercial molecular line probe assay was used to detect resistance to isoniazid and rifampin (GenoType MTBDR*plus* assay; Hain Lifescience, Nehren, Germany). In the absence of bacteriologic confirmation, children were treated empirically according to the drug susceptibility test results of the likely source cases' isolates.

Children received individualized treatment regimens, based on WHO recommendations and in accordance with their drug susceptibility test results or that of their source case, where known [4]. Approved tablets of MFX (moxifloxacin hydrochloride 400-mg tablets; Dr Reddy's Laboratories Ltd, Hyderabad, India) were used. All HIV-infected children received trimethoprim-sulfamethoxazole and cART consisting of 2 nucleoside reverse transcriptase inhibitors (lamivudine, stavudine, or abacavir) and either efavirenz (4 of 6 children) or lopinavir/ritonavir (2 of 6 children).

### Pharmacokinetic Investigation

Children receiving MFX 7.5–10 mg/kg once daily (according to WHO and South African MDR tuberculosis treatment guidelines) underwent pharmacokinetic sampling 2–8 weeks following initiation of tuberculosis treatment, when MFX would be expected to be at steady state. On the day of sampling, exact 10 mg/kg doses were calculated for each child. Tablets were cut and weighed accordingly (to the nearest 0.1 mg), and given either whole or crushed and suspended in water, according to patient tolerance. All tuberculosis drugs were administered with a small amount of water in the morning after a minimum of 4 hours of fasting; a standard breakfast was offered 1 hour later. HIV-infected children were given cART with breakfast. Blood samples were collected predose (time 0), and at 1, 2, 4, 8, and either 6 or 11 hours after dosing, in ethylenediaminetetraacetic acid-containing vacutainer tubes, centrifuged, and plasma was separated and frozen within 30 minutes at  $-80^{\circ}\text{C}$ .

MFX concentrations were determined using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay, at the Division of Clinical Pharmacology, University of Cape Town. The assay was validated according to US Food and Drug Administration and European Medicines Agency guidelines. Plasma samples were extracted and chromatographic separation achieved on a Gemini-NX 5 µm C18, 50 mm × 2.1 mm analytical column. An AB Sciex API 3000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode, monitoring the transition of the protonated molecular ions at mass-to-charge ratio (*m/z*) 402.2 to the product ions at *m/z* 384.2 for MFX, and monitoring the transition of the protonated molecular ions at *m/z* 406.3 to the product ions at *m/z* 388.2 for the stable isotope-labeled internal standard, MFX-d4. The calibration curve fitted a linear (weighted by 1/concentration) regression over the range 0.0628–16.1 µg/mL.

MFX pharmacokinetic measures were calculated using non-compartmental analysis (NCA). Stata 12.1 SE software (StataCorp 2011, College Station, Texas) was used for NCA and other analyses.  $C_{\max}$  and time until  $C_{\max}$  ( $T_{\max}$ ) were taken directly from the concentration-time data. The area under the curve from 0–8 hours ( $AUC_{0-8}$ ) was calculated using the linear trapezoidal rule. The  $T_{1/2}$  was denoted as  $\ln(2)/K_{el}$ , where  $K_{el}$  (elimination rate constant) is the negative slope of the log-linear regression of  $\geq 3$  final data points.  $K_{el}$  and  $T_{1/2}$  were not evaluated in patients with  $< 3$  concentration data points during the elimination phase. The area under the curve from 0–24 hours ( $AUC_{0-24}$ ) was calculated

using exponential extrapolation from the patient's final concentration data point at either 8 or 11 hours.

### Statistical Analysis

Demographic and clinical characteristics were summarized using descriptive statistics. MFX pharmacokinetic measures were reported as medians and interquartile ranges (IQRs). The  $AUC_{0-8}$ ,  $C_{\max}$ , and  $T_{\max}$  were compared by HIV status, nutritional status (weight-for-age *z* score [WAZ]  $\leq -2$  vs WAZ  $> -2$ ) using British growth charts, and administration method (crushed vs whole tablets) using the Wilcoxon rank-sum test. A multivariable linear regression model was generated to determine which covariates were associated with  $AUC_{0-8}$ . The clinically relevant variables HIV, nutritional status, and age were included in the model. Estimated pharmacodynamic indices ( $AUC_{0-24}/MIC$  [minimum inhibitory concentration] and  $C_{\max}/MIC$ ) were calculated using an approximate MFX antimicrobial concentration that inhibits growth of 90 % of the strains ( $MIC_{90}$ ) of 0.5 µg/mL based on published studies [5, 23].

### Assessment of Toxicity

All children had clinical safety assessments monthly for the first 6 months of treatment, then every 2 months until treatment was completed. Laboratory assessment was done every 2 months and included blood cell count (for children on cART and linezolid), liver function tests, creatinine, and thyroid function (for children on ethionamide or para-aminosalicylic acid). Toxicity and drug adverse effects were determined using combined data sources including chart and routine clinical review, laboratory investigation, and parental and children's report. Standard Division of AIDS (National Institute of Allergy and Infectious Diseases) criteria were used to grade severity of adverse events. Adverse events were considered MFX-related if they were possibly, probably, or definitely drug-related, and likely due to MFX or if they were not otherwise able to be attributed to another specific drug. Adverse events reported at consecutive follow-up visits without intervening asymptomatic periods were considered a single adverse event. Person time was calculated from the baseline study assessment until treatment completion or at the last available study visit if the child withdrew from the study or was still on treatment at the time of analysis.

A 12-lead electrocardiogram (ECG) was completed on the pharmacokinetic sampling day at 3 hours after drug dosing. ECGs were evaluated using a standard approach by a single pediatric cardiologist, with the corrected QT (QTc) interval calculated using the method described by Fridericia [24]; a QTc  $> 450$  ms was classified as prolonged.

Written informed consent was obtained from parents/legal guardians and written informed assent from children. The study was approved by the Health Research Ethics Committees, Stellenbosch University (reference number N11/03/059A) and

**Table 1. Demographic and Clinical Characteristics of Children (n = 23)**

Characteristic	No. (%)
Male sex	9 (39.1)
Race	
Black	13 (56.5)
Mixed ethnicity	10 (43.5)
Previous tuberculosis episode or treatment	11 (47.8)
Known current tuberculosis source case	12 (52.2)
Certainty of tuberculosis diagnosis <sup>a</sup>	
Bacteriologically confirmed	20 (87.0)
Probable tuberculosis	3 (13.0)
Tuberculosis disease type	
PTB only	14 (60.9)
EPTB only	3 (13.0)
PTB and EPTB	6 (26.1)
HIV-infected	6 (26.1)
Weight-for-age <i>z</i> score $< -2.0^b$	3 (13.0)

Abbreviations: EPTB, extrapulmonary tuberculosis; HIV, human immunodeficiency virus; PTB, pulmonary tuberculosis.

<sup>a</sup> According to Graham et al [22].

<sup>b</sup> Calculated using 1990 British growth charts.



the University of Cape Town, South Africa (reference number 200/2012).

## RESULTS

Twenty-three children, with a median age of 11.1 years (IQR, 9.2–12.0 years) and median weight of 28.9 kg (range, 21.4–66 kg), were included; 6 of 23 (26.1%) were HIV-infected. Three children, all HIV-infected, were underweight for age (UWA). Clinical and demographic features are summarized in Table 1. Pulmonary tuberculosis was predominant (20 children [87%]). Antituberculosis medication used included ethambutol, pyrazinamide, amikacin, kanamycin, MFX, ethionamide, terizidone, para-aminosalicylic acid, high-dose isoniazid, linezolid, clofazimine, amoxicillin-clavulanate, and rifampin. The median number of antituberculosis drugs given was 7 (IQR, 6–7).

Table 2 shows summary MFX pharmacokinetic measures. The interindividual variability of MFX serum concentrations was high, especially during the absorption phase (Figure 1). MFX concentrations at 0 hour were below the limit of quantification in 3 participants and were taken as zero when generating the NCA parameters. When  $K_{el}$  was available,  $AUC_{0-24}$  was extrapolated.  $K_{el}$  could only be evaluated in 12 of 23 children. These 12 children did not differ from the 11 children without  $K_{el}$  calculation regarding  $AUC_{0-8}$  or  $C_{max}$ .  $T_{max}$  occurred faster in children with  $K_{el}$  calculation (data not shown).

Table 3 shows pharmacokinetic measures stratified by HIV status, nutritional status, and administration method. Serum concentration-time curves following an oral MFX dose of 10 mg/kg stratified by HIV status are presented in Figure 2. HIV infection and UWA were associated with lower MFX  $AUC_{0-8}$ , whereas  $C_{max}$  and  $T_{max}$  did not differ by nutritional status. There was a nonsignificant trend toward a lower  $C_{max}$  in

HIV-infected children ( $P = .08$ ). Crushing of tablets was associated with more rapid absorption of MFX, whereas  $C_{max}$  or  $AUC$  did not differ by administration method. Neither sex nor race had an influence on MFX pharmacokinetic measures (data not shown). In a simple linear regression model,  $AUC_{0-8}$  was reduced by  $6.85 \mu\text{g} \times \text{h/mL}$  (95% confidence interval [CI],  $-11.15$  to  $-2.56 \mu\text{g} \times \text{h/mL}$ ) in HIV-infected children, and for each unit decrease in WAZ,  $AUC$  was reduced by  $2.39 \mu\text{g} \times \text{h/mL}$ . In a multivariable regression model,  $AUC_{0-8}$  was reduced by  $5.62 \mu\text{g} \times \text{h/mL}$  (95% CI,  $-10.94$  to  $-.30 \mu\text{g} \times \text{h/mL}$ ) among patients with HIV infection compared with uninfected patients, adjusting for age and nutritional status.

Children were followed for a median of 236 days (IQR, 142–541 days). Table 4 shows all adverse events noted as well as adverse effects potentially related to MFX. The main adverse effects potentially related to MFX were gastrointestinal (nausea and vomiting;  $n = 12$ ), headache ( $n = 5$ ), elevated alanine aminotransferase (ALT) ( $n = 4$ ), and arthralgia ( $n = 4$ ).

In 13 of 23 children, ECG data were available. Mean QTc interval was 403 ms (standard deviation, 30 ms). None had a QTc interval  $>450$  ms.

## DISCUSSION

This is the first study to our knowledge investigating the pharmacokinetics and safety of MFX in children with tuberculosis. Exposure to MFX was lower than that achieved with a standard dose of 400 mg in adults receiving MFX for MDR tuberculosis (Supplementary Table 1) despite the slightly higher dose given to children (median dose of 6.7–8 mg/kg in adults vs 10 mg/kg in children) in our study.

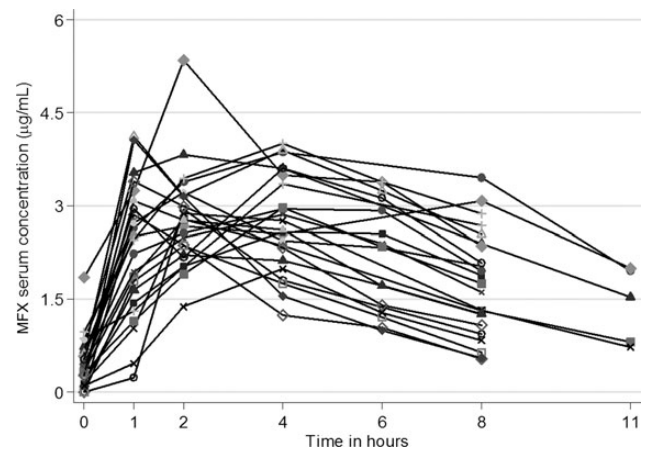
A considerably lower  $AUC$  in children compared with adults has also been described for levofloxacin, ofloxacin, and many

**Table 2. Summary Statistics for Pharmacokinetic Measures in Children Following an Oral Moxifloxacin Dose of 10 mg/kg**

Pharmacokinetic Measure	No.	Moxifloxacin
$C_{max}$ , $\mu\text{g/mL}$	23	3.08 (2.85–3.82)
$T_{max}$ , h	23	2.0 (1.0–8.0)
CL/F, L/h	12	10.53 (7.23–14.14)
Vd, L	12	70.61 (57.53–77.70)
$C_0$ , $\mu\text{g/mL}$	12	4.27 (3.38–4.86)
$T_{1/2}$ , h	12	4.14 (3.45–6.11)
$AUC_{0-8}$ , $\mu\text{g} \times \text{h/mL}$	23	17.24 (14.47–21.99)
$AUC_{0-24}$ , $\mu\text{g} \times \text{h/mL}$	12	23.31 (19.24–42.30)

All pharmacokinetic measures are reported as median and interquartile range, except for  $T_{max}$ , which is reported as median and range.

Abbreviations:  $AUC_{0-8}$ , area under the curve from 0–8 hours;  $AUC_{0-24}$ , area under the curve from 0–24 hours;  $C_0$ , concentration at time 0; CL, clearance;  $C_{max}$ , maximum serum concentration; F, fraction absorbed;  $T_{1/2}$ , half-life;  $T_{max}$ , time until  $C_{max}$ ; Vd, volume of distribution.



**Figure 1.** Individual serum concentrations in children 7–15 years of age following an oral moxifloxacin (MFX) dose of 10 mg/kg/day.

**Table 3. Pharmacokinetic Measures by HIV Status, Nutritional Status, and Administration Method of Moxifloxacin in Children (n = 23)**

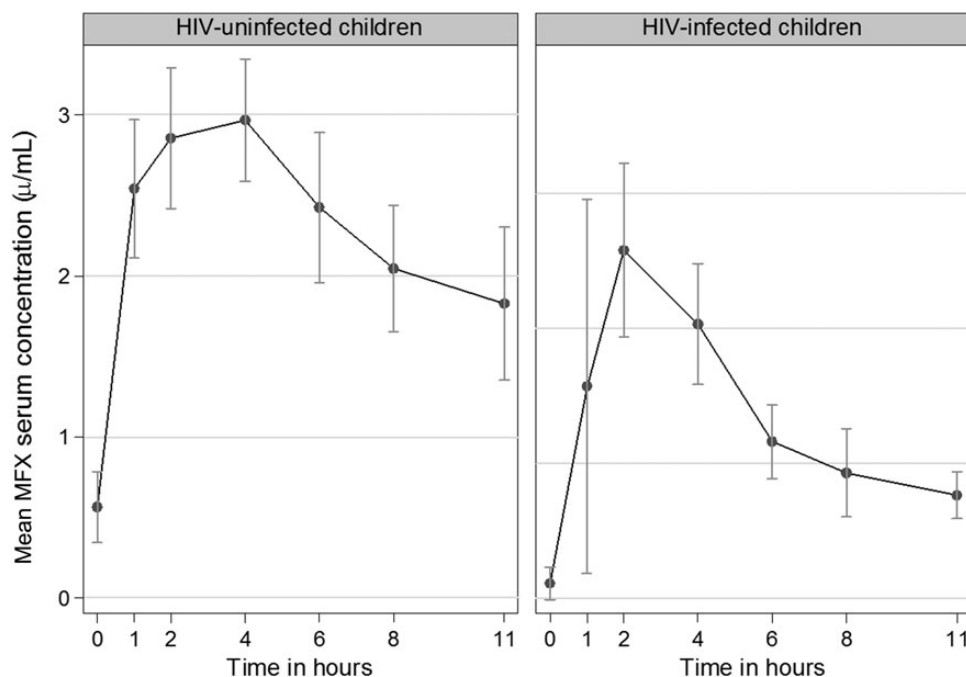
Characteristic	$C_{max}$ , $\mu\text{g/mL}$			$T_{max}$ , h			$AUC_{0-8}$ , $\mu\text{g} \times \text{h/mL}$		
	No.	Median (IQR)	<i>P</i> Value	No.	Median (Range)	<i>P</i> Value	No.	Median (IQR)	<i>P</i> Value
<b>HIV-status</b>									
HIV-infected	6	2.83 (2.36–2.94)		6	2.0 (1.0–4.0)		6	13.19 (11.89–15.90)	
HIV-uninfected	17	3.21 (2.95–3.82)	.080	17	4.0 (1.0–8.0)	.334	17	19.98 (16.71–25.21)	.003
<b>WAZ</b>									
$\geq -2.0$	20	3.08 (2.87–3.71)		20	2.0 (1.0–8.0)		20	18.76 (16.43–23.60)	
$< -2.0$ (UWA)	3	2.78 (1.99–4.06)	.411	3	2.0 (1.0–4.0)	.594	3	14.47 (9.95–15.90)	.045
<b>Administration</b>									
Whole	20	2.96 (2.82–3.71)		20	3.0 (1.0–8.0)		20	17.36 (14.93–23.60)	
Crushed	3	3.21 (3.08–4.06)	.294	3	1.0 (1.0–2.0)	.047	3	17.24 (14.47–19.53)	.715

Abbreviations:  $AUC_{0-8}$ , area under the curve from 0–8 hours;  $C_{max}$ , maximum serum concentration; HIV, human immunodeficiency virus; IQR, interquartile range;  $T_{max}$ , time until  $C_{max}$ ; UWA, underweight for age; WAZ, weight-for-age z score.

other first- and some second-line antituberculosis agents, and has been attributed to a more rapid elimination of drugs in children [25–28]. In 12 children, in whom it was possible to calculate, the  $T_{1/2}$  of 4 hours was roughly half of that in adults (7–10 hours). However, our data may be biased toward demonstrating more rapid elimination, as the  $T_{1/2}$  could not be calculated in children with a later  $T_{max}$  and potentially slower elimination. Although drug elimination is expected to be more rapid in

children because of allometric scaling [29], the degree of difference from adult values is surprising given that these relatively older children should be metabolically mature and have pharmacokinetics more closely approximating that of adults.

In general, adult activity of phase II enzymes is achieved within the first years of life, although some enzyme isoforms may exceed adult values during childhood [30]. Additionally, the extrapolated  $AUC_{0-24}$  may be underestimated as the



**Figure 2.** Effect of human immunodeficiency virus (HIV) on moxifloxacin (MFX) serum concentrations in children aged 7–15 years following an oral dose of 10 mg/kg/day.

**Table 4. Adverse Events in Children Receiving Treatment for Multidrug-Resistant Tuberculosis Including Moxifloxacin**

Adverse Event	All Adverse Events Any Grade (1, 2, 3, 4)							Adverse Effects Any Grade Possibly, Probably, Definitely Related to Moxifloxacin						
	No. of Patients With Event	Grade 1	Grade 2	Grade 3	Grade 4	Total No. of Events	Event Rate/ PY	No. of Patients With Event	Grade 1	Grade 3	Grade 3	Grade 4	Total No. of Events	Event Rate/ PY
Arthralgia	5	6	1	0	0	7	0.341	4	4	1	0	0	5	0.243
Arthritis	0	0	0	0	0	0	...	0	0	0	0	0	0	...
Pain other than traumatic injury	7	9	1	0	0	10	0.487	1	1	0	0	0	1	0.049
Headache	8	10	1	1	0	12	0.584	5	4	1	0	0	5	0.243
Neurosensory alteration	1	1	0	0	0	1	0.049	0	0	0	0	0	0	...
Insomnia	0	0	0	0	0	0	...	0	0	0	0	0	0	...
Fatigue/malaise	3	2	1	0	0	3	0.146	1	1	0	0	0	1	0.049
Nausea	15	22	1	0	0	23	1.119	9	9	0	0	0	9	0.438
Vomiting	11	13	1	0	0	14	0.681	3	3	0	0	0	3	0.146
Anorexia	2	2	0	0	0	2	0.097	0	0	0	0	0	0	...
Cutaneous reaction	2	2	0	0	0	2	0.097	1	1	0	0	0	1	0.049
Pruritus	6	8	0	0	0	8	0.389	2	4	0	0	0	4	0.195
Acute systemic allergic reaction	0	0	0	0	0	0	...	0	0	0	0	0	0	...
Elevated ALT	6	4	2	1	1	8	0.389	4	2	2	1	0	5	0.243
Bilirubin	1	0	1	0	0	1	0.049	1	0	1	0	0	1	0.049

Grading of adverse events according to Division of AIDS criteria. 23 patients followed for a median time of 236 days (interquartile range, 142–541 days); total person years = 20.55.

Abbreviations: ALT, alanine aminotransferase; PY, person-years.

extrapolation does not account for a 2-compartment model, which has been described for MFX [13].

In our study, HIV-infected and UWA children had lower MFX concentrations. The multivariate model suggests that this association of UWA with lower exposures may be due to confounding by HIV status. HIV infection has been associated with reduced absorption of antituberculosis agents in adults and children [27, 31] due to drug–drug interactions or gastrointestinal disturbances. In our study,  $C_{max}$  in HIV-infected children seemed to be lower than in HIV-uninfected children, potential evidence for reduced absorption. Our search did not identify any study investigating drug–drug interactions of anti-retrovirals and MFX. MFX is not metabolized by the cytochrome P450 system, but other interactions, such as has been described with rifampin [11], are possible.

Because of the lack of child-friendly formulations for many second-line tuberculosis drugs, it is common practice to crush tablets and administer these with water. The effect of such practices on the bioavailability has not been evaluated for any of the fluoroquinolones. Our data indicate that absorption occurs more rapidly if MFX tablets are crushed (median  $T_{max}$ , 1.0

hour vs 3.0 hours) with no influence on the AUC; however, the number of participants is small and additional evaluation is warranted. When crushed, MFX tablets are bitter and rarely tolerated by children. Child-friendly formulations of fluoroquinolones are urgently needed.

Fluoroquinolones have concentration-dependent bactericidal activity, with the AUC/MIC ratio most closely associated with in vivo efficacy against *M. tuberculosis* in mice [7]. Proposed targets for the fluoroquinolones against *M. tuberculosis* are  $AUC_{0-24}/MIC$  ratio of >100 or  $C_{max}/MIC$  of 8–10 [7, 8]; however, their clinical relevance remains unclear, particularly for children who typically have paucibacillary tuberculosis. In our study, MFX MIC of isolates from the children could not be determined, and the MFX MIC<sub>90</sub> of 0.5 µg/mL was taken from published data from the same study setting [23]. Children failed to achieve the proposed pharmacodynamic targets, with a median  $AUC_{0-24}/MIC$  of 46.6 (IQR, 38.5–84.6) (n = 12) and a median  $C_{max}/MIC$  of 6.2 (IQR, 5.7–7.6) (n = 23).

In vitro, *M. tuberculosis* resistance development depends on the fluoroquinolone concentration, and insufficient exposures may fail to prevent development of resistance [32]. However,

the limited clinical data suggest that children treated with currently recommended doses of MFX have better outcomes than adults with MDR tuberculosis [20, 33, 34].

Nevertheless, with increasing numbers of pediatric MDR tuberculosis, especially in the context of HIV, antituberculosis treatment needs be optimized to further improve outcome, shorten the duration of treatment, and to prevent emergence of resistance. Because fluoroquinolones are the backbone of existing MDR tuberculosis therapy and will remain fundamentally important in novel regimens that may be shorter and injectable-sparing, their optimal use in children with MDR tuberculosis is critically important.

Overall, MFX with prolonged dosing was well tolerated. Gastrointestinal intolerance, headache, and mildly elevated ALT were the most frequent adverse effects. Our conservative approach may reflect an overestimation of MFX adverse effects in these children receiving multidrug regimens; adverse effects that may have been associated with other drugs of the antituberculosis treatment, but could not be convincingly attributed to them, were considered possibly MFX related. Except for 1 child with grade 3 ALT elevation, all adverse effects were mild and did not require cessation of therapy.

Use of fluoroquinolones in children has been limited because of their potential to induce arthropathy in juvenile animals [35]; however, no unequivocal documentation of similar fluoroquinolone-induced arthropathy in children has been observed [36, 37]. Five children in our study reported mild arthralgia; none had clinical evidence of arthritis. All resolved spontaneously without adjusting or stopping MFX. Although arthralgia/arthritis requiring cessation of fluoroquinolones has been reported in children with MDR tuberculosis receiving MFX therapy [20, 34], our report adds to a growing body of evidence showing the long-term use of currently recommended doses of fluoroquinolones to be safe [19, 38, 39]. This might partly be due to the low drug exposure in children; safety for higher, adult-equivalent doses should be evaluated.

Fluoroquinolones are known to prolong the QT interval, with MFX having the largest effect [40]. It is reassuring that we did not observe any QTc prolongation; however, we evaluated only a small number of participants and lacked a prefluoroquinolone baseline QTc assessment for comparison. Further evaluation in children is warranted given that in the future, MFX may be combined with novel tuberculosis drugs also known to cause QT prolongation, such as bedaquiline and delamanid.

Our study is limited by the modest number of children and the resulting difficulty in separating the effects of important covariates such as HIV status, nutritional status, or drug administration method as well as the influence of concomitant antituberculosis and antiretroviral medication. We were also limited by the lack of later sampling time points, which

precluded our ability to characterize drug elimination in some participants. Future studies with larger study cohorts using population pharmacokinetic modeling techniques could overcome these limitations.

In conclusion, we demonstrate low MFX serum concentrations in children 7–15 years of age with MDR tuberculosis following an oral dose of MFX 10 mg/kg bodyweight. MFX was well tolerated with long-term administration. To approximate serum concentrations found in adults on standard doses, higher dosing of MFX may be required in children. Evaluation of safety and pharmacokinetics with higher doses as well as with child-friendly formulations should be considered.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (<http://cid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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## Chapter 6

### **The safety data of the second-line anti-tuberculosis drugs ethionamide, ofloxacin, levofloxacin and moxifloxacin in children with tuberculosis**

The importance of ethionamide (ETH) and the fluoroquinolones in the treatment of drug-resistant (DR) and drug-susceptible (DS) tuberculosis (TB) has been highlighted in chapter 3. Because these are widely used in current anti-tuberculosis regimens and are also included as components of future regimens, I have selected ETH and the 3 most frequently used fluoroquinolones (ofloxacin [OFX], levofloxacin [LFX] and moxifloxacin [MFX]) for investigation of safety in children.

#### **6.1. Effects of ethionamide on thyroid function in children with tuberculosis**

For ETH, hypothyroidism is a known reversible adverse effect that has not been systematically studied in children with TB. Children are a vulnerable group where development, especially brain development, might be negatively influenced by hypothyroidism. I hypothesized that ETH at the routine current dose recommendations is safe to use with respect of thyroid function in children with TB.

In order to answer this important question, I performed a retrospective folder review. All children with MDR-TB or TB meningitis treated with a regimen that included ETH (15-20mg/kg/day) in Tygerberg Children's Hospital or BHCD between January 2008 and February 2010 were included. Routine investigations of thyroid function (serum thyroid-stimulating hormone (TSH) and free thyroxine (fT4) levels) and clinical assessment were completed as per fixed clinical schedule and recommended standard of care. In binominal measurements, comparison was made using the Chi-Square test or Mann-Whitney test in ordinal measurements, respectively.



A total of 137 children (median age 2.9 years) were enrolled; 104 children (76%) were treated for pulmonary TB, 44 (32%) were HIV-infected. Abnormal thyroid function tests were found frequently, in 58% of children, with at least 41% of those with abnormalities likely due to ETH (340). HIV infection (OR 3.27, CI 1.23-8.67,  $p=0.015$ ) and co-medication with PAS (OR 9.17, CI 2.29-36.73,  $p=0.001$ ) increased the odds of having hypothyroidism. This study revealed a high frequency of hypothyroidism in children treated with ETH and indicated the need for regular thyroid function test monitoring in children on long-term ETH treatment, especially in the case of HIV co-infection and concomitant use of PAS. The study is limited by its retrospective nature and limited data on potential alternative diagnoses. The impact of abnormal thyroid function tests and the need and influence of thyroxine supplementation on treatment outcome requires assessment in children with TB. Future prospective studies, investigating the cause and the evolution of hypothyroidism in children with TB are needed.

## Abnormal thyroid function tests in children on ethionamide treatment

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### SUMMARY

Ethionamide (ETH) treatment may cause hypothyroidism. Clinical data, serum thyroid stimulating hormone (TSH) and free thyroxine (fT4) levels were retrospectively assessed in 137 children receiving anti-tuberculosis treatment including ETH. Abnormal thyroid function tests (TFTs) were recorded in 79 (58%) children: elevated serum TSH and suppressed fT4 ( $n = 30$ ), isolated elevated serum TSH ( $n = 20$ ), isolated low serum fT4 ( $n = 28$ ) and isolated low TSH ( $n = 1$ ). The risk for bio-

chemical hypothyroidism was higher for children on regimens including para-aminosalicylic acid and in human immunodeficiency virus infected children. TFT abnormalities are frequent in children on ETH and are mainly due to primary hypothyroidism or euthyroid sick syndrome.

**KEY WORDS:** ethionamide; children; tuberculosis; thyroid function

ETHIONAMIDE (ETH) is a second-line drug commonly used in the treatment of tuberculous meningitis (TBM) and drug-resistant tuberculosis (TB). Hypothyroidism has been reported as an adverse effect of ETH in adults.<sup>1</sup> ETH, which is structurally similar to the thyrostatic drug methimazole, seems to inhibit thyroid hormone synthesis by the inhibition of organification.<sup>2</sup> From in vitro studies, it appears that ETH also inhibits the uptake of iodine into thyroid cells.<sup>1</sup> Para-aminosalicylic acid (PAS) may also cause hypothyroidism by inhibiting thyroid peroxidase.<sup>3,4</sup> Another common thyroid function abnormality expected in chronic diseases, including TB, is that associated with the euthyroid sick syndrome (ESS).<sup>5,6</sup> Nevertheless, there are several reports on primary hypothyroidism associated with ETH.<sup>1,7</sup> Based on these concerns, routine thyroid function monitoring for children on ETH treatment was initiated at our clinical paediatric TB services.

### METHODS

A retrospective folder review was completed to investigate the occurrence of abnormal thyroid function tests (TFTs) in children treated with anti-tuberculosis regimens including ETH and PAS in Tygerberg Children's Hospital (TCH) and the Brooklyn Hospital for Chest Diseases (BHCD) in Cape Town, South Africa

(January 2008–February 2010). Ethical approval was obtained from the Institutional Review Board of Stellenbosch University.

ETH was given at a daily dosage of 15–20 mg per kg body weight and PAS at a dosage of 150 mg/kg. Human immunodeficiency virus (HIV) infected children were routinely treated with highly active anti-retroviral therapy (HAART).

Thyroid stimulating hormone (TSH) and free thyroxine (fT4) levels were measured using two different assays: ARCHITECT *i2000* System® (Abbott Laboratories, Abbott Park, IL, USA) and ADIVA® Centaur™ analyzer (Bayer Diagnostics, Tarrytown, NY, USA). TSH and fT4 values were considered abnormal if they were >5% beyond the reference ranges provided by the respective laboratories.

Comparisons between groups were made using the  $\chi^2$  test (in binominal measurements) or the Mann-Whitney test (in ordinal measurements), using SPSS version 17.0 (Statistical Package for Social Sciences, Chicago, IL, USA).

### RESULTS

A total of 137 children (median age 2.9 years, range 4 months–15.8 years) were enrolled: 104 (76%) were diagnosed with pulmonary and 33 (24%) with extrapulmonary TB (11 tuberculous meningitis [TBM],

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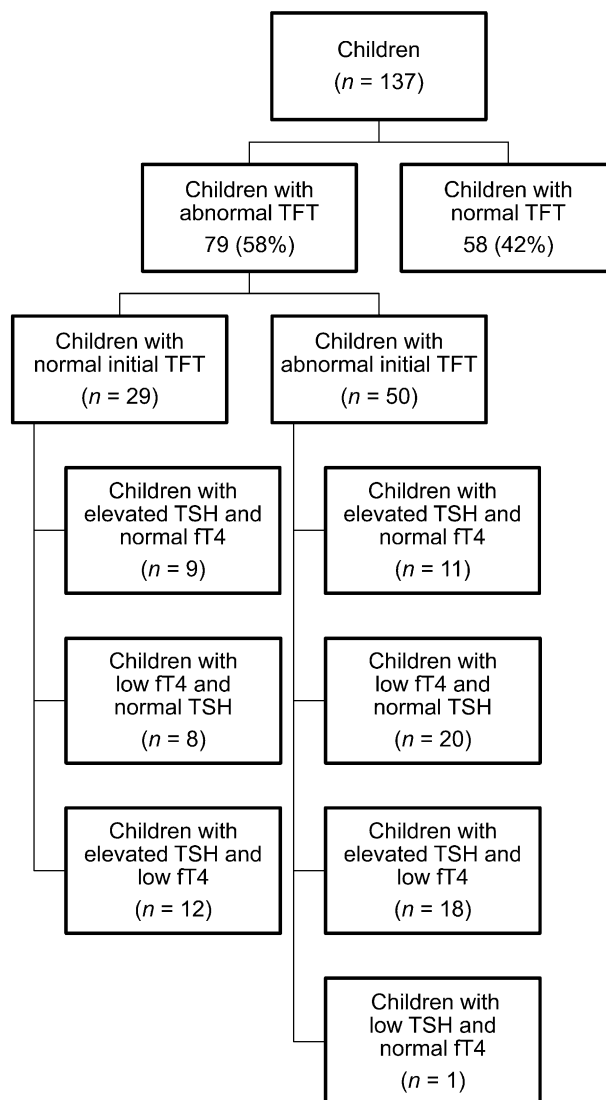
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7 lymph-node TB, 15 others). Nineteen children (14%) were treated for mono-resistant TB (either isoniazid or rifampicin [RMP]), 106 (68%) for multidrug- or polydrug-resistant TB. A positive culture for TB was obtained in 53 children (39%).

TFT measurements were repeated every 55–90 days, and between 1 and 8 times in a single child. The median (interquartile) duration of ETH treatment at the first four TFT measurements was respectively 70 (27–124), 148 (85–212), 217 (143–319) and 302 (213–392) days.

In 79 (58%) children, TSH and/or fT4 values were >5% above/below the reference range at some time point during treatment (Figure). Four patterns of thyroid function abnormalities were identified (Table): high serum TSH and low serum fT4, high serum TSH (with normal fT4), low serum TSH (with normal fT4) and an isolated low serum fT4. Of the 16 children (12%) receiving both ETH and PAS, 13 (81%) had abnormal TFTs (Table).

Eighteen children (13%) were given thyroxine treatment by the attending clinician. Forty-four (32%) were HIV-infected; of these, 33 (75%) had at least one abnormal TFT compared to 45/93 (48%) non-HIV-infected children (odds ratio [OR] 3.63, 95% confidence interval [CI] 1.61–8.19,  $P = 0.001$ ; Table). Children with high TSH and low fT4 (biochemical primary hypothyroidism,  $n = 30$ ) showed no differences in age ( $P = 0.676$ ), sex ( $P = 0.886$ ), diagnosis of TBM ( $P = 0.208$ ), type of TB (pulmonary vs. extrapulmonary TB,  $P = 0.493$ ) or concomitant treatment with RMP ( $P = 0.812$ ) compared to children with normal TFT ( $n = 58$ ). Children with biochemical primary hypothyroidism were more likely to be on regimens including PAS (OR 9.17, CI 2.29–36.73,  $P = 0.001$ ) and be HIV-infected (OR 3.27, CI 1.23–8.67,  $P = 0.015$ ) than children with normal TFT.



**Figure** Overview of thyroid hormone measures obtained in children on ethionamide treatment ( $n = 137$ ). TFT = thyroid function test; TSH = thyroid stimulating hormone; fT4 = free thyroxine.

**Table** Characteristics of children with normal and abnormal thyroid function test results, classified in four groups based on TSH and fT4 values (most abnormal results only;  $n = 137$ )

	TSH high, fT4 low <i>n</i> (%)	TSH high, fT4 normal <i>n</i> (%)	TSH normal, fT4 low <i>n</i> (%)	TSH low, fT4 normal <i>n</i> (%)	TSH and fT4 normal <i>n</i> (%)
Number*	30 (22)	20 (15)	28 (20)	1 (<1)	58 (42)
Age, years, median [range]	2.2 [0.3–13.7]	4.5 [0.3–14.2]	3.6 [0.5–13.3]	1.8	2.6 [0.4–15.8]
Sex					
Female	16 (53)	13 (65)	14 (50)	1 (100)	30 (52)
Male	14 (46.7)	7 (35)	14 (50)	0	28 (48)
Nutritional status for age					
Normal	19 (63)	12 (60)	19 (67)	1 (100)	39 (67)
Underweight	11 (37)	8 (40)	9 (32)	0	19 (33)
Goitre	1 (3)	0	0	0	4 (7)
On PAS	10 (33)	0	3 (11)	0	3 (5)
On RMP	9 (30)	9 (45)	8 (29)	0	16 (28)
On INH	29 (97)	20 (100)	18 (100)	1 (100)	54 (93)
HIV-infected	13 (43)	7 (35)	13 (46.4)	0	11 (19)
TBM	3 (10)	3 (15)	3 (11)	0	2 (3)

\*Percentage given of all children participating in study; otherwise percentages are given for specific groups. TSH = thyroid stimulating hormone; fT4 = free thyroxine; PAS = para-aminosalicylic acid; RMP = rifampicin; INH = isoniazid; HIV = human immunodeficiency virus; TBM = tuberculous meningitis.

## DISCUSSION

TFTs were frequently abnormal in hospitalised children treated for TB with an ETH-containing regimen. The most common abnormality was a high serum TSH and a low serum fT4, indicating primary hypothyroidism. The second most common TFT abnormality identified in our study was a suppressed serum fT4 with a normal serum TSH. The differential diagnosis includes ESS, central hypothyroidism and a potential drug effect.<sup>6</sup> Typically, during progression of ESS, a drop in both total and free serum T3 and T4, without an elevation of serum TSH, may occur. Total T4 is affected first, followed by a drop in fT4.<sup>6</sup>

A drug-related adverse effect is likely if primary hypothyroidism developed during treatment. In 41% of the children in our study with impaired TFT, the initial TFT was within normal limits, suggesting TB treatment as a likely cause of hypothyroidism.

There are several reports of hypothyroidism in adults treated with ETH.<sup>1</sup> Most of these cases presented with clinical hypothyroidism which was then confirmed biochemically (mainly raised serum TSH). Limited data have been published on adverse effects of ETH in the treatment of children: in a single report on children treated for multidrug-resistant TB, hypothyroidism, based on elevated serum TSH, occurred in 3/38 children (8%).<sup>7</sup> In our study, only one child with biochemical hypothyroidism also had a goitre. To make a clinical diagnosis of hypothyroidism in children with TB is challenging, as many symptoms of hypothyroidism, such as fatigue or loss of appetite, are common symptoms of TB and also possibly of anti-tuberculosis treatment.

In our study, children on a regimen that contained both PAS and ETH were twice as likely to develop primary hypothyroidism as if they were on ETH only, suggesting a significant clinical additive effect of PAS.

We found evidence of HIV-associated abnormal TFTs in children on ETH. ESS has frequently been reported among HIV-infected children with advanced disease,<sup>8,9</sup> and stavudine has been identified as a risk factor for hypothyroidism in HIV-infected patients.<sup>8</sup> In our study, primary hypothyroidism was more likely in the presence of HIV co-infection. In 20 children, isolated elevated serum TSH levels were found. This could be due to true subclinical hypothyroidism, it could be drug-related or it could be due to the recovery phase of ESS.<sup>10</sup> In the latter, an elevated TSH is, however, short-lived and not very high (usually <10 mU/l). This was the case in 18 children, indicating that the recovery phase of ESS is the most likely cause.

This study is limited by its retrospective nature, the use of two laboratories, the lack of fT3 levels in the workup, and limited data on potential alternative diagnoses. Furthermore, age-related reference ranges are not available for all methods and populations.<sup>11,12</sup>

In conclusion, this study shows that TFT abnormalities in children on anti-tuberculosis treatment that includes ETH are common. This risk is higher in HIV-infected children or when PAS is included in the treatment regimen. Prospective studies are needed to determine the clinical significance of these findings and the potential role of thyroxine therapy in children on anti-tuberculosis treatment.

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## R É S U M É

Un traitement à l'éthionamide (ETH) peut provoquer de l'hypothyroïdie. On a évalué de manière rétrospective les données cliniques, les niveaux sériques d'hormone stimulante de la thyroïde (TSH) ainsi que de thyroxine libre (fT4) chez 137 enfants bénéficiant d'un traitement antituberculeux comportant l'ETH. On a noté des anomalies de la fonction thyroïdienne (TFT) chez 79 enfants (58%) : une élévation du TSH sérique et une suppression du fT4 ( $n = 30$ ), une élévation isolée du TSH

( $n = 20$ ), un taux sérique isolé faible du fT4 ( $n = 28$ ) et un taux isolé faible du TSH ( $n = 1$ ). Le risque d'hypothyroïdie biochimique est plus élevé chez les enfants qui ont un régime incluant de l'acide para-aminosalicylique ainsi que chez les enfants infectés par le virus de l'immunodéficience humaine. Les anomalies de la TFT sont fréquentes chez les enfants sous ETH et sont dues principalement à une hypothyroïdie primaire ou à un syndrome de maladie euthyroïdienne.

## R E S U M E N

El tratamiento con etionamida (ETH) puede dar lugar a hipotiroidismo. Se estudiaron en forma retrospectiva los datos clínicos, la determinación sérica de tirotrópina y de tiroxina libre (fT4) en 137 niños que recibían tratamiento antituberculoso con ETH, entre otros medicamentos. Se observaron cifras anormales en las pruebas de función tiroidea (TFT) en 79 niños (58%): alta concentración sérica de tirotrópina y supresión de fT4 ( $n = 30$ ), niveles bajos de fT4 aislados ( $n = 28$ ) y bajas con-

centraciones aisladas de tirotrópina ( $n = 1$ ). El riesgo de presentar hipotiroidismo bioquímico fue mayor en los niños con pautas terapéuticas que comportaban ácido *p*-aminosalicílico y en los niños infectados por el virus de la inmunodeficiencia humana. Los trastornos de las TFT son frecuentes en los niños que reciben ETH y se deben principalmente un hipotiroidismo primario o al síndrome del eutiroides enfermo.

## 6.2. Safety, including cardiotoxicity, in children with tuberculosis on fluoroquinolone therapy

Fluoroquinolones are known to prolong the QT-interval with moxifloxacin (MFX) having the greatest potential, but this has never been investigated in children. Information on this adverse effect is highly relevant, because the fluoroquinolones might not only become first-line agents, but will also remain part of optimized background therapy in regimens including new drugs, such as bedaquiline and the nitro-imidazoles (e.g. delamanid). These new drugs have shown to cause QT prolongation, potentially leading to an additive cardiotoxic effect in combination with fluoroquinolones.

Children enrolled in the pharmacokinetic studies on ofloxacin (OFX), levofloxacin (LFX) and MFX (chapter 5) were systematically monitored for adverse effects including cardiotoxic effects (360, 377). The analysis of the broader range of potential adverse events other than cardiotoxic effects of OFX and LFX is currently being prospectively evaluated in larger prospective cohort studies and is beyond the scope of this PhD thesis (see appendix).

For MFX, clinical safety assessments using combined data sources including chart and routine clinical review, laboratory investigation, parental and children's report during MFX therapy were performed. Standard Division of Acquired Immunodeficiency Syndrome (NIH US; DAIDS) criteria were used to grade severity of adverse events. To assess cardiotoxicity of all three fluoroquinolones, a 12-lead electrocardiogram (ECG) was performed. Due to logistical reasons, ECGs were only obtained in children receiving fluoroquinolone as part of MDR-TB preventive therapy. An ECG was performed on each of the pharmacokinetic assessment days at 3 hours after drug dosing, to coincide with the likely fluoroquinolone  $t_{max}$ . ECGs were evaluated using a standardized approach by a single paediatric cardiologist, with the corrected QT-interval (QTc) calculated using the method described by Fridericia (378) with a QTc >450ms or a change from baseline of >60 ms classified as prolonged (360).

In children receiving OFX and LFX the mean QTc was 361ms (SD 37ms) for OFX and 369ms (SD 22ms) for LFX. No children in either group had a QTc >450 ms. In 6 children, all in the preventive therapy group, baseline ECGs before initiation of any fluoroquinolone therapy were obtained. In one child in the LFX group, a QTc



prolongation of more than 60ms from baseline (QTc 342ms) occurred with a QTc of 403ms being still within the normal range. In 5 children receiving either OFX or LFX ECG abnormalities other than QTc prolongation were documented that could not be related to fluoroquinolone therapy (377).

Children on MFX were followed for a median of 236 days (IQR 142-541 days). Main adverse effects potentially related to MFX were gastrointestinal (nausea and vomiting; n=12), headache (n=5), elevated alanine-aminotransferase (ALT; n=4) and arthralgia (n=4). In 13/23 children on MFX ECG data was available. Mean QT<sub>c</sub> was 403ms (SD 30ms). None had a QT<sub>c</sub> -interval >450ms.

Although fluoroquinolones are known to prolong the QT-interval with MFX having the largest effect (chapter 4)(379), we did not observe any concerning QTc prolongation in children; although the largest cohort to date, we evaluated a modest number of participants and in the majority of children lacked a pre-fluoroquinolone baseline QTc assessment for comparison (359, 360). Further evaluation in children is warranted given that in future fluoroquinolones (esp. MFX) may be combined with novel TB drugs which are also known to cause QT prolongation, such as bedaquiline and delamanid (380, 381), which are both under evaluation in children. More information is provided in the articles included.

## Chapter 7: Conclusions and future directions

The global burden of tuberculosis (TB) in children remains high and TB has high morbidity and mortality especially amongst young children. Despite this burden of disease, TB in children has been a neglected disease and has only in the last decade started receiving the necessary attention. The emerging epidemic of multidrug-resistant TB (MDR-TB) is also a threat for children – maybe even more than for adults, as information on the use of second-line drugs in children is very limited (5, 382), and data on the use of new drugs is absent.

Pharmacokinetics, drug doses and safety of anti-tuberculosis drugs are generally poorly studied in children, even of those developed more than 6 decades ago. Evidence-based dosing and data on safety of drugs in children are critical for optimizing treatment and developing acceptable formulations.

The first literature review in this thesis confirmed that information on the optimal dosing of isoniazid (INH), the first-line anti-tuberculosis drug most extensively used for preventive therapy and TB treatment, in the youngest and most vulnerable age group of children (children <2 years of age) is still limited. My study on the pharmacokinetics of INH provided supportive evidence for the new World Health Organization (WHO) dosing recommendation of INH given at 10mg/kg (range 7-15 mg/kg) in South African children <2 years of age (excluding neonates) (118). Further pharmacokinetic and safety studies are needed to determine whether young children in other populations with different distributions of NAT2-acetylator status and possible different risk for INH-induced toxicity require different dosages of INH.

Several paediatric pharmacokinetic studies have shown that higher doses of rifampicin (RMP) than the previously recommended WHO doses of 10mg/kg (range 8-12 mg/kg) are needed to achieve serum concentrations comparable to those in adults after a dose of 600mg/day (55, 98, 120, 172, 182-187). I have provided evidence that a dose of at least 15 mg/kg daily is needed in children <2 years of age to achieve adult target  $C_{max}$  of 8-24µg/ml (118, 160). It has been shown that activity against *Mycobacterium tuberculosis* as well as the development of RMP drug resistance is concentration-dependent and therefore high doses of RMP up to 35mg/kg have currently been

evaluated in adults in order to optimize RMP dose and to potentially shorten TB treatment regimens (383). Doses of RMP 30-35mg/kg showed favourable extended early bactericidal activity compared to lower RMP doses with no increase in hepatotoxicity (383). There is robust evidence that severe RMP hepatotoxicity is rare and is not dose-dependent in children (122). Therefore, if a higher RMP dose is shown to be beneficial in further adult studies, doses achieving similar RMP concentrations to the higher adult dose eventually chosen also need to be evaluated in children. This is especially relevant in young children who have an increased risk for central nervous system (CNS) TB, as penetration of RMP across the blood-brain barrier is only modest (170).

Pyrazinamide (PZA) drug concentrations, different to INH and RMP, reach similar  $C_{max}$  at the same mg/kg body weight dose in adults and children (223-225). However, information on the pharmacokinetics in young children (<2 years) is limited. There is uncertainty regarding the optimal pharmacokinetic or pharmacodynamic target for PZA in adults and children. Should it be confirmed that a PZA  $C_{max}$  of <35µg/ml is indeed associated with a poorer treatment outcome as shown by Chideya et al. (22), PZA doses of at least 30-35mg/kg would be required in children including children <2 years of age (174, 219). Again, this needs to be studied in a larger number of children with different genetic backgrounds.

The data presented in this thesis provides supportive evidence for the implementation of the revised WHO guidelines for first-line anti-tuberculosis treatment in young children. Following the new WHO dosing recommendations for INH, RMP and PZA, fixed-dose combinations are currently in development. These will be most likely manufactured in a dispersible tablet containing INH 50mg, RMP 75mg and PZA 150mg (384). If ongoing studies demonstrate superiority of higher rifampicin concentrations, the rifampicin compound might be increased to e.g. 100mg. The relatively small drug amounts in each tablet and the dispersible formulation will simplify dosing in young children and will avoid crushing and splitting of tablets with unknown effect on drug bioavailability. Nevertheless, in simulations based on a population pharmacokinetic model, these fixed-dose combinations led to INH and PZA exposures lower than in adults, especially in young children (174). Studies of such new fixed-dose combinations administered according to weight-banded dosing will also need to be done in different

settings. The pharmacokinetics of first-line agents in infants below 12 months of age are currently under investigation (385).

Further research is ongoing to develop appropriate child-friendly formulations of the current standard first-line TB treatment (386), and also to examine the effect of the revised WHO dose recommendations and the influence on concomitant treatment with antiretroviral therapy in children (DATiC NCT01637558; Treat Infant TB; and PK-PTBHIV01, SHINE trial, ISRCTN63579542).

Drug-resistant TB, especially MDR-TB, is increasing globally, also in children, as children are typically infected by adult drug-resistant source cases and do not acquire mycobacterial drug resistance. Therefore pharmacokinetic studies to confirm the dosing and safety of the second-line agents in children have become a matter of urgency.

Of the second-line anti-tuberculosis drugs, I prioritized the study of ethionamide (ETH) (which is similar to prothionamide (PTH)) and the 3 most frequently used fluoroquinolones (and currently the best second-line TB drugs) ofloxacin (OFX), levofloxacin (LFX) and moxifloxacin (MXF).

By reviewing the literature, I highlighted gaps in the current knowledge on dosing and safety of ETH/PTH in children. With the first study on ETH pharmacokinetics I provided supporting evidence for the current dosing recommendation of ETH 15-20mg/kg in children with TB. In this study I found that young children and HIV-infected children were at risk for lower ETH serum concentrations, but that the mean drug exposure was still within range of the adult  $C_{max}$  reference target (2.5µg/ml) (310).

Along with pharmacokinetic data on ETH, I showed for the first time, that there is a high frequency of thyroid function abnormalities in children treated with ETH, indicating the need for careful thyroid function test monitoring in children on long-term ETH treatment, especially in case of HIV co-infection and concomitant use of para-aminosalicylic acid (PAS). The study was limited by its retrospective nature and the inability to evaluate the clinical impact of the findings on TB treatment outcome.

Future studies on the optimal pharmacodynamic target(s) for ETH/PTH in anti-tuberculosis treatment and pharmacokinetic and safety studies in a larger number of children are warranted. The focus should be on the most vulnerable groups: young and

HIV-infected children. Careful attention needs to be given to other relevant clinical covariates.

MXF and LFX at higher doses (1,000mg) have been identified as the two most potent fluoroquinolones against *M. tuberculosis* in adults. Because these newer fluoroquinolones are not readily available in resource-limited, TB high-burden countries, the less active but inexpensive OFX is still widely used.

My literature review on the use of fluoroquinolones in childhood TB revealed that the strong bactericidal and sterilizing activity, favourable pharmacokinetics, and toxicity profile have made the fluoroquinolones the most important component of existing MDR-TB treatment regimens not only in adults but also in children (387). Although current evidence on MXF-containing regimens does not yet support their use in the shortening of TB treatment for drug-susceptible TB (DS-TB), future regimens including higher MXF doses and/or combining MXF with novel anti-tuberculosis agents may still show promise in shortening regimens (388). Data on the possible role of fluoroquinolones in treatment shortening for paediatric DS-TB, including TB meningitis, is needed.

There is limited data on fluoroquinolone pharmacokinetics and safety in children with TB. My pharmacokinetic studies on OFX, LFX and MXF in children have demonstrated that children eliminate fluoroquinolones faster than adults, leading to substantially lower drug exposure with failure to achieve the proposed primary pharmacodynamic target for fluoroquinolones of  $AUC/MIC > 100$  at the currently recommended doses (359, 360). Failure to approximate adult exposures or to achieve pharmacodynamic targets has important implications for current and future dosing recommendations in children for these essential anti-tuberculosis drugs. While  $C_{max}$  is considered more relevant for the development of resistance (389),  $AUC/MIC$  is associated with treatment success (71). Limitations of my pharmacokinetic studies were the modest sample size and the lack of well defined pharmacodynamic targets for treatment of childhood TB. No serious adverse effects, especially no cardiotoxic effect with prolongation of the QTc-interval, occurred in the children included in these studies. Although numbers were modest and a baseline ecg not always available, this is reassuring and adds to the body of evidence of safe use of fluoroquinolones in childhood TB.

Studies of LFX for MDR-TB preventive therapy (TB-CHAMP, V-Quin) and for the treatment of TB meningitis (TBM-KIDS study) are currently being planned in both adults

and children. For the foreseeable future, LFX and MFX are likely to be used in children and information on their effective use in childhood TB is critical. Their role in future treatment shortening trials of MDR-and DS-TB will also remain important.

For the second-line drugs, no child friendly formulations or lower dose strength tablets are available, making dosing in children challenging. In daily practice, TB medicines are routinely crushed and mixed with different fluids and foods to facilitate their administration to young children. This causes a high risk of inaccurate dosing – both over-and under-dosing. Additionally, information on the influence of these procedures on stability and bioavailability of the anti-tuberculosis agents is lacking. The Sentinel Project on Pediatric Drug-Resistant Tuberculosis is conducting a laboratory-based study to evaluate the stability and availability of ETH, LFX, cycloserine, and linezolid when mixed with different foods; results are still pending.

For ethionamide, a dose of 15-20mg/kg seems to be appropriate in children. To adequately dose younger children a tablet strength lower than the standard 250mg ETH tablet is needed. In India, 125mg ETH tablets are already available and a prototype of a scored dispersible ETH tablet is in development (356).

For the fluoroquinolones, data necessary to develop appropriately dosed paediatric formulations is still lacking. Nevertheless, given the available data, the use of LFX and MFX is recommended over OFX. For LFX, a dose of 15-20mg/kg/d and for MFX of 10mg/kg/d seems appropriate (49, 390). In the case of MFX, standard 400mg tablets are not scored and very bitter when crushed. MFX use is therefore generally restricted to older children, therefore excluding the most vulnerable group of children. A tablet strength of 100-150mg for LFX and of 100mg for MFX is proposed. Issues of acceptability and palatability also warrants further drug investigation.

Development of child-friendly formulations of first- and second-line agents is critical; Fixed drug combinations (FDC) in order to reduce tablet burden are available for the first-line agents, but have not been developed for MDR anti-tuberculosis treatment. MDR treatment regimens differ in different countries/regions due to susceptibility patterns and availability of second-line agents. As a child-friendly formulation, dispersible tablets are preferred over liquids, because liquids are bulky, not easy to store, frequently come in breakable bottles and often need refrigeration after opening. New formulations need



to be informed by rigorous pharmacokinetic and safety studies in children with different genetic backgrounds. Once developed, their availability to those most in need, that is, children in high-burden, low resource settings, needs to be ensured.

Taken together, paediatric pharmacokinetic studies have revealed that higher doses of first- and secondline anti-tuberculosis agents compared to adults are needed to achieve comparable blood concentrations. These higher doses (that might even be increased depending on efficacy results of ongoing adult studies) need to be thoroughly investigated, including their safety in children with TB. Therefore, intensive sampling pharmacokinetic studies as well as sparse sampling studies in a larger group of children with different genetic backgrounds, different ages, different nutritional status, HIV-infected and HIV-uninfected with and without receiving ARTs need to be undertaken and mathematical modeling (population pharmacokinetic models) be applied in order to optimally characterize the pharmacokinetics in childhood TB. For safety analysis, children need to be monitored on a regular basis- clinically as well as with laboratory investigations. Valuable endpoints for efficacy of anti-tuberculosis treatment in childhood TB are difficult to define. The disease diversity in childhood TB is high and the natural history of these manifestations vary considerably, making it difficult to judge the success of treatment. Because sputum cultures are frequently negative, evaluation of treatment outcome often rely on radiological and clinical improvement. For study purposes children need to be followed up until the end of treatment and optimally until one year thereafter.

Once the pharmacokinetics of the anti-tuberculosis agents (and their child-friendly formulations) have been better defined in children, the actual MIC distribution of clinical *M. tuberculosis* strains should be established, because of the variability of MICs from region to region. This would guide the most appropriate drug doses needed to achieve proposed pharmacodynamic targets and doses could be individualised accordingly. The pharmacokinetic studies in children revealed a high inter-individual variability in drug concentrations. This is especially of concern in second-line agents where the margin between efficacy and toxicity is narrow. Nevertheless, also for INH individualized dosing has been shown to be beneficial for treatment success and toxicity (391). Because the traditional venipuncture-based method is currently not feasible for routine care purposes, new therapeutic TB drug monitoring like dried blood spot assays are currently being evaluated (Pediatric Tuberculosis: enabling early detection of children

at-risk for treatment failure (Dried-blood spot sub-study), PI Anneke Hesselning). Assays further research (48). The feasibility of such monitoring in routine care settings should still be evaluated.

### **Impact on policy and practice**

My research generated supportive evidence for the current for determination of drugs or their metabolites in saliva or urine could also be a focus for WHO dose recommendations of INH, RMP and PZA in children younger than two years of age and for ETH in children younger than 12 years of age. This data can enter population pharmacokinetic models, aiming to identify and quantify sources of variability and facilitating sparse-sampling strategies, which are highly relevant in children.

Influenced by my pharmacokinetic study, LFX is currently being evaluated at a higher dose of LFX 20mg/kg in children in the same study setting, and may be evaluated at higher doses in future, including in an international TB meningitis trial. International LFX dose recommendations have now been increased from 7.5-10 mg/kg to 15-20mg/kg based on these results. For MFX, the dose is still 7.5-10mg/kg, but aiming for the high end of the range is recommended – further evaluation is needed at higher doses and will be studied in recently funded trials.

The study I have conducted has increased awareness about hypothyroidism occurring during ETH therapy, and routine testing of thyroid function is now being recommended in international MDR-TB treatment guidelines for children (392).

In conclusion, my research has identified and addressed critical gaps in the current knowledge in the management of children with both drug-susceptible and drug-resistant TB. I have provided essential evidence on both the dosing and safety of first- and second-line anti-tuberculosis agents, informing international treatment guidelines for childhood TB. More data is needed in a larger number of children with different genetic backgrounds, with and without HIV co-infection and with higher drug doses. Novel more child-friendly treatment regimens and child-friendly formulations are urgently needed to further optimize anti-tuberculosis treatment in children.

## Appendices

### Other contributing works

**1. Thee S**, Detjen A, Quarcoo D, Wahn U, Magdorf K. Ethambutol in paediatric tuberculosis: aspects of ethambutol serum concentration, efficacy and toxicity in children. *Int J Tuberc Lung Dis.* 2007 Sep;11(9):965-71 (54).

In case of drug-resistant TB or „adult-type“ disease, ethambutol (EMB) forms part of anti-tuberculosis treatment in children. Information on dosing of EMB is limited. I investigated the pharmacokinetics of EMB following an oral dose of 15mg/kg, 25mg/kg and 35mg/kg. EMB serum concentrations were lower than those in adults receiving a similar dose and dosing according to body surface has been suggested. Even at a dose of EMB 35mg/kg ocular toxicity was rare.

**2. Thee S**, Detjen A, Magdorf K et al.: Pyrazinamide serum levels in childhood tuberculosis, *Int J Tuberc Lung Dis.* 2008 Sep;12(9):1099-101 **(224)**

Pyrazinamide (PZA) serum concentrations in 34 children aged 1 to 14 years were measured either after oral administration of PZA alone or in combination therapy with isoniazid (INH) and rifampicin (RMP). It was shown that with a dose of PZA 30mg/kg adequate serum concentrations are reached in children of different ages with and without concomitant treatment with INH and RMP.

**3. Thee S**, Detjen A, Magdorf K et al.: Rifampicin serum levels in childhood tuberculosis. *Int J Tuberc Lung Dis.* 2009 Sep; 13(9):1106-11. **(55)**

Rifampicin (RMP) serum concentrations were measured in 27 children 2-14years of age following an oral dose of RMP 10mg/kg with or without concomitant treatment with EMB. RMP serum concentrations were lower compared to those in adults receiving a similar oral dose with even lower RMP serum concentrations following combination therapy with EMB.

**4. Thee S**, Detjen A, Wahn U, Magdorf K.: Isoniazid pharmacokinetic studies of the 1960s: considering a higher isoniazid dose in childhood tuberculosis. *Scand J Infect Dis.* 2010 Apr;42(4):294-8. (44)

This paper presents data from 2 prior studies, investigating isoniazid (INH) serum levels in children of different age groups. It was shown that doses of INH 5mg/kg bodyweight lead to lower serum concentrations than those recommended for anti-tuberculosis therapy in adults. Dosing according to bodyweight (200mg/m<sup>2</sup>) appears to be more appropriate.

5. Seddon JA, **Thee S**, Jacobs K, Ebrahim A, Hesselning AC, Schaaf HS. Hearing loss in children treated for multidrug-resistant tuberculosis. *J Infect.* 2013 Apr;66(4):320-9. doi: 10.1016/j.jinf.2012.09.002. Epub 2012 Sep 6. (393):

In this retrospective report 97 children treated for multidrug-resistant TB with an injectable drug were assessed. Hearing loss occurred in 23 children (24%) and 7/11 children classified as having hearing loss using audiometry had progression of hearing loss after finishing the injectable drug.

6. Zvada SP, Denti P, Donald PR, Schaaf HS, **Thee S**, Seddon JA, Seifart HI, Smith PJ, McIlleron HM, Simonsson US: Population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in children with tuberculosis: in silico evaluation of currently recommended doses. *J Antimicrob Chemother.* 2014 May;69(5):1339-49

Previously published data from 76 South African children with TB were used to describe the population pharmacokinetics of RMP, INH and PZA. Simulations based models suggest that with the new WHO dosing guidelines and using available paediatric fixed-dose combinations, children will receive adequate RMP exposures compared with adults, but with a larger degree of variability. PZA and INH exposures in many children will be lower than in adults.

7. Garcia-Prats T, Draper HR, **Thee S**, Dooley KE, McIlleron HM, Seddon JA, Wiesner L, Castel S, Schaaf HS, Hesselning AC: The pharmacokinetics and safety of ofloxacin in children with drug-resistant tuberculosis. Accepted at AAC, AAC01404-15.

The pharmacokinetics and safety of ofloxacin (OFX) in children <15 years routinely receiving ofloxacin for MDR-TB treatment or preventive therapy at a dose of 20mg/kg were assessed. Children with MDR-TB disease underwent long-term safety monitoring. Eighty-five children (median age 3.4years) were included. OFX was safe and well tolerated in children with MDR-TB, but exposures were well below reported adult values.

## Ethambutol in paediatric tuberculosis: aspects of ethambutol serum concentration, efficacy and toxicity in children

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### SUMMARY

**SETTING:** Ethambutol (EMB) is used as a fourth drug in paediatric anti-tuberculosis treatment. In current recommendations the dosage of EMB is calculated per kg body weight.

**OBJECTIVE:** To present two studies investigating an appropriate EMB dosage in children, and observational data on its toxicity and efficacy.

**DESIGN:** EMB serum levels in children of different age groups were determined after single oral administration of EMB alone as well as after EMB combined with rifampicin, and optimal dosages were established. The efficacy and toxicity of these EMB dosages were examined retrospectively.

**RESULTS:** EMB serum levels were lower than those expected in adults receiving a similar oral dose, due to dif-

ferent pharmacokinetics and pharmacodynamics in childhood. Thereafter, children were treated with EMB doses calculated by body surface (867mg/m<sup>2</sup>). Ocular toxicity occurred in 0.7% of cases and relapses in 0.8%.

**CONCLUSION:** Current recommended EMB dosages in childhood tuberculosis lead to subtherapeutic serum levels. It appears to be more valid to calculate the EMB dosage on the basis of body surface rather than body weight, leading to higher dosages especially in younger children. With these dosages, therapeutic serum levels are reached in all age groups, leading to a high efficacy of anti-tuberculosis treatment without increased ocular toxicity.

**KEY WORDS:** anti-tuberculosis agents; drug therapy pharmacokinetics; ethambutol; children

ETHAMBUTOL (EMB) was first recommended for use in the treatment of tuberculosis (TB) in 1966. In dosages used for therapy, EMB has bacteriostatic activity against *Mycobacterium tuberculosis*.<sup>1-3</sup> The main advantages of EMB are its low toxicity, low costs and administration by the oral route. In children, EMB is included in an anti-tuberculosis regimen when there is an increased likelihood of the disease being caused by organisms resistant to isoniazid (INH) or when the child has 'adult-type tuberculosis'.<sup>4-6</sup> Especially in countries with a high TB burden, there are very few alternatives to the use of EMB if a fourth drug is needed in the treatment of childhood TB.<sup>5,6</sup>

Very few reports have been published on the pharmacokinetics, pharmacodynamics, efficacy and the resulting adequate dosage of EMB in children. Current recommendations for the dosage of EMB in children vary from 15 to 25 mg/kg body weight.<sup>2,5-11</sup> A sufficiently high tissue level is essential for the efficacy of all chemotherapeutics. The minimum inhibition concentration (MIC) of EMB for *M. tuberculosis* in vitro is 0.5-2.0 µg/ml, depending upon the media

used.<sup>2,5-7,12,13</sup> In vivo, the target range of EMB serum concentration is 2.6 µg/ml for daily doses.<sup>6,12,14</sup>

EMB distributes well and rapidly through the body, but the pharmacokinetics and pharmacodynamics differ between children and adults.<sup>6,15</sup> In children, the extravascular space is much larger in relation to the body weight than in adults, changing with age and resulting in a different age-dependent drug distribution.<sup>16-18</sup>

Two studies to determine the ideal dosage of EMB in the treatment of TB in children were performed at the Department of Pediatric Pneumology of the Chest Hospital Heckeshorn, Berlin, Germany, in 1971 and 1973.<sup>19,20</sup> These nationally published findings were never made accessible to the international medical community, and they are thus presented here. In the first study, EMB was given to children in single doses of 15 mg/kg followed by 25 mg/kg; in the second study, doses of 35 mg/kg EMB were given alone and later in combination with 10 mg/kg rifampicin (RMP). The EMB dosage was higher than the currently recommended dosage (between 15 and 25 mg/kg EMB).<sup>5,6,8,9,11,19,20</sup>

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EMB is a relatively well tolerated compound, with side effects observed in 3–6% of adults.<sup>10,21</sup> Its most important adverse effect is ocular toxicity,<sup>1,2,10,22</sup> because of the difficulty of identifying this rare but serious side effect in young children, national and international guidelines currently recommend that EMB should only be given with caution to children aged <5 years.<sup>5,6,11</sup>

The results reported here add to the data about EMB in the treatment of childhood TB. We also present follow-up data regarding ocular toxicity and the efficacy of EMB in children treated at our department until 1981.

## PATIENTS AND METHODS

During the time of the studies all children diagnosed with TB for the first time at our department were included. They had not received any previous anti-tuberculosis medication before inclusion in the studies. Both studies were approved by the ethics committee of the Free University Berlin; parents gave informed written consent for participation.

Twenty children were enrolled in the first study, and 28 children in the second.<sup>19,20</sup> Any history of visual disturbance was a criterion for exclusion. Children were divided into three age groups: 2 to <6 years, 6 to <10 years and 10–14 years.

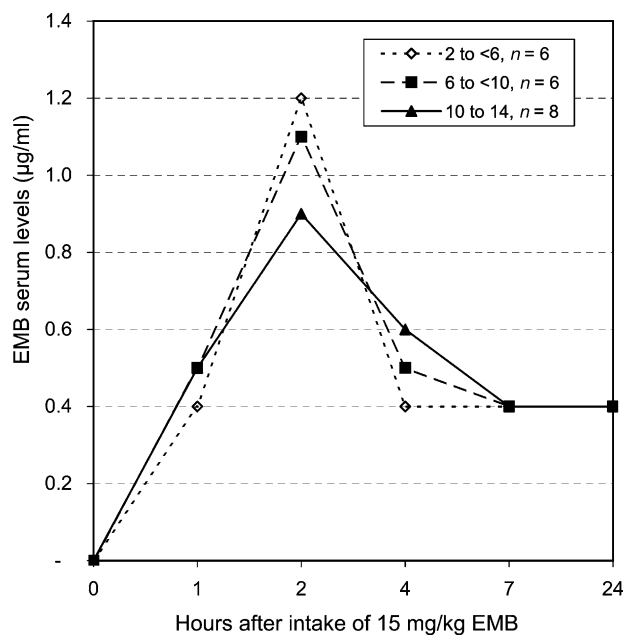
In the 1971 study, all children received a single dose of 15 mg/kg EMB. After an adequate wash-out time of 1 week, they received a single dose of 25 mg/kg EMB. In the 1973 study, 35 mg/kg EMB was given, and after the wash-out time the same children received 35 mg/kg EMB plus 10 mg/kg RMP.<sup>20</sup>

Blood was taken by venipuncture before the medication and 1, 2, 4, 7 and 24 h thereafter. The samples were centrifuged within 30 min. Serum and blood were separated and frozen at  $-18^{\circ}\text{C}$ . EMB was determined in the serum by a modified microbiological vertical diffusion test with the rapid growing mycobacterial ATCC 607 strain, as previously described.<sup>23</sup> After the study measurements, standard combined anti-tuberculosis treatment was initiated.

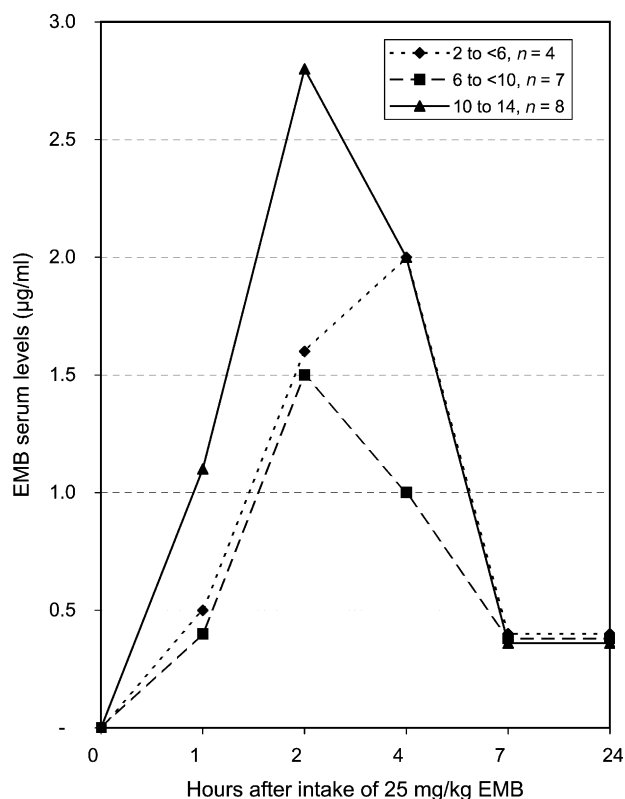
## RESULTS

Mean EMB serum levels after oral application of 15 mg/kg body weight EMB are shown in Figure 1, and serum levels after intake of 25 mg/kg are shown in Figure 2. After 15 mg/kg EMB, none of the children reached the serum target range of 2  $\mu\text{g}/\text{ml}$ . After 25 mg/kg EMB, detectable serum levels were achieved over an extended period (up to 7 h). While in children aged 6–10 years the serum levels stayed below 2  $\mu\text{g}/\text{ml}$ , the youngest children reached serum EMB levels of 2  $\mu\text{g}/\text{ml}$  after 4 h and the eldest group (10–14 years) already exceeded this level after 2 h.

Finally, the EMB dose of 25 mg/kg was calculated per body surface as well as per body weight (Table 1).



**Figure 1** Mean serum levels of EMB ( $\mu\text{g}/\text{ml}$ ) in children at different age groups after the intake of 15 mg/kg body weight EMB in a single dose before breakfast. Source: Hussels and Otto.<sup>19</sup> EMB = ethambutol.



**Figure 2** Mean serum levels of EMB ( $\mu\text{g}/\text{ml}$ ) in children at different age groups after the intake of 25 mg/kg body weight EMB in a single dose before breakfast. Source: Hussels and Otto.<sup>19</sup> EMB = ethambutol.

Differences in the actual doses are explained by the smallest drug capsules available, containing 100 mg EMB and 50 mg RMP. The dosage calculated by body surface varies more than the dosage calculated by



**Table 1** Comparison of dosage calculation by mg/kg body weight and mg/m<sup>2</sup> body surface in the different age groups compared to standard dose adults

EMB doses	Age, years			Adults (standard dose)
	2–6	6–10	10–14	
Calculated by mg/kg body weight				
Min	24.0	24.0	22.2	—
Max	25.9	25.3	25.7	—
Mean	24.7	24.6	24.6	25.
Calculated by mg/m <sup>2</sup> body surface				
Min	555.6	625.0	688.1	—
Max	614.0	707.5	846.9	—
Mean	587.5	652.6	755.7	867.1

Source: Hussels and Otto.<sup>19</sup>  
EMB = ethambutol.

body weight in the different age groups; younger children receive smaller doses per m<sup>2</sup> than adults or older children.

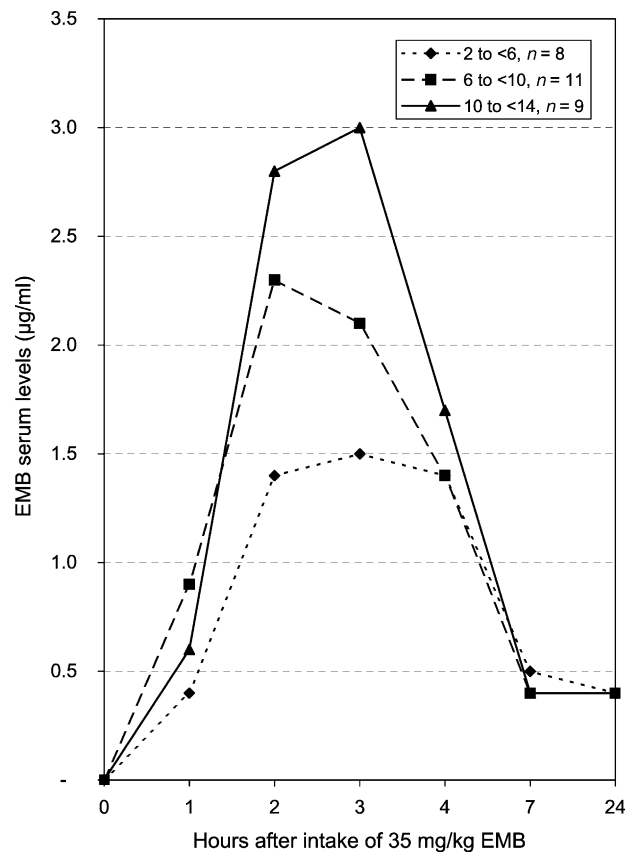
Figure 3 and Table 2 show pharmacokinetic data from the second study after a dose of EMB 35 mg/kg.<sup>20</sup> With this dose, children in age groups 6 to <10 and 10–14 years exhibited higher mean levels of EMB than after 25 mg/kg.<sup>20</sup> In children in the age group 2 to <6 years, the mean values did not reach 2.0 µg EMB/ml at any time. The time until maximum serum concentrations were reached as well as the serum concentrations themselves differed widely between individual children.

EMB serum levels were then measured after combined administration of 35 mg/kg EMB and 10 mg/kg RMP (Table 3 and Figure 4).<sup>20</sup> In children aged between 2 and <6 years, average EMB serum levels exceeded 2 µg/ml 3 h and 4 h after administration of the combined drugs; mean serum levels of EMB in all age groups were higher than after the single application of EMB. This difference in average serum levels reached statistical significance in the 2–6 year age group 4 h after EMB and RMP were given in combination ( $P < 0.02$ ), and was still close to statistical significance after 5 h ( $P < 0.05$ ), but was not generally significant due to the wide variations in standard deviation.

**Table 2** Mean values of EMB serum levels (µg/ml) after administration of 35 mg/kg EMB. Mean values and maximum ( $C_{max}$ ) serum levels observed in every child during the experiment were calculated. The time intervals until maximum serum levels were reached were also averaged and designated  $T_{max}$ .

Age, years	n	EMB serum levels (µg/ml) before and after oral administration mean (min–max)							$C_{max}$ (min–max)	$T_{max}$ (min–max)	Calculated mean doses	
		Before	After								mg/m <sup>2</sup> body surface	mg/kg body weight
			1 h	2 h	3 h	4 h	7 h	24 h				
2–<6	8	<0.4	0.4 (<0.4–1.5)	1.4 (<0.4–3.1)	1.5 (<0.4–2.5)	1.4 (0.8–3.5)	0.5 (<0.4–1.2)	<0.4	2.3 (0.6–3.5)	186 (60–240)	837 (732–925)	34.8 (32.7–35.7)
6–<10	11	<0.4	0.9 (<0.4–5.4)	2.3 (<0.4–8.5)	2.1 (0.7–3.9)	1.4 (0.6–3.0)	<0.4 (0.4–1.0)	<0.4	3.0 (0.9–3.5)	180 (120–240)	901 (810–972)	34.9 (34.5–35.4)
10–14	9	<0.4	0.6 (<0.4–2.7)	2.8 (<0.4–7.0)	3.0 (1.6–5.9)	1.7 (0.8–9.4)	<0.4 (<0.4–1.5)	<0.4	4.0 (1.5–9.4)	180 (120–300)	1070 (973–1234)	35.0 (34.7–35.5)

Source: Hussels, Kroenig and Magdorf.<sup>20</sup>  
EMB = ethambutol.



**Figure 3** Mean values of EMB serum concentrations (µg/ml) after administration of 35 mg/kg EMB. Source: Hussels, Kroenig and Magdorf.<sup>20</sup> EMB = ethambutol.

*EMB toxicity*

During the time of the described studies, no child was identified with any change in visual acuity or colour vision.<sup>19,20</sup> To detect decreased colour vision, Panel D15-Desaturé or Ishihara tables were used in older children,<sup>24</sup> while those aged 2–6 years underwent only funduscopy and testing of colour vision (Panel D15-Desaturé).

Following these studies, until 1981, 567 children treated with EMB in our department were examined for ocular toxicity.<sup>15</sup> As the standard regimen children received a triple course of anti-tuberculosis drugs

**Table 3** Maximum values of ethambutol serum levels ( $C_{max}$ ) after combined administration of 35 mg/kg EMB and 10 mg/kg RMP and time until these serum levels are reached ( $T_{max}$ )

	Age, years		
	2 to <6	6 to <10	10 to <14
$T_{max}$ , minutes (min–max)	210 (180–300)	168 (120–240)	156 (120–240)
$C_{max}$ , $\mu\text{g/ml}$ (SD)	3.0 ( $\pm 0.4$ )	2.8 ( $\pm 0.5$ )	6.6 ( $\pm 1.4$ )

Source: Hussels, Kroenig and Magdorf.<sup>20</sup>  
EMB = ethambutol; RMP = rifampicin; SD = standard deviation.

consisting of INH, RMP and EMB for 6 months, followed by INH and RMP for a further 6 months, and then INH monotherapy for 1 year. During this period, the EMB dosage was calculated in our department by body surface based on the standard dosage in adults of 867 mg EMB/m<sup>2</sup> body surface.<sup>19</sup> Daily dosage in toddlers was equivalent to about 35 mg/kg bodyweight, and decreased stepwise to a dosage of about 25 mg/kg in adolescents.

Ocular toxicity was monitored every 4 weeks during treatment, including funduscopy and perimetry. To detect decreased colour vision, Panel D15-Desaturé or Ishihara tables were used in older children.<sup>24</sup> Children aged 3–6 years only received funduscopy and testing of colour vision (Panel D15-Desaturé). Of 567 chil-

dren examined, decreased colour vision was only identified in four children (0.7%); anti-tuberculosis treatment of a 10-year-old with a daily dosage of 22 mg/kg EMB had to be stopped after 3 months; two other children (aged 4 and 8 years) had received a daily dosage of 35 mg/kg EMB for 3 and 2 months, respectively. Data are no longer available for the fourth child. Although at that time all children were treated with higher doses than currently recommended, the percentage of cases of optic toxicity stayed below 1% (0.7%).

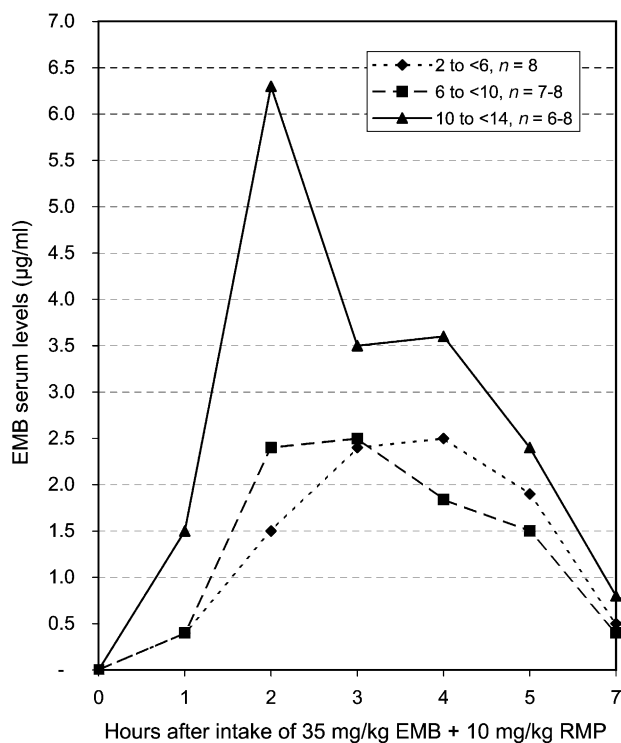
## DISCUSSION

The data presented suggest that in children aged <10 years an oral dosage of 15–25 mg/kg EMB can lead to serum levels below the MIC for *M. tuberculosis* of 2  $\mu\text{g/ml}$ .<sup>12,21</sup> Similar findings have been reported by other authors.<sup>6,14,25,26</sup> As shown above, the EMB serum levels reached after oral administration increase with age. Children aged between 10 and 14 years displayed a sufficiently high serum EMB level after oral administration of 25 mg/kg. With an oral dose of 35 mg/kg, children aged 6–10 years also had serum EMB levels above 2  $\mu\text{g/ml}$ . In children aged 2–6 years, sufficient serum levels were measured after the 25 mg/kg dose, but not after 35 mg/kg. As adsorption varies widely between individuals, these contrary findings might be due to the small number examined in this age group (four children). In comparison, maximum serum concentrations of EMB in adults after an oral dose of 15, 25 and 50 mg/kg are 2–4, 4–6 and 8  $\mu\text{g/ml}$ , respectively.<sup>1,14,16,27</sup>

In combined EMB/RMP treatment (35 mg/kg EMB + 10 mg/kg RMP), adequate serum levels were also reached in children aged 2–6 years (8 children examined). In the group aged 10–14 years, EMB serum levels after combined treatment were unusually high.<sup>20</sup> Consequently, it is not necessary to increase the dosage of EMB in the age group 10–14 years to 35 mg/ml when EMB is given in combination with RMP. While there are other data on pharmacokinetics of EMB in children used as monotherapy, none compare the serum levels of EMB in combined treatment. Our data suggest an interaction between EMB and RMP leading to higher EMB serum concentrations in all age groups, although these differences do not reach statistical significance.

The association of EMB serum levels and age might be explained by the larger extravascular space relative to body weight in younger children.<sup>16,17</sup> The body compartments grow disproportionately, resulting in a difference in volume of distribution between children and adults.<sup>16</sup> As intra- and extracellular EMB concentrations are the same, serum concentrations give a good estimate of therapeutic efficacy and are very useful in optimising drug therapy.<sup>21,26</sup>

Not only does distribution differ between children and adults, but so does the absorption and elimination of EMB.<sup>13,14</sup> After oral application in adults, max-



**Figure 4** Mean values of EMB serum concentrations after combined administration of 35 mg/kg EMB and 10 mg/kg RMP. As serum levels could not be measured in every child every hour, the number of examined patients differ. The reasons for the drop out are no longer available. Source: Hussels, Kroenig and Magdorf.<sup>20</sup> EMB = ethambutol.

imum serum concentrations of EMB are reached after approximately 2 h.<sup>13</sup> In children aged <6 years, peak serum levels are found approximately 4 h after ingestion of 25 mg/kg or 35 mg/kg EMB. This is in line with the findings of Zhu et al., who reported an adsorption delay in children receiving daily doses of EMB and also described an decrease of elimination (lengthening of half-life  $t_{1/2}$ ) with age.<sup>14</sup> These results suggest not only lower serum concentrations but also a shortened period of maximum serum concentrations in young children as compared to adults. The efficacy of EMB is time-dependent, and thus the duration of effective serum levels also has to be considered when establishing optimal EMB dosage in children.<sup>3,26</sup> Taking into account body distribution and pharmacokinetic parameters of EMB in children, it would appear to be better to calculate the dosage in relation to body surface rather than body weight. However, these calculations might not always be convenient in daily practice, especially under the conditions often found in developing countries. On the basis of our calculations using body surface, we therefore consider an EMB dosage of 35 mg/kg for children aged <10 and of 25 mg/kg EMB for children aged  $\geq 10$  years as adequate.

Finding the optimum dosage means a balance between efficacy and toxic effects. Efficacy of EMB in adult pulmonary TB is evaluated by negativity of sputum culture.<sup>12,28,29</sup> In children, the efficacy of anti-tuberculosis regimens is measured by clinical improvement, the number of relapses or the reduction of bacteria in sputum cultures, although TB in children is mostly paucibacillary and cultures are often initially negative.<sup>29</sup> Of 567 children treated with standard combination therapy for TB in the local department until 1981, 364 children were followed up for at least 2 years after the end of treatment. Only three relapses of TB (0.8%) were observed during this period.<sup>15</sup>

Regarding side effects, the use of EMB with higher dosages always raises the concern of ocular toxicity leading to optic neuritis, causing decreased visual acuity, blurred vision and the loss of red/green colour vision. Ocular toxicity is related to dosage and duration of treatment and is fortunately usually reversible after cessation of therapy.<sup>8,22,30</sup>

In adults, ocular toxicity has been associated with EMB daily doses >25 mg/kg and hence higher serum concentrations; it occurs in 3–6% of patients.<sup>14,31</sup> Leibold showed a dose-dependent toxicity of EMB in adults. Of 59 patients with EMB dosages >35 mg/kg/day, 11 developed ocular toxicity, but only two did so when receiving EMB dosages below 30 mg/kg/d.<sup>30</sup>

Several studies that included ophthalmological follow-up have been performed in children using daily EMB dosages between 13 and 25 mg/kg.<sup>32–34</sup> Apart from one case (an 11-year-old child) with slight oedema of the optic disk after 7 months of treatment, no changes in visual evoked potentials or any sign of optic neuritis were reported in these studies.<sup>32–34</sup> As

shown in our studies, maximum serum concentrations after ingestion of EMB are generally lower in children than in adults. In a recently published review, Donald et al. assumed that the rare EMB toxicity is due to the considerably lower serum concentrations reached in children.<sup>5,6</sup> Although children in our department were treated with EMB dosages of up to 35 mg/kg/day in combined treatment, the frequency of visual side effects stayed below 1% (0.7%; 4/567 children).<sup>15</sup> This indicates that children who are exposed to the serum concentrations similar to those among adults are not exposed to a higher risk of ocular toxicity. However, some cases of optic neuritis have been reported with low doses in adults, showing that there is no so-called 'safe' dosage of EMB, but rather some kind of drug-incompatibility independent of dose-related toxicity.<sup>12,13,35</sup> Although EMB toxicity occurs quite rarely, it has to be kept in mind that in developing countries, where EMB finds its main application, regular monitoring of vision cannot be guaranteed.

Limitations of our studies are the small number of patients in whom serum concentrations were measured. The investigations were conducted more than 30 years ago, and some details of the methods and recruitment of the patients are no longer available.

## CONCLUSION

Our results add to the sparse pharmacokinetic data for the use of EMB in children. An adequate dosage is essential for the efficacy of anti-tuberculosis treatment, especially in the era of multidrug-resistant tuberculosis. Due to differences in pharmacokinetics and pharmacodynamics, EMB serum levels in children are lower than those in adults receiving similar oral mg/kg doses. Therefore, recent recommendations for oral dosage of EMB in childhood might be too low. For the efficient treatment of childhood TB, our data indicate that the dosage should be calculated according to body surface area based on the standard dosage in adults of 867 mg EMB/m<sup>2</sup>, leading to an equivalent dosage of 25 mg/kg EMB in children aged  $\geq 10$  years and 35 mg/kg in children aged <10 years. It has been demonstrated that these comparatively high dosages are efficient without increasing the rate of ocular toxicity. More studies with larger numbers of patients are needed on the pharmacokinetics and side effects of EMB in different paediatric age groups.

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## RÉSUMÉ

**CONTEXTE :** L'éthambutol (EMB) est utilisé comme quatrième médicament dans le traitement antituberculeux en pédiatrie. Dans les recommandations actuelles, le dosage d'EMB est calculé par kilogramme de poids corporel.

**OBJECTIF :** Ce travail présente deux études investigant un dosage approprié d'EMB chez les enfants ainsi que des données observationnelles sur sa toxicité et son efficacité.

**SCHEMA :** Les niveaux sériques d'EMB chez les enfants de différents groupes d'âge ont été déterminés après administration unique de l'EMB ainsi qu'après EMB en combinaison avec rifampicine ; on a élaboré les dosages

optimalisés. L'efficacité et la toxicité des dosages d'éthambutol ont été examinées rétrospectivement.

**RÉSULTATS :** Les niveaux sériques d'EMB sont plus bas que ceux attendus chez les adultes recevant une dose orale similaire, par suite de différences pharmacocinétiques et pharmacodynamiques chez l'enfant. Ensuite, les enfants ont été traités par des doses d'EMB calculées en fonction de la surface corporelle (867 mg/m<sup>2</sup>). On a observé une toxicité oculaire dans 0,7% des cas et des rechutes dans 0,8%.

**CONCLUSION :** Les dosages d'EMB actuellement recommandés pour la tuberculose de l'enfant entraînent des

niveaux sériques sous-thérapeutiques. Il semble plus valable de calculer la dose d'EMB sur la base de la surface corporelle plutôt que sur le poids corporel, ce qui entraîne des dosages plus élevés, particulièrement chez les enfants

plus jeunes. Grâce à ces dosages, les taux sériques thérapeutiques sont atteints dans tous les groupes d'âge, ce qui entraîne une efficacité élevée du traitement antituberculeux sans accroissement de la toxicité oculaire.

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#### RESUMEN

**MARCO DE REFERENCIA:** En pediatría, el etambutol (EMB) se emplea como cuarto medicamento en el tratamiento de la tuberculosis. En las recomendaciones vigentes, la posología del EMB se calcula por kilo de peso corporal.

**OBJETIVO:** En este artículo se presentan dos estudios que investigan la dosis adecuada de EMB en niños y los datos observados sobre su toxicidad y eficacia.

**MÉTODO:** Se determinó la concentración sérica de EMB en niños de diferentes grupos de edad, después de una administración única y exclusiva de EMB y después de suministrar EMB combinado con rifampicina y se definieron luego las posologías óptimas. La eficacia y la toxicidad de estas pautas posológicas se analizaron en forma retrospectiva.

**RESULTADOS:** Las concentraciones séricas de EMB fue-

ron inferiores a aquellas previstas en adultos que reciben dosis equivalentes, debido a una farmacocinética y a una farmacodinámica diferentes en los niños. A partir de este momento, el tratamiento de los niños comportó dosis calculadas según la superficie corporal (867 mg por m<sup>2</sup>). Se observó toxicidad ocular en 0,7% y recaídas en 0,8% de los casos.

**CONCLUSIÓN:** Las pautas posológicas de EMB vigentes en pediatría conducen a concentraciones séricas insuficientes. Pareciera más válido calcular la posología del EMB según la superficie corporal y no según el peso, con el fin de alcanzar dosis más altas, en particular en los niños pequeños. Con estas pautas terapéuticas se alcanzan concentraciones séricas óptimas en todos los grupos de edad y se obtiene una alta eficacia del tratamiento antituberculoso sin aumentar la toxicidad ocular.



## Pyrazinamide serum levels in childhood tuberculosis

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### SUMMARY

Pyrazinamide (PZA) is one of the first-line drugs in anti-tuberculosis treatment. In the present study, PZA serum levels in 34 children aged 1 to 14 years were measured either after oral application of PZA alone or after combination therapy with isoniazid and rifampicin. Serum levels did not differ statistically with age, in PZA monotherapy or in combination therapy. With a dosage of 30 mg/kg

PZA, efficient serum levels were reached. Because PZA is distributed uniformly in the body, serum levels are related to body weight, and a dose of 30 mg/kg bodyweight is appropriate in children.

**KEY WORDS:** childhood tuberculosis; pyrazinamide; serum levels; pharmacokinetics

PYRAZINAMIDE (PZA) is an essential component in the initial phase of chemotherapy regimes for tuberculosis (TB). Although *Mycobacterium bovis* is intrinsically resistant to PZA, the drug has highly specific intra- and extracellular activity against *M. tuberculosis* and is valued particularly for its sterilising effect and bactericidal activity.<sup>1</sup>

In adults, PZA is well absorbed and well distributed when taken orally; it is hydrolysed by liver enzymes and excreted primarily by glomerular filtration. Although in general liver metabolism and glomerular filtration are assumed to be well developed by the age of 2 years, differences in pharmacokinetics in children compared to adults, for example due to adsorption and distribution, are yet to be evaluated, and no data are available to date concerning children at different ages.

A study investigating PZA serum levels in children, performed in 1983 at the Department of Paediatric Pneumology and Allergology, Heckeshorn, Berlin, Germany, was published only nationally.<sup>2</sup> PZA serum levels were measured after oral medication with PZA alone or in combination with rifampicin (RMP) and isoniazid (INH) to examine whether the resultant PZA serum levels differ in children of different age groups.

### MATERIALS AND METHODS

Thirty-four children aged between 1 and 14 years receiving treatment for pulmonary tuberculosis (without extra-pulmonary manifestations) at the Department of

Paediatric Pneumology and Allergology, Heckeshorn, Berlin, Germany, were enrolled in the study. The study was approved by the ethics committee of the Free University Berlin. Parents gave informed written consent for participation.

Twenty-one children received only PZA orally at 30 mg/kg body weight and another 13 children also received INH (5–10 mg/kg) and RMP (10–15 mg/kg). Individual doses were administered with 100 mg PZA tablets and given on an empty stomach before breakfast. Venipuncture was performed at 2, 3, 4, 8 and 24 h after medication. Samples were centrifuged within 30 min; PZA serum levels were determined by mass fragmentography with a detection limit of 1.5 µg/ml.<sup>3</sup> A minimal inhibition concentration (MIC) of 16–32 µg/ml PZA was considered effective against *M. tuberculosis*.<sup>4</sup>

For further analysis, children were separated into three age groups: <6 years (monotherapy  $n = 8$ , combination therapy  $n = 4$ ), 6–10 years ( $n = 6$ ,  $n = 6$ ) and 10–14 years ( $n = 7$ ,  $n = 3$ ).

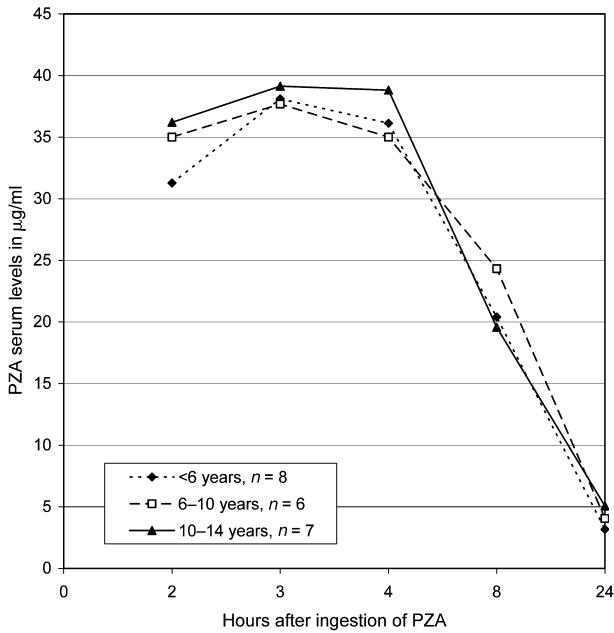
### RESULTS

Mean serum levels after oral application of a single dose of 30 mg/kg body weight PZA alone (Figure 1) were not statistically different between the age groups at any time. Group analysis was performed using Student's *t*-test. Peak serum levels ( $C_{max}$ ) were reached after 3 h in all age groups, with mean maximum serum levels of 38.1 µg/ml in children aged <6 years, 37.7 µg/ml in children aged 6–10 years and 39.1 µg/ml in those

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Hours after ingestion	PZA serum levels in children (µg/ml)		
	<6 years mean (SD)	6-10 years mean (SD)	10-14 years mean (SD)
2	31.3 (2.3)	35.0 (4.2)	36.2 (12.2)
3	38.1 (9.3)	37.7 (8.6)	39.1 (8.0)
4	36.1 (7.4)	35.0 (8.0)	38.8 (9.5)
8	20.4 (6.7)	24.3 (10.6)	19.6 (3.4)
24	3.2 (1.1)	4.1 (2.6)	5.1 (2.8)

**Figure 1** PZA serum levels in children after an oral dose of 30 mg/kg in monotherapy. PZA = pyrazinamide; SD = standard deviation.

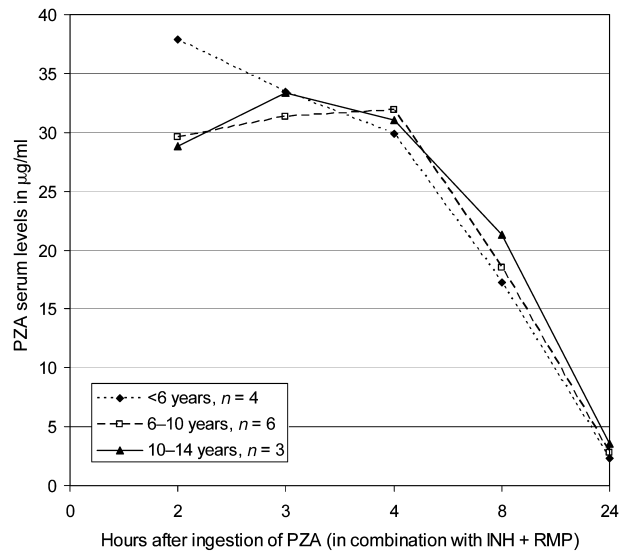
aged 10–14 years. After combination treatment with RMP and INH (Figure 2), peak serum levels were reached after 3 h in children aged ≥6 years and after 2 h in children aged <6 years. Peak serum levels in the youngest age group (37.9 µg/ml) were higher than in children aged 6–10 years (31.3 µg/ml) and 10–14 years (33.3 µg/ml), although the differences were not statistically significant.

PZA serum levels of all age groups after oral application of 30 mg/kg PZA alone were higher than levels after combination treatment. However, these differences did not reach statistical significance.

Severe side effects were not observed. Some children showed a slight elevation of serum uric acid. The number of children with side effects is no longer available.

The volume of distribution for PZA in combination therapy was 20.5 l/kg (standard deviation [SD] 9.9), normalised per kg body weight 0.8 l/kg (SD 0.2) and in monotherapy 18.3 l/kg (SD 8.2), normalised 0.9 l/kg (SD 0.3). There was no statistical difference between the age groups.

Regardless of whether PZA was administered alone or in combination, serum levels stayed above the MIC for *M. tuberculosis* (16–32 µg/ml) for more than 6 h in all age groups.



Hours after ingestion	PZA serum levels in children (µg/ml)		
	<6 years mean (SD)	6-10 years mean (SD)	10-14 years mean (SD)
2	37.9 (5.6)	29.6 (11.5)	28.8 (3.9)
3	33.4 (3.5)	31.3 (7.9)	33.3 (2.3)
4	29.9 (1.0)	31.9 (6.4)	31.0 (1.3)
8	17.3 (4.3)	18.5 (4.6)	21.3 (1.3)
24	2.3 (0.9)	2.8 (1.3)	3.5 (1.0)

**Figure 2** PZA serum levels in children after an oral dose of 30 mg/kg in combination treatment (RMP 10–15 mg/kg + INH 5–10 mg/kg). PZA = pyrazinamide; RMP = rifampicin; INH = isoniazid; SD = standard deviation.

## DISCUSSION

The data presented show that efficient serum levels are reached in children of all age groups with a PZA dosage of 30 mg/kg body weight, with no statistically significant difference between PZA administered alone and PZA given in combination with RMP and INH. In adults, PZA is absorbed almost entirely from the gastrointestinal tract and penetrates well into body tissues, reaching comparable levels in serum, cerebrospinal fluid and body tissue.<sup>5,6</sup> Assuming a one-compartment model, PZA serum levels are related to body weight rather than body surface, and the disproportionate growth of body compartments in children does not have an important impact on serum levels of PZA.<sup>5</sup> Our results confirm that the same dose of PZA per kg body weight can be administered in children until the age of 14 years.

Compared to adults, in whom peak serum levels are found after 1–2 h, PZA is absorbed more slowly in children.<sup>7,8</sup> Consistent with our findings, delayed absorption of ≥3 h was previously reported in children.<sup>7</sup> However, a recently published study found the PZA absorption time to be comparable in children and adults.<sup>9</sup> Between the youngest and the oldest children in our study, there was no difference in time until maximum serum concentrations were reached in PZA monotherapy.

Maximum concentrations of PZA serum levels differ widely between individuals. In our study, mean maximum serum concentrations were not statistically different between the age groups, but were slightly lower than the PZA serum levels reported in adults receiving a similar dose per kg body weight.<sup>5,7</sup> Mean maximum PZA serum levels in children were above 32 µg/ml in all age groups, and therefore well above the MIC for *M. tuberculosis*.<sup>4</sup>

Boulahbal et al. reported that PZA peak serum levels in adults were up to 40% lower when administered in combination than in monotherapy, and attributed this to modifications in intestinal absorption of PZA or in liver enzyme induction.<sup>10</sup> In our study in children, PZA serum levels were also slightly lower when PZA was given in combination therapy, but these differences did not reach statistical significance. Regarding the efficacy of PZA at the site of infection, PZA is only active in vitro against *M. tuberculosis* in acidic environments. The surface of a pulmonary cavity, for example, is basic, while in caseous tissue and in macrophages the environment is acidic.<sup>2</sup> There is nevertheless some evidence that PZA does kill mycobacteria in the walls of pulmonary cavities despite the alkaline environment in vivo, raising uncertainty about the MIC of PZA.<sup>11</sup>

## CONCLUSION

PZA serum levels after an oral dosage of 30 mg/kg do not differ between children of all age groups up to the age of 14 years suffering from TB, regardless of whether PZA is given alone or in combination with INH and RMP. The question as to whether efficient serum levels can be achieved with lower oral dosages of PZA of 25 mg/kg (range 20–30), as currently recommended by the World Health Organization for adults and children, was not discussed in this study.<sup>12</sup> There

seems to be evidence that even dosages as low as 15 mg/kg might be efficient in children, but large studies in children are scarce.<sup>9</sup>

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## RÉSUMÉ

Le pyrazinamide (PZA) fait partie du traitement de première ligne de la tuberculose. Dans cette étude, les niveaux sériques de PZA ont été mesurés chez 34 enfants âgés de 1 à 14 ans, soit après administration du seul PZA par voie orale, soit après un traitement combiné comportant également l'isoniazide et la rifampicine. Il n'y a pas eu de différences significatives des niveaux sériques ni en fonc-

tion de l'âge, ni en fonction du traitement par PZA en monothérapie ou en combinaison. Avec une dose de PZA de 30 mg/kg, on atteint des niveaux sériques efficaces. Comme le PZA se distribue de manière uniforme dans l'organisme, les niveaux sériques sont en relation avec le poids corporel et une dose de 30 mg/kg de poids corporel est appropriée chez les enfants.

## RESUMEN

La pirazinamida (PZA) es un medicamento de primera línea en el tratamiento de la tuberculosis. En el presente estudio se midió la concentración sérica de PZA en 34 niños entre 1 y 14 años, después de su ingestión oral aislada o en asociación con isoniazida y rifampicina. No se observó variación estadísticamente significativa de la concentración sérica de PZA con la edad en la monoter-

pia ni en el tratamiento mixto. Con una dosis de 30 mg/kg, se alcanzaron concentraciones séricas eficientes. Puesto que la PZA se distribuye en forma uniforme en el cuerpo, existe una correlación entre la concentración sérica y el peso corporal y una dosis de 30 mg/kg es apropiada en los niños.

## Rifampicin serum levels in childhood tuberculosis

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### SUMMARY

**BACKGROUND:** Rifampicin (RMP) is an essential drug in paediatric anti-tuberculosis treatment. The current World Health Organization (WHO) guidelines recommend an oral dosage of 10 (8–12) mg per kg body weight.

**OBJECTIVE:** To present a study investigating RMP serum levels in children after oral medication of RMP alone and after combination treatment with ethambutol (EMB).

**DESIGN:** RMP serum levels in children of different age groups were determined after a single oral administration of 10 mg/kg RMP alone as well as after combination with 35 mg/kg EMB.

**RESULTS:** RMP serum levels were lower than those ex-

pected in adults receiving a similar oral dose. RMP serum levels in combination treatment were even lower than in monotherapy.

**CONCLUSION:** Currently recommended RMP dosages in childhood tuberculosis lead to serum levels lower than those recommended for adults, probably due to different pharmacokinetics and pharmacodynamics in children. In children, it appears to be more valid to calculate RMP dosage on the basis of body surface area rather than body weight, leading to higher dosages especially in younger children.

**KEY WORDS:** childhood tuberculosis; rifampicin; serum levels; pharmacokinetics

WITH AN ESTIMATED 1 million cases worldwide each year, paediatric tuberculosis (TB) is a serious health problem.<sup>1</sup> Rifampicin (RMP) is an important pillar in modern short-course treatment regimens, and RMP resistance is a disaster for both patients and control programmes.<sup>2</sup> RMP has moderate early bactericidal activity (EBA) against *Mycobacterium tuberculosis*, but in combination with isoniazid (INH), its sterilising ability is unique.<sup>3</sup> RMP is well absorbed from the gastrointestinal tract and distributes extensively throughout the body despite a protein binding of 80%.<sup>4,5</sup> It is metabolised in the liver mainly by acetylation to 25-O-desacetyl-rifampicin, which is also active against *M. tuberculosis* and excreted through the bile and, to a lesser extent, through urine.<sup>4–7</sup> In adults, serum levels of the order of 10 µg/ml occur 2 h after a single oral dose of 600 mg RMP, and are associated with efficacy in clinical studies.<sup>4,5,8,9</sup> As the principles of anti-tuberculosis treatment in children do not differ from those for adults, children should be exposed to the same serum levels.<sup>10</sup> The RMP dosage for children currently recommended by the World Health Organization (WHO) and the American Thoracic Society (ATS) is respectively 10 (8–12) and 10–20 mg per kg bodyweight.<sup>11–13</sup>

Children experience significant changes during growth, not only in height and weight, but also in the relative size of the body compartments as well as in their ability to absorb, metabolise and excrete drugs, leading to pharmacokinetics that differ from those in adults. There is a lack of pharmacokinetic data on anti-tuberculosis drugs in children and fundamental uncertainties about age-appropriate RMP dosage.<sup>14</sup>

A study in children investigating RMP serum levels performed in 1973 at the Department of Paediatric Pneumology and Allergology, Chest Hospital Heckeshorn, Berlin, Germany, was published only nationally at the time.<sup>15</sup> These findings were never made accessible to the international medical community, and they are thus presented here.

RMP serum levels were measured in children of different age groups after oral medication with RMP alone and in combination with ethambutol (EMB). EMB pharmacokinetics were also studied, and the data on EMB were published previously.<sup>16</sup>

### MATERIALS AND METHODS

Previously untreated children diagnosed with pulmonary TB at the Department of Paediatric Pneumology

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and Allergology, Chest Hospital Heckeshorn, Berlin, Germany, were included in the study in 1973. Twenty-seven children aged between 2 and 14 years were enrolled in the study, which was approved by the ethics committee of the Free University Berlin. Parents gave informed written consent for participation. Serum levels were studied after the first dose of RMP was given. After completion of all study measurements, standard multidrug anti-tuberculosis treatment was initiated.

In the first part of the study, children received a single dose of RMP orally at 10 mg/kg body weight. In the second part, 10 mg/kg RMP in combination with 35 mg/kg EMB was given as a single dose after an adequate wash-out time of 1 week. Tablets were administered on an empty stomach after overnight fasting. Venipuncture was performed at 1, 2, 3, 4, 5, 7 and 24 h after medication. Samples were centrifuged within 30 min and the serum stored at  $-18^{\circ}\text{C}$ .

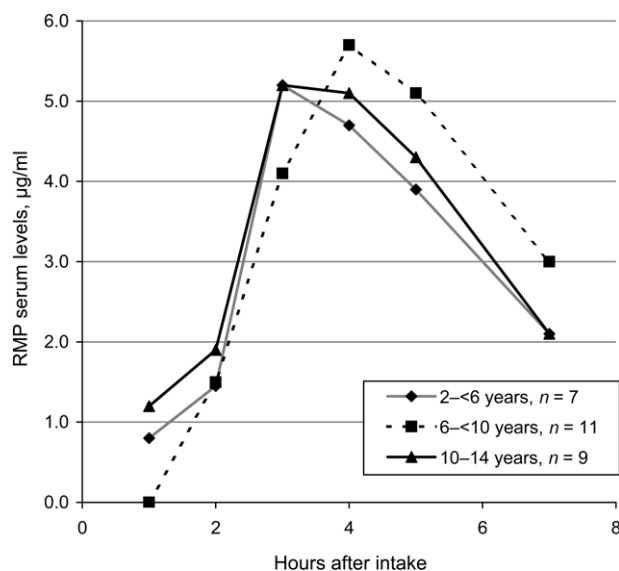
RMP serum levels were measured by a microbiological method based on the agar diffusion disc technique in a modification previously described.<sup>17</sup> This was the standard technique 30 years ago, at the time the study was performed. A staphylococcus strain highly sensitive to RMP was used as the indicator strain. As staphylococcus strains are less sensitive to the main metabolite of RMP, desacetyl-rifampicin, than *M. tuberculosis*, assays with *Staphylococcus aureus* slightly underestimate the total activity against *M. tuberculosis*.<sup>18</sup> As the *S. aureus* strain was EMB-resistant, combination treatment had no influence on measurements of RMP, and RMP levels could also be determined when both drugs were given in combination. For further analysis, children were separated into three age groups: 2–<6 years ( $n = 7$ ), 6–<10 years ( $n = 11$ ) and 10–14 years ( $n = 9$ ).

The mean RMP serum levels of all children were calculated for each time point. The means of the maximum serum levels ( $C_{\text{max}}$ ) as well as the time until these levels were reached ( $T_{\text{max}}$ ) were determined. Group analysis was performed using Student's *t*-test. The area under the serum concentration-vs.-time curve from time 0 to 7 h ( $\text{AUC}_{0-7\text{h}}$ ) was determined by the linear trapezoidal rule.

## RESULTS

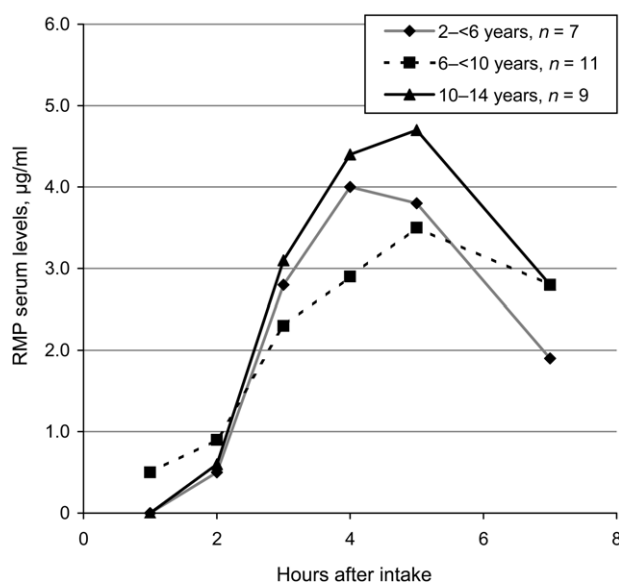
The mean RMP serum levels after oral application of 10 mg/kg are shown in Figure 1 and the mean serum levels after combined administration of RMP (10 mg/kg) plus EMB (35 mg/kg) are shown in Figure 2. Tables 1 and 2 show the  $C_{\text{max}}$  and standard deviations (SDs),  $T_{\text{max}}$ , mean half-life and the  $\text{AUC}_{0-7\text{h}}$  of RMP after single and combined treatment, respectively.

The mean serum levels of the different age groups showed a high variability, with mean maximum serum levels of 6.5–7.1  $\mu\text{g}/\text{ml}$  in monotherapy and 4.5–5.4  $\mu\text{g}/\text{ml}$  in combination therapy. Mean maximum



**Figure 1** Mean serum levels of RMP among children in different age groups after the intake of RMP 10 mg/kg body weight in a single dose. RMP = rifampicin.

serum concentrations in children aged <6 years after single and after combined drug administration tended to be lower than those found in the older children, although these differences fail to reach statistical significance. Compared to monotherapy, the mean maximum serum levels in combined therapy were lower in all age groups. These differences in maximum serum levels were up to 2.0  $\mu\text{g}/\text{ml}$ , but again fail to reach statistical significance, probably due to the small sample size and a high variability in individual serum concentrations (data not shown). The mean  $T_{\text{max}}$  was



**Figure 2** Mean serum levels of RMP among children in different age groups after combined administration of RMP 10 mg/kg + EMB 35 mg/kg body weight. RMP = rifampicin; EMB = ethambutol.

**Table 1** Mean RMP serum levels and SDs among children in different age groups after intake of RMP 10 mg/kg in a single dose

	2–<6 years (n = 7) µg/ml (SD)	6–<10 years (n = 11) µg/ml (SD)	10–14 years (n = 9) µg/ml (SD)
Hours after ingestion			
1	0.8*	<0.4*	1.2*
2	1.5*	1.5*	1.9*
3	5.2 (1.9)	4.1 (1.3)	5.2 (0.2)
4	4.7 (0.9)	5.7 (1.1)	5.1 (0.6)
5	3.9 (0.6)	5.1 (0.9)	4.3 (0.7)
7	2.1 (0.3)	3.0 (0.4)	2.1 (0.2)
24	<0.4	<0.4	<0.4
C <sub>max</sub>	6.5 (1.2)	7.1 (1.2)	6.6 (0.8)
T <sub>max</sub> , h [min–max]	3.8 [3.0–5.0]	4.0 [4.0–5.0]	3.5 [1.5–5.0]
t <sub>1/2</sub> , h	2.1	2.6	1.9
AUC <sub>0–7h</sub> , µgh/ml	20.15	21.75	22.75

\*As variability was high, SDs were not calculated.

RMP = rifampicin; SD = standard deviation; C<sub>max</sub> = maximum serum level; T<sub>max</sub> = time to maximum serum level; t<sub>1/2</sub> = elimination half-life; AUC<sub>0–7h</sub> = area under the serum concentration vs. time curve from time 0 to time 7 h.

3.5–4.3 h in all children, with only minor differences between the age groups and independently of whether monotherapy or combination therapy was received. Again, variability was high.

The elimination half-life was 1.9–2.6 h in monotherapy and 2.1–2.5 h in combination therapy. Children aged >10 years seemed to eliminate RMP faster than those aged <6 years. The AUC<sub>0–7h</sub> in all age groups on monotherapy was greater than that for combination therapy. In monotherapy, the AUC<sub>0–7h</sub> rose with age, while in combination therapy the age group 6–10 years had a lower AUC<sub>0–7h</sub> than the young-

**Table 2** Mean RMP serum levels and SDs after ingestion of 10 mg/kg RMP in combination with 35 mg/kg EMB in children in different age groups

	2–<6 years (n = 7) µg/ml (SD)	6–<10 years (n = 11) µg/ml (SD)	10–14 years (n = 9) µg/ml (SD)
Hours after ingestion			
1	<0.4*	0.5*	<0.4*
2	0.5*	0.9*	0.6*
3	2.8 (1.0)	2.3 (0.7)	3.1 (0.6)
4	4.0 (1.1)	2.9 (0.6)	4.4 (0.8)
5	3.8 (0.9)	3.5 (0.7)	4.7 (0.7)
7	1.9 (0.3)	2.8 (0.8)	2.8 (0.4)
24	<0.4	<0.4	<0.4
C <sub>max</sub>	4.5 (1.1)	5.3 (0.7)	5.4 (0.6)
T <sub>max</sub> , h [min–max]	4.0 [3.5–5.0]	3.7 [2.0–5.0]	4.3 [3.0–5.0]
t <sub>1/2</sub> , h	2.5	2.2	2.1
AUC <sub>0–7h</sub> , µgh/ml	14.90	13.60	17.95

\*As variability was high, SDs were not calculated.

RMP = rifampicin; SD = standard deviation; EMB = ethambutol; C<sub>max</sub> = maximum serum level; T<sub>max</sub> = time to maximum serum level; t<sub>1/2</sub> = elimination half-life; AUC<sub>0–7h</sub> = area under the serum concentration vs. time curve from time 0 to time 7 h.

est children, whereas children aged >10 years had the highest AUC<sub>0–7h</sub>.

## DISCUSSION

In this study, RMP serum levels after oral intake of RMP alone and in combination with EMB in children of different age groups are described.

In children aged between 2 and 14 years, after a dosage of 10 mg/kg RMP is given, RMP serum levels are lower than those observed in adults after a standard dose of 600 mg. RMP serum levels in children are even lower if RMP is given in combination with EMB. RMP serum levels in adults after a standard oral dose of 600 mg RMP are in the range of 8–24 µg/ml 2 h postdose.<sup>19,20</sup> Serum levels in children in single or in combination therapy found in our study are even below the lower limit.

As low drug concentrations reduce therapeutic efficacy, higher doses of RMP than those currently recommended by the WHO (8–12 mg/kg) might therefore be necessary.<sup>11</sup>

Lower RMP serum levels after oral administration in children have been reported previously.<sup>4,5,21,22</sup> Serum levels in children treated with 10 mg/kg body-weight were found to correspond to one third to one tenth of that in adults receiving 600 mg, and thus were similar to those in adults treated with a dose of 250 mg RMP.<sup>4</sup> On the other hand, serum levels of 9–11 µg/ml were found in another study in children where 10 mg/kg RMP was used as prophylaxis against *Haemophilus influenzae* type b disease.<sup>23</sup> This might be partly explained by the use of RMP suspension in this study, which could lead to improved RMP absorption. As a special in-house preparation for the RMP suspension was used, its pharmaceutical formulation might also differ from commercially available products, with possible impact on its pharmacokinetics and pharmacodynamics.

To our knowledge, no other studies have compared RMP serum levels in children after monotherapy with combination therapy with EMB. Similar to our findings in children, a decreased RMP area under the curve (AUC) has been reported in adults when the drug is given in combination with EMB.<sup>22</sup> Findings of serum levels of RMP given in combination therapy with INH are conflicting: both higher and lower RMP levels, as well as no change, have been described.<sup>22–26</sup> However, in combination therapy with INH and pyrazinamide (PZA), serum concentrations of RMP do not seem to differ from those with monotherapy.<sup>24,26,27</sup>

In the study, only a two-drug combination of RMP+EMB was examined to exclude the influence from other drugs, although in anti-tuberculosis treatment RMP is rarely given in combination with EMB alone. As the differences in RMP serum levels in mono- and combination therapy with EMB are not statistically significant, no conclusion can be drawn about



the clinical relevance of the lower RMP serum levels in combination therapy found in our study.

After oral intake of RMP, absorption from the intestinal tract is almost complete in adults, and peak serum levels are found after 2 h.<sup>4,19,28</sup> In children, absorption seems to be delayed, and peak serum levels were found after 4 h in our study, in line with previously reported findings.<sup>4</sup>

Unlike RMP absorption, there seems to be no major difference in elimination half-life ( $t_{1/2}$ ) between adults and children. In adults, the  $t_{1/2}$  is dose-dependent, and ranges from 2.3 h to 5.1 h, which is in line with our results and previously described findings of an average  $t_{1/2}$  of 2.9 h in children after a 10 mg/kg dose of RMP.<sup>7,23</sup>

To explain these lower serum levels in children, different pharmacokinetic aspects have to be considered. Food has been shown to significantly reduce the absorption of RMP.<sup>29</sup> However, the children in our study received medication after overnight fasting.

Many more factors, such as gastric pH, gastric emptying, intestinal transit time, functional absorptive area, metabolic capacity and carrier mechanisms or drug transporters in the gastrointestinal tract, influence gastro-intestinal absorption of a drug.<sup>19,30</sup> However, as gastric pH, gastric emptying and intestinal transit time reach adult values within the first year of life, they would have a marginal influence on serum levels in older children compared to adults.<sup>31</sup> A decrease in the functional absorptive area of the intestine in TB patients has also been considered to explain reduced serum levels.<sup>19</sup> A prehepatic metabolism of RMP was described previously, probably localised in the gut wall in adults.<sup>32</sup> However, very little is known about the metabolic capacity or maturation of drug transporters in the gut wall in children and their influence on the quantity and time of absorption.<sup>30</sup>

Not only has delayed absorption been described in children but also a bioavailability of only 50% after oral administration of RMP.<sup>33</sup> RMP is well distributed throughout the body. As about 25% of the drug is ionised at a physiological pH, while the molecule as a whole is lipid-soluble, RMP concentrations in the various tissues of the human body differ.<sup>6,7</sup>

Observed differences in peak serum levels have mainly been attributed to age-related differences in extracellular body water compartments, which decrease from 45–60% in newborns to approximately 20% in adulthood.<sup>4,7,22,31</sup>

Although RMP is a strong inducer of the cytochrome P450 system, RMP itself is mainly metabolised in the liver by B-esterases.<sup>34,35</sup> Most liver enzymes mature after the first year of life.<sup>31</sup> As the half-life for serum levels and the appearance of RMP in the urine are similar in children and adults, maturation of these two systems probably has only a minor influence on RMP serum levels.<sup>4–6</sup>

The low maximum serum concentrations found in

this study raise further questions regarding the clinical efficacy of RMP at a dosage of 10 mg/kg in childhood TB. In an in vitro study, it was shown that the efficacy of RMP is dependent on concentration rather than time.<sup>36</sup> This was also shown to be valid in vivo by a concentration-dependent drop in colony-forming units of *M. tuberculosis* in the sputum of patients with pulmonary TB as well as in clinical trials with different doses of RMP.<sup>3,37,38</sup> The EBA of a 1200 mg RMP dose was almost double that found at a dose of 600 mg RMP in adults.<sup>39</sup> Clinical outcome, as manifested by a lower rate of sputum conversion and a higher rate of treatment failures, was less good in patients with TB treated with dosages of 450 mg RMP per day than in patients treated with 600 mg per day.<sup>38</sup> RMP serum levels of 6–7 µg/ml, as found among children in our study, correspond to expected serum levels in adults after a dose of only 300–450 mg.<sup>4</sup>

Comparison of study results might be difficult because of the different analytical methods used. In the present study, a microbiological agar diffusion technique was used for the determination of RMP concentration, which was found to yield results that are essentially identical to those of high-performance liquid chromatography used today.<sup>22</sup> The staphylococcal strains used in the microbiological assay in this study are less sensitive to the main active metabolite of RMP, desacetyl-rifampicin, than *M. tuberculosis*. In adults, the metabolite concentrations are about 10% of those of the parent drug, and the total activity against *M. tuberculosis* might therefore be slightly underestimated in our study.<sup>18</sup> The amount of desacetyl-rifampicin of the parent drug in children is not known.

In children as well as in adults, there is considerable intra- and inter-individual variation in RMP serum levels, limiting the significance of data obtained from small study groups.<sup>4,5,15</sup> Taken together, a uniform dosage of 10 mg/kg RMP does not appear to be appropriate in children of all age groups, and especially not in children aged <6 years. In adults, RMP serum levels in continuous treatment are lower than at the beginning of treatment due to self-induction of RMP metabolism.<sup>4</sup> As serum levels in this study in children were measured at the beginning of treatment, it implies that they would be even lower in continuous treatment. It is possible that even a single dose of RMP induces its own metabolism; this could explain in part the lower RMP concentrations in combination therapy, where RMP is given for the second time.

As many physiological functions, including the water compartments at various ages, are proportional to body surface area (BSA), dosing according to body surface might be more appropriate.<sup>40</sup> An RMP dosage of 300 mg/m<sup>2</sup> BSA given to children at the age of 3 months to 2.9 years leads to mean RMP serum levels of 9.1 µg/ml, which is close to the desired serum level of 10 µg/ml.<sup>29</sup> We therefore assumed that efficient



serum levels should be achieved with an RMP dosage in children corresponding to the adult value of 350 mg/m<sup>2</sup> BSA (standard BSA in adults of 1.73 m<sup>2</sup> and a standard RMP dose of 600 mg), although these suggestions need to be validated by further studies. These calculations lead to a dosage of 15 mg/kg RMP in toddlers and young children, decreasing to 10 mg/kg RMP in adolescents.<sup>41</sup> ATS dosage recommendations of up to 20 mg/kg are even higher.<sup>13</sup>

Higher dosages always raise concerns about side effects. The most common side effects of RMP include gastrointestinal intolerance, elevation of liver enzymes and, in intermittent regimens, which are rarely applied to children, a flu-like syndrome.<sup>35,42</sup> RMP also turns urine, stool, sweat and plasma red. In adults, side effects are observed more frequently in doses above 900 mg.<sup>27</sup>

Reports of side effects of RMP in children are scarce. After an intravenous dosage of RMP of 20 mg/kg given to children, adverse effects (mainly cutaneous reactions) were observed frequently (five of nine patients).<sup>42</sup> In standard oral anti-tuberculosis regimens in children, RMP seems to be well tolerated.<sup>37</sup> After the single dose of RMP and EMB, none of the children in our study showed any side effect. In 567 children treated in our department between 1970 and 1980 for TB with a three- or four-drug regimen consisting of RMP, INH, EMB and PZA, the efficacy and toxicity of anti-tuberculosis treatment was evaluated retrospectively.<sup>41</sup> Only minor hepatotoxic side effects corresponding to RMP were found in 1.8% of the children.<sup>41</sup>

## CONCLUSION

According to our study, the currently recommended RMP doses in childhood TB result in lower serum levels than in adults, running the risk of lower clinical efficacy.<sup>11–13,20</sup> Doses based on body surface (350 mg/m<sup>2</sup>) might be more adequate for children. Larger studies are needed to validate these data and to base RMP doses in children on well-founded evidence.

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## R É S U M É

**CONTEXTE :** La rifampicine (RMP) est un médicament essentiel dans le traitement antituberculeux chez les enfants. Selon les recommandations actuelles de l'Organisation mondiale de la Santé, le dosage oral est de 10 (8–12) mg par kg de poids corporel.

**OBJECTIF :** Investiguer les niveaux sériques de RMP chez les enfants après administration orale de RMP seule ou après traitement en combinaison avec l'éthambutol (EMB).

**SCHEMA :** Les niveaux sériques de RMP chez les enfants de différents groupes d'âge ont été déterminés après une administration unique de 10 mg/kg de RMP seule ainsi qu'après sa combinaison avec 35 mg/kg d'EMB.

**RÉSULTATS :** Les niveaux sériques de RMP sont plus

faibles que ceux attendus chez les adultes recevant une dose orale similaire. Les niveaux sériques de RMP dans les traitements en combinaison sont même plus faibles qu'en cas de monothérapie.

**CONCLUSION :** Les dosages de RMP actuellement recommandés pour la tuberculose de l'enfant entraînent des taux sériques plus faibles que ceux recommandés chez les adultes, probablement en raison de différences pharmacocinétiques et pharmacodynamiques chez l'enfant. Chez les enfants, il semble plus valable de calculer la dose de la RMP sur base de la surface corporelle plutôt que sur base du poids corporel, ce qui entraînerait des doses plus élevées, particulièrement chez les jeunes enfants.

## R E S U M E N

**MARCO DE REFERENCIA :** La rifampicina (RMP) es un medicamento esencial en el tratamiento de la tuberculosis (TB) en los niños. Según las recomendaciones vigentes de la Organización Mundial de la Salud, la dosis oral es de 10 mg (8–12 mg) por kilogramo de peso corporal.

**OBJETIVO :** Analizar las concentraciones séricas alcanzadas con la administración oral de RMP sola o asociada con etambutol (EMB) en una población infantil.

**MÉTODOS :** Se determinaron las concentraciones séricas de RMP en niños de diferentes grupos de edad, después de la administración de una dosis única de 10 mg/kg de RMP y de una asociación con 35 mg/kg de EMB.

**RÉSULTADOS :** Las concentraciones séricas de RMP

fueron inferiores a las previstas en adultos que reciben una dosis oral equivalente. Con la administración combinada, las concentraciones de RMP fueron aún más bajas que con la monoterapia.

**CONCLUSIÓN :** Las dosis recomendadas actualmente para los niños en el tratamiento de la TB originan concentraciones séricas inferiores a las recomendadas en los adultos, probablemente en razón de las diferencias de la farmacocinética y la farmacodinamia. Pareciera que en los niños, el cálculo de la dosis de RMP en función de la superficie corporal fuese más adecuado que con respecto al peso ; esto implica pautas posológicas más altas, sobre todo en los niños más pequeños.

## ORIGINAL ARTICLE

**Isoniazid pharmacokinetic studies of the 1960s: considering a higher isoniazid dose in childhood tuberculosis**STEPHANIE THEE<sup>1</sup>, A. ANNE DETJEN<sup>1</sup>, ULRICH WAHN<sup>1</sup> & KLAUS MAGDORF<sup>1,2</sup>*From the <sup>1</sup>Department of Paediatric Pneumology and Immunology, Charité, Universitätsmedizin Berlin, and <sup>2</sup>Department of Paediatric Pneumology and Allergology, Helios Klinikum Emil von Behring, Chest Hospital Heckeshorn, Berlin, Germany***Abstract**

Isoniazid (INH) is one of the most important drugs for the treatment of tuberculosis (TB). In the current international recommendations there is still disagreement on the optimal dosage of INH in childhood TB. This paper presents data from 2 studies, one performed in 1960 and the other in 1961, investigating INH serum levels in children of different age groups, not yet presented internationally. Doses were calculated according to bodyweight (BW) as well as according to body surface area (BSA). In the first study, INH serum levels at different time points in children of different age groups were determined after oral INH administration at 5 mg/kg BW. In the second study, INH serum levels were measured once, 4 h after subcutaneous application of 5 mg/kg BW and once, 4 h after subcutaneous application of INH 200 mg/m<sup>2</sup> BSA 1 week later. These data was compared to adult data on INH collected prior to these studies. After application of 5 mg/kg BW oral dose, INH serum levels were much lower in children than in adults at all time points, especially in children younger than 8 y. In contrast, after dosing according to 200 mg/m<sup>2</sup> BSA, similar serum levels were achieved in children and adults. Dose recommendations of INH 5 mg/kg BW in childhood TB lead to lower serum concentrations than those recommended for adults. In children, it appears to be more appropriate to calculate the INH dose on the basis of body surface area rather than bodyweight.

**Introduction**

Isoniazid (INH) is one of the most important drugs for the treatment of tuberculosis (TB) caused by drug-susceptible Mycobacterium tuberculosis. It is valued for its high early bactericidal activity in the initial phase of treatment, as well as for its important role in preventing drug resistance during the intensive phase of treatment [1]. It is also recommended for preventive chemotherapy of TB infection in children [2].

INH is completely absorbed after oral administration, with peak serum levels after 1–2 h and distributed widely intra- and extracellularly in a compartment corresponding to the total body water [3]. INH is not appreciably bound to plasma protein. It is mainly acetylated in the liver and the small intestine to acetyl-isoniazid. The capacity for acetylation is genetically determined and a trimodality in elimination of INH has been demonstrated [4]. Therefore in humans there are slow, intermediate and fast

acetylators. Only little INH is excreted unmetabolized in the urine.

Although INH has been used for half a century in the treatment of childhood TB, there is still disagreement on the dose recommendations for children, partly due to the limited data on INH pharmacokinetics in children. Very recently the World Health Organization (WHO) increased the recommended daily oral dose of INH in children to 10–15 mg/kg bodyweight (BW), being the same dose recommended by the Centers for Disease Control and Prevention (CDC) [5,6]. Current British Thoracic Society (BTS) guidelines recommend only 5 mg/kg BW for both adults and children [7]. Several studies have suggested higher doses of INH [8–11]. In a recently published study, McIlleron et al. state that younger children require INH doses of 8–12 mg/kg BW to achieve INH concentrations similar to those in adults after a 5 mg/kg BW dose [10].

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In the light of the ongoing discussion, we present data from 2 pharmacokinetic studies performed in the early 1960s at our institute – the Chest Hospital Heckeshorn, Berlin [12,13]. These findings have not previously been accessible to the international medical community.

## Methods

Both studies included previously untreated children diagnosed with pulmonary TB at the Department of Paediatric Pneumology and Allergology, Chest Hospital Heckeshorn, Berlin, Germany. The studies were approved by the Ethics Committee of the Free University Berlin. Parents gave written informed consent for participation. Serum levels were studied after the first and second dose of INH.

After completion of all study measurements, standard multidrug treatment was initiated. At the time of the study the standard combination therapy was INH, para-aminosalicylic acid (PAS) and streptomycin.

Blood samples were centrifuged within 30 min and the serum stored at  $-18^{\circ}\text{C}$ . INH levels were measured by a biological method based on the agar diffusion technique in a modification previously described [14]. The highly sensitive *Mycobacterium tuberculosis* H37Ra was used as indicator strain [14]. At the time of the study this was the standard technique. In contrast to the chemical and radiochemical methods used today, the biological method measured all biologically active INH.

In the first study, 20 children aged between 1 and 13 y were enrolled [12]. Children received a single dose of INH orally at 5 mg/kg BW. Tablets were administered on an empty stomach after an overnight fast. Venipuncture was performed before medication and 1, 2, 4 and 6 h after medication. For analysis, children were separated into 3 age groups: 1 to <4 y ( $n=7$ ), 4 to <8 y ( $n=7$ ) and 8–13 y ( $n=6$ ). In a previous study, data for 24 adults also receiving INH 5 mg/kg were collected at the same institute [12]. The area under the serum concentration-versus-time curve from time 0 to 6 h ( $\text{AUC}_{0-6}$ ) was determined by the linear trapezoidal rule.

In the second study, another 25 children aged between 1 and 13 y were enrolled [13]. In the same children, INH was given subcutaneously at a dose according to bodyweight as well as according to body surface area (BSA). Subcutaneous application was a common form of drug administration in children at the time of the study. In the first part, children received a dose of 5 mg/kg BW and in the second part, after an adequate wash-out time of 1 week, the dose was 200 mg/m<sup>2</sup> BSA. This is based on the standard dosage in adults of 200 mg/m<sup>2</sup>,

which corresponds to a dosage of 5 mg/kg BW in an adult of 70 kg [13]. The children did not receive other anti-tuberculosis treatment during this week. None of the children were acutely ill and all were clinically stable. Again, data from adults ( $n=22$ ) collected in previous studies at our institute were added.

In both parts of the second study (INH dose of 5 mg/kg BW and 200 mg/m<sup>2</sup> BSA), INH serum levels were measured only once, 4 h after medication. The time point of 4 h was chosen because in previous data on INH pharmacokinetics in adults collected at the Chest Hospital Heckeshorn, Berlin, it was shown that inter-individual differences are less after 4 h than at other time points [12]. Also, at the time of the studies the discrimination between slow and fast acetylators of INH was based on the INH serum level 4 h after medication. Genetic determination of acetylator status was not yet available.

## Results

Mean serum levels of INH in children of different age groups as well as adults after oral intake of 5 mg/kg BW at different time points are shown in Figure 1; the corresponding  $\text{AUC}_{0-6}$  are shown in Table I. The curve progression in adults and children showed similar results, but INH serum levels were much lower in children than in adults at all time points, especially in children younger than 8 y. Accordingly, the AUC for 0–6 h was much smaller in younger children. As only the mean values of the INH serum levels were still available for this presentation, an analysis for statistical significance could not be made.

Table II shows the mean INH serum levels 4 h after subcutaneous medication. In comparison to adults, INH serum levels in children after dosing 5 mg/kg BW were only about half of the values found in adults after the same per kg dose. In contrast, after

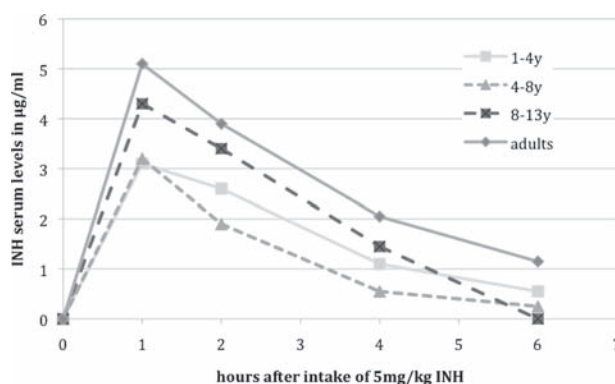


Figure 1. Mean serum levels of isoniazid (INH; µg/ml) after oral intake of 5 mg/kg bodyweight in adults and children of different age groups.



Table I. Area under the curve (AUC, µg/h/ml) after oral medication of isoniazid 5 mg/kg bodyweight.

Age	1–4 y (n=7)	4–8 y (n=7)	8–13 y (n=6)	Adults (n=24)
AUC <sub>0–6</sub>	9.75	7.4	13.2	16.2

dosing 200 mg/m<sup>2</sup> BSA, similar serum levels were achieved in children as in adults.

Figure 2 shows a dosing curve based on our calculations of 200 mg INH/m<sup>2</sup> BSA for children weighing up to 50 kg.

**Discussion**

The presented data from 2 previous studies performed in 1960 and 1961 show that serum levels of INH in children after oral or subcutaneous dosing according to BW are lower than in adults receiving the same per kg dose. Similar serum levels in children and adults can be achieved if the paediatric dose is calculated according to BSA (200 mg/m<sup>2</sup> BSA).

As can be seen in Figure 1, INH serum levels in children after an oral dose of 5 mg/kg BW INH are lower than in adults at all time points measured. The mean maximum serum concentration is reached in all groups after 1 h and conforms to previous reports [3,15].

If INH is given subcutaneously at the same mg/kg dose, INH serum levels also differ between children and adults. There are no further data comparing INH pharmacokinetic parameters after oral and subcutaneous dosing. However, in the study of Olson et al., after intramuscular administration of INH in children, nearly equivalent plasma concentrations to the oral dose were reached [15]. Nevertheless, some uncertainty remains regarding whether or not subcutaneous and oral application of INH lead to the same serum levels, as large studies comparing these

2 application forms have never been performed. Due to INH acetylation in the gut wall, INH serum levels after oral intake might be lower than after parenteral medication [3]. In the 2 study groups presented, the 4 h INH serum levels were similar whether INH was given orally or subcutaneously.

Lower INH serum levels in children compared to adults have been described previously [8,10,11]. They have been explained by the larger liver mass relative to whole bodyweight in children, leading to a higher hepatic metabolic capacity than in adults [8,11]. In infants the metabolism of INH is further influenced by the ongoing maturation of hepatic and renal functions. Furthermore, the body compartments grow at different rates, resulting in different volumes of distribution between children and adults. Younger children have a larger volume of distribution of INH in comparison to older children and adults [3,8,10]. INH distributes in a compartment comparable to total body water. During growth, the proportion of total body water to bodyweight changes from 70% at birth to 50% in adulthood [16,17]. As total body water closely correlates to BSA, INH dosing according to BSA has been suggested [10,18,19].

The presented data show that after subcutaneous application, INH serum levels comparable to adults can be achieved in young children if the INH dose is calculated according to BSA rather than BW. However, these calculations might not always be convenient in daily practice, especially under conditions often found in developing countries. On the basis of our calculations using BSA, we created a graph on which the INH dose for each BW can simply be read off (Figure 2) [19]. This corresponds approximately to a dose of 8–10 mg/kg BW in children aged 0–5 y, 7–8 mg/kg BW for age 6–9 y, 6–7 mg/kg BW for age 10–14 y and 5–6 mg/kg BW for age 15–18 y. These calculations are based on data after subcutaneous

Table II. Mean isoniazid levels 4 h after subcutaneous isoniazid medication according to bodyweight (5 mg/kg) and body surface area (200 mg/m<sup>2</sup>).

Age	n	Mean INH levels 4 h after subcutaneous medication with:	
		5 mg/kg BW	200 mg/m <sup>2</sup> BSA
1–13 y (all children)	25	0.99	1.82
1–4 y	2	- <sup>a</sup>	- <sup>a</sup>
4–8 y	13	1.10	1.93
8–13 y	10	1.14	1.99
adults	22	2.05	2.05

INH, isoniazid; BW, bodyweight; BSA, body surface area.

<sup>a</sup>Data not calculated.

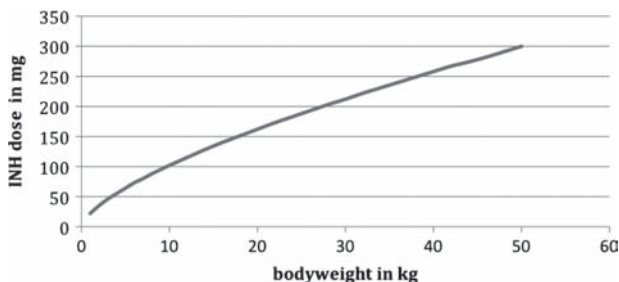


Figure 2. Isoniazid (INH) dose according to 200 mg INH/m<sup>2</sup> body surface area for children up to 50 kg bodyweight. (As no difference in serum levels could be found after oral and subcutaneous application of INH 5 mg/kg, we adapted these calculations based on data after subcutaneous application of INH 200 mg/m<sup>2</sup> to the oral route.)

application of INH. In our studies in children, the INH serum levels after oral and subcutaneous application were similar. We therefore assume that these calculations are valid for oral intake of INH as well. It has also previously been shown for ethambutol that dosing according to BSA is more appropriate in childhood tuberculosis [20]. Recently it has been proposed that weight to the power of  $\frac{3}{4}$  should be used for calculating paediatric drug doses [21]. It remains to be shown whether these calculations lead to INH serum levels equivalent to those achieved in adults.

Studies indicate a dose-related response to INH with a maximum early bactericidal activity at a dose of 300 mg (4–6 mg/kg BW) in adults [22]. According to extensive pharmacokinetic studies in adults, the proposed INH peak concentration after oral intake is 3–5 µg/ml [23–25]. In the presented study, this is barely achieved in children younger than 8 y after oral intake of INH 5 mg/kg BW.

A microbiological method was used for the determination of INH serum levels. This method might overestimate INH concentrations because both INH and metabolites with anti-TB activity were measured [3,14,26]. In the presented investigations INH metabolites were not measured separately. Hence, INH serum levels in children younger than 8 y after an oral dose of 5 mg/kg BW might actually be lower than indicated by these presented studies.

INH is mainly eliminated through acetylation by the N-acetyltransferase 2 (NAT2) enzyme system, which shows a trimodal distribution [3,4,11]. Individuals may be homozygotic fast, heterozygotic fast or homozygotic slow acetylators of INH, also leading to differences in INH exposure in children [4,11]. In homozygotic fast acetylators, lower INH serum levels are achieved than in slow acetylators. The time of exposure to therapeutic serum levels is shorter in fast acetylators due to the faster elimination of INH [11]. At the time of the presented studies, determination of NAT2 genotype was not available and a phenotypical differentiation of the acetylator status was not performed in this study group.

Whether the acetylator status should be taken into consideration for dosing of INH in adults as well as in children is still part of the ongoing discussion [10,27].

Limitations of the presented studies are the small number of patients in which serum concentrations were measured. Especially in young infants who have potentially important differences in physiology to older children (e.g. maturation of metabolic enzymes), the number of patients was limited. The investigations were conducted nearly 50 y ago and some of

the patient data necessary for statistical analysis are no longer available. Nevertheless, this data contributes to our knowledge of INH pharmacokinetics in childhood TB and to the ongoing discussion on dose regimens. Due to the small number of pharmacokinetic studies on INH in childhood, an agreement on INH dose has still not been reached. More data are urgently needed for children across ranges of age, weight and body composition in order to establish the superiority of surface area-based dosing for the oral route.

## Conclusions

In children younger than 8 y an INH dose of 5 mg/kg BW, as recommended by some international authorities, given either orally or subcutaneously, leads to lower serum levels than in adults receiving the same per kg dose [6,7,12,13]. The efficacy of maximum serum levels of 3–5 µg/ml in adults has been shown in extensive studies [22–24]. With an INH dose of 200 mg/m<sup>2</sup> BSA (in this study given subcutaneously), serum levels comparable to those in adults are also achieved in younger children.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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# Hearing loss in children treated for multidrug-resistant tuberculosis

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## KEYWORDS

Hearing;  
 Audiology;  
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**Summary Objective:** The aminoglycosides and polypeptides are vital drugs for the management of multidrug-resistant (MDR) tuberculosis (TB). Both classes of drug cause hearing loss. We aimed to determine the extent of hearing loss in children treated for MDR-TB.

**Methods:** In this retrospective study, children (<15 years) admitted to Brooklyn Chest Hospital, Cape Town, South Africa, from January 2009 until December 2010, were included if treated for MDR-TB with injectable drugs. Hearing was assessed and classified using audiometry and otoacoustic emissions.

**Results:** Ninety-four children were included (median age: 43 months). Of 93 tested, 28 (30%) were HIV-infected. Twenty-three (24%) children had hearing loss. Culture-confirmed, as opposed to presumed, diagnosis of TB was a risk factor for hearing loss (OR: 4.12; 95% CI: 1.13–15.0;  $p = 0.02$ ). Seven of 11 (64%) children classified as having hearing loss using audiometry had progression of hearing loss after finishing the injectable drug.

**Conclusions:** Hearing loss is common in children treated for MDR-TB. Alternative drugs are required for the treatment of paediatric MDR-TB.

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**Abbreviations:** MDR, multidrug-resistant; DR, drug-resistant; TB, tuberculosis; WHO, World Health Organization; PTA, pure tone audiometry; OAE, otoacoustic emission; DPOAE, distortion product otoacoustic emission; BCH, Brooklyn Chest Hospital; IM, intramuscular; ASHA, American Speech and Hearing Association; OR, odds ratio; CI, confidence intervals; IQR, inter-quartile range.

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## Introduction

Multidrug-resistant (MDR) tuberculosis (TB) is an evolving global challenge with the World Health Organization (WHO) estimating there to be over 650,000 prevalent cases of MDR-TB in 2010.<sup>1</sup> MDR-TB is caused by *Mycobacterium tuberculosis* resistant to at least isoniazid and rifampicin.<sup>2</sup> As paediatric TB constitutes 15–20% of all TB cases in high burden settings,<sup>3,4</sup> a large number of children are likely to be affected by MDR-TB. Due to the paucibacillary nature of the pathology, few cases of paediatric MDR-TB have traditionally been accurately diagnosed or appropriately treated, but with the imminent roll-out of molecular diagnostic tests,<sup>5,6</sup> these numbers are likely to rise. In addition to the difficulties in diagnosis, treatment is frequently required for greater than twelve months and is associated with significant adverse effects.<sup>7–9</sup>

The aminoglycosides (amikacin and kanamycin), together with capreomycin (a polypeptide), are classified as group two drugs by WHO. These injectable second-line agents are vital for the management of MDR-TB.<sup>2</sup> Although strains resistant to rifampicin but susceptible to isoniazid can be treated with slightly less intense regimens, these rifampicin mono-resistant (RMR) cases are usually treated as MDR-TB in most National TB Programmes. This is due to the limitations of modern molecular diagnostic tests which either do not test for isoniazid resistance<sup>10</sup> or miss a significant proportion of cases which have phenotypic resistance.<sup>11</sup> In most circumstances rifampicin resistance is seen as a surrogate for multidrug resistance.

Both the aminoglycosides and polypeptides are known to have adverse effects that include renal and eighth cranial nerve impairment.<sup>12–14</sup> The effects on the kidneys are thought to be temporary but those on the vestibulo-cochlear system are permanent.<sup>15,16</sup> Hearing loss related to injectable TB drug use usually starts in the high frequencies and if treatment continues, there is progression to the lower frequencies required for communication; however, in some cases severe hearing loss can develop acutely. Hearing is vital not only for effective communication but also for neurological development. Children with hearing deficits have delayed developmental and communication milestones compared to children with normal hearing.<sup>17–19</sup>

Hearing testing for children is performed for two reasons. The first is to identify and quantify hearing loss to enable the provision of support, education, training and hearing aids. The second is to identify hearing loss early, when it is mild and only at high frequencies, so that treatment, where possible, can be changed to prevent further damage. The testing of hearing is challenging in children. Pure tone audiometry (PTA) is the method of choice for testing adults and allows the testing of different frequencies and amplitudes in both ears independently.<sup>20</sup> PTA is only possible in children on therapy who are able to understand commands and co-operate with testing, which effectively precludes its use in children younger than five years. As young children are at high risk of developing TB following infection and as young children bear the brunt of the epidemic in many settings,<sup>21</sup> this means that many children are excluded from this form of testing. Auditory brainstem response (ABR) testing is the optimal testing methodology for young children<sup>22</sup> but is only available in

South Africa in specialist centres. Otoacoustic emission (OAE) testing can assess cochlear patency in younger children and is widely available. OAEs are not fully validated for quantifying hearing loss and do not provide as comprehensive an assessment as PTA or ABR. Although the technology is improving for OAEs, with newer tests able to provide diagnostic evaluations, due to the difference in testing methodology it is not possible to directly compare OAE and PTA. In some studies correlation has been shown to be good between the two types of testing in children,<sup>23</sup> but in others, significant discrepancies are seen.<sup>24</sup> OAE in South Africa is currently used as a screening test.

The frequency and severity of hearing loss is unknown in children treated for MDR-TB with injectable medications. Some data are available for children given these injectable drugs as short antibiotic courses for the treatment of other bacterial infections.<sup>13,25</sup> Some data regarding ototoxicity are available for adults treated for MDR-TB,<sup>26</sup> but few studies have examined the adverse effects of injectable drugs in children treated for MDR-TB. The aim of this study was to determine the frequency and extent of hearing loss in children treated with an aminoglycoside or polypeptide as part of an MDR-TB regimen.

## Methods

### Setting and standard of care

The Western Cape Province of South Africa had a TB notification rate of 976 per 100,000 in 2009.<sup>27</sup> Amongst children routinely diagnosed with culture-confirmed TB at a tertiary hospital in the Province, 8.9% were identified as MDR.<sup>28</sup> Children with MDR- and RMR-TB present to various regional health centres but once diagnosed and stabilized all children requiring injectable TB medications are transferred to Brooklyn Chest Hospital (BCH). BCH is a specialist TB hospital with a sixty bed paediatric capacity.

MDR-TB (or RMR-TB) is diagnosed as confirmed or presumed disease. A confirmed diagnosis is made when *M. tuberculosis* is isolated using liquid culture with demonstrated resistance.<sup>28</sup> A presumed diagnosis is made if the child had symptoms, signs and/or radiology highly suggestive of TB in the presence of either a drug-resistant source case or when the child was failing first-line TB therapy. Routine hearing testing for children treated with injectable TB medications was introduced in 2008. Children are assessed prior to starting injectable drugs and then monthly. If there are challenges to testing or if abnormalities are found, testing is carried out every two weeks. Where hearing loss is determined, treatment with the injectable medication is (if possible without compromising treatment efficacy) stopped or switched to an alternative medication. Children with severe hearing loss are referred for hearing aids and educational support. Children are treated for MDR- and RMR-TB with amikacin (20 mg/kg once daily via intramuscular [IM] injection) for between four and six months. Children treated for isolates resistant to amikacin are treated with capreomycin (20 mg/kg once daily IM) or streptomycin (20 mg/kg once daily IM) dependent on drug susceptibility test results. Amikacin is used in preference to kanamycin due to the availability of

smaller ampoule size available (less wastage), its lower minimal inhibitory concentration compared to kanamycin and capreomycin, and the anecdotal impression that injections are less painful.

### Study population

This retrospective study included all children routinely admitted to BCH from January 2009 until December 2010, aged 0–15 years, if they had been a) diagnosed with confirmed or presumed MDR- or RMR-TB, were b) treated with an injectable TB drug for at least a month, and c) had received at least one audiological assessment.

### Audiological assessments

Children were assessed using a combination of otoscopy, tympanometry, PTA (including conditioned play audiometry) and/or distortion product otoacoustic emissions (DPOAEs). Otoscopy was used to ensure that there were no anatomical abnormalities and that the external ear canal was clear of occluding wax, foreign bodies or obstruction. A Welch Allyn 262 tympanometer (MFI Medical Equipment Inc. San Diego, USA) was used to assess middle ear function using a 226 Hz probe tone. The probe was placed into the child's ear canal ensuring a tight seal with no leakage. Static compliance between 0.2 and 1.8 cm<sup>3</sup>, middle ear pressure between +100 and –150 dekapascals, and ear canal volume of 0.2–2.0 cm<sup>3</sup> were used. If a type B tympanogram (indicating possible middle ear infection) was noted, the audiologist would notify the attending physician. A five day course of oral antibiotics was usually prescribed before reassessment. If the problem persisted, the child was referred to the ear, nose and throat team.

PTA was performed in a sound-proof booth with calibrated equipment. The AC40 dual channel audiometer (Interacoustics, Assens, Denmark) and the MA51 audiometer (MAICO Diagnostics GmbH, Berlin, Germany) were used. Pure tone air conduction hearing thresholds were obtained for children between six and fifteen years of age, for each ear by testing the octave bands from 250 Hz to 8 kHz. Audiologists followed the modified Hughson-Westlake procedure<sup>29</sup> (i.e. 10 dB down, 5 dB up, repeated twice to reliably determine hearing threshold). Stimuli were presented in the following order: 1 kHz, 2 kHz, 4 kHz, 8 kHz, repeated at 1 kHz, then 500 Hz and 250 Hz. If there was a difference of 20 dB between consecutive frequencies the audiologist would test half octave frequencies, i.e. 750 Hz, 1.5 kHz, 3 kHz and 6 kHz. For participants younger than six years, either conditioned play audiometry or DPOAE were performed. For descriptive purposes we considered thresholds of <25 dB as normal, 26–40 dB as mild, 41–55 dB as moderate, 56–70 dB as moderately severe, 71–90 dB as severe and >90 dB as profound hearing impairment.<sup>30,31</sup>

DPOAEs were obtained using an OtoRead™ machine (Interacoustics, Assens, Denmark). A rubber-tipped probe was placed in the external ear canal to create a tight seal. Two simultaneous pure tone signals were then presented to each ear at two different primary frequencies ( $f_1$  and  $f_2$ , where  $f_2 > f_1$ ) with  $f_1:f_2$  ratio of 1.22 and an intensity of 65 dB Sound Pressure Level (SPL) and 55 dB SPL

respectively. Frequencies 2 kHz, 4 kHz, 6 kHz, 8 kHz, 10 kHz and 12 kHz were tested. In order for a child to pass the DPOAE, the emission amplitude needed to be 6 dB or greater above the noise floor. If a child was unable to be tested for any reason, or if the test was abnormal, they were re-tested two weeks later. If the child passed the DPOAE, then they were assessed monthly. DPOAE results were classified as pass, fail or unable to test.

### Data collection

BCH admission records were reviewed to identify all patients treated for MDR- and RMR-TB over the study period. Records were compared with data from the audiology department to determine which of the patients had received audiological testing. Clinical records were reviewed to determine the dosage and duration of injectable treatment, demographic and clinical details, as well as audiological and laboratory data.

### Data classification and analysis

We drew a distinction between hearing deficit and hearing loss. Hearing deficit describes the absolute impairment in hearing experienced by a child at treatment completion whereas hearing loss is a measured deterioration in hearing function between two assessments. Children could therefore have hearing deficit at the end of treatment but if previous assessments were not carried out, hearing loss could not be determined. Conversely, it was possible for children to have hearing deficit at the beginning and at the end of treatment, but to experience no hearing loss between assessments.

Hearing deficit assessed by PTA was classified as, at the last hearing assessment, a threshold of greater than or equal to 25 dB at any tested frequency, in the presence of normal tympanograms. When testing using OAEs, a classification of hearing deficit was made if the child failed the assessment in the presence of normal tympanograms. When assessed using PTA, hearing loss was classified according to the American Speech and Hearing Association (ASHA) guidelines: a) an increase in pure tone thresholds of greater than or equal to 20 dB at any one test frequency, b) an increase of greater than or equal to 10 dB at any two adjacent test frequencies, or c) a loss of three consecutive frequencies.<sup>20,32,33</sup> A diagnosis of hearing loss using OAE was made if the child failed the assessment in the presence of normal tympanograms having passed a previous assessment. The classification of hearing deficit and hearing loss used is shown in Table 1.

Risk factors for hearing loss were determined by comparing the frequency or mean/median value for children with hearing loss (determined by both PTA and OAE) vs. children without. Chi square (or Fisher's Exact) tests, student *t*-tests or Mann Whitney tests were used; odds ratios (OR) and 95% confidence intervals (CIs) calculated.

### Ethical considerations

Ethical approval for this retrospective study was obtained from the Ethics Committees of Stellenbosch University

**Table 1** Classification of hearing deficit and hearing loss using otoacoustic emissions and pure tone audiometry.

		Otoacoustic emissions	Pure tone audiometry
Hearing deficit	No hearing deficit	<ul style="list-style-type: none"> <li>• A normal OAE in the last month of therapy or after completing injectable medication</li> </ul>	<ul style="list-style-type: none"> <li>• All frequencies better than 25 dB in the last month of or after completing injectable medication with no subsequent abnormal tests</li> </ul>
	Hearing deficit	<ul style="list-style-type: none"> <li>• An abnormal OAE in the presence of normal tympanograms</li> </ul>	<ul style="list-style-type: none"> <li>• Any frequency greater than 25 dB at any point before, during, or after therapy in the presence of normal tympanograms</li> </ul>
	Unable to classify	<ul style="list-style-type: none"> <li>• Abnormal tympanograms</li> <li>• Unable to test child due to noise or child unable to co-operate</li> <li>• Normal OAE but performed before the last month of therapy</li> </ul>	<ul style="list-style-type: none"> <li>• Abnormal tympanograms</li> <li>• Unable to test child due to noise or child unable to co-operate</li> </ul>
Hearing loss	No hearing loss	<ul style="list-style-type: none"> <li>• A normal OAE in the last month of therapy or after completing injectable medication</li> </ul>	<ul style="list-style-type: none"> <li>• A normal PTA (all frequencies better than 25 dB) in the last month of or after completing injectable medication with no subsequent abnormal tests or</li> <li>• No significant deterioration (as determined by ASHA criteria)<sup>1</sup> between an audiogram performed before or within the first month of therapy and one performed after within the last month of therapy with no subsequent deterioration</li> </ul>
	Hearing loss	<ul style="list-style-type: none"> <li>• A normal OAE documented before or during therapy followed by an abnormal OAE in the presence of normal tympanograms</li> </ul>	<ul style="list-style-type: none"> <li>• A significant deterioration (as determined by the ASHA criteria)<sup>1</sup> between an audiogram performed before or during therapy and one performed later during therapy or after completing therapy in the presence of normal tympanograms</li> </ul>
	Unable to classify	<ul style="list-style-type: none"> <li>• Normal final OAE but performed before the last month of therapy</li> <li>• Abnormal tympanograms</li> <li>• Abnormal OAE throughout therapy</li> <li>• Unable to test child due to noise or child unable to co-operate</li> </ul>	<ul style="list-style-type: none"> <li>• An abnormal audiogram without an earlier audiogram for comparison</li> <li>• A normal final audiogram (all frequencies better than 25 dB) before the last month of therapy</li> <li>• Abnormal tympanograms</li> <li>• Unable to test child due to noise or child unable to co-operate</li> </ul>

OAE: otoacoustic emission; PTA: pure tone audiometry; ASHA: American Speech and Hearing Association.

(which oversees paediatric research at BCH) and the London School of Hygiene and Tropical Medicine.

## Results

### Patient characteristics

Ninety-four children were included in the study from 113 who were started on injectable treatment for MDR-TB (Fig. 1). Median age was 43 months (inter-quartile range [IQR]: 20–110). Forty-five (48%) were boys and 30 (32%) had evidence of extrapulmonary TB. Children were generally malnourished with weight-for-age z-scores a mean of 1.48 standard deviations below the reference mean and median body mass index of 15.5 kg/m<sup>2</sup> (IQR: 14.5–17.3). Fifty-two (55%) had a culture-confirmed diagnosis and the majority (62 children; 66%) were treated for MDR-TB. The other children either had disease with more extensive resistance or were started on treatment for MDR-TB but were later confirmed to have less resistant organisms. Twenty-eight children (out of 93 tested; 30%) were HIV-infected

of which 20 (71%) were already on antiretroviral therapy at the start of TB treatment. Most children ( $n = 82$ ; 87%) were treated with amikacin (Table 2).

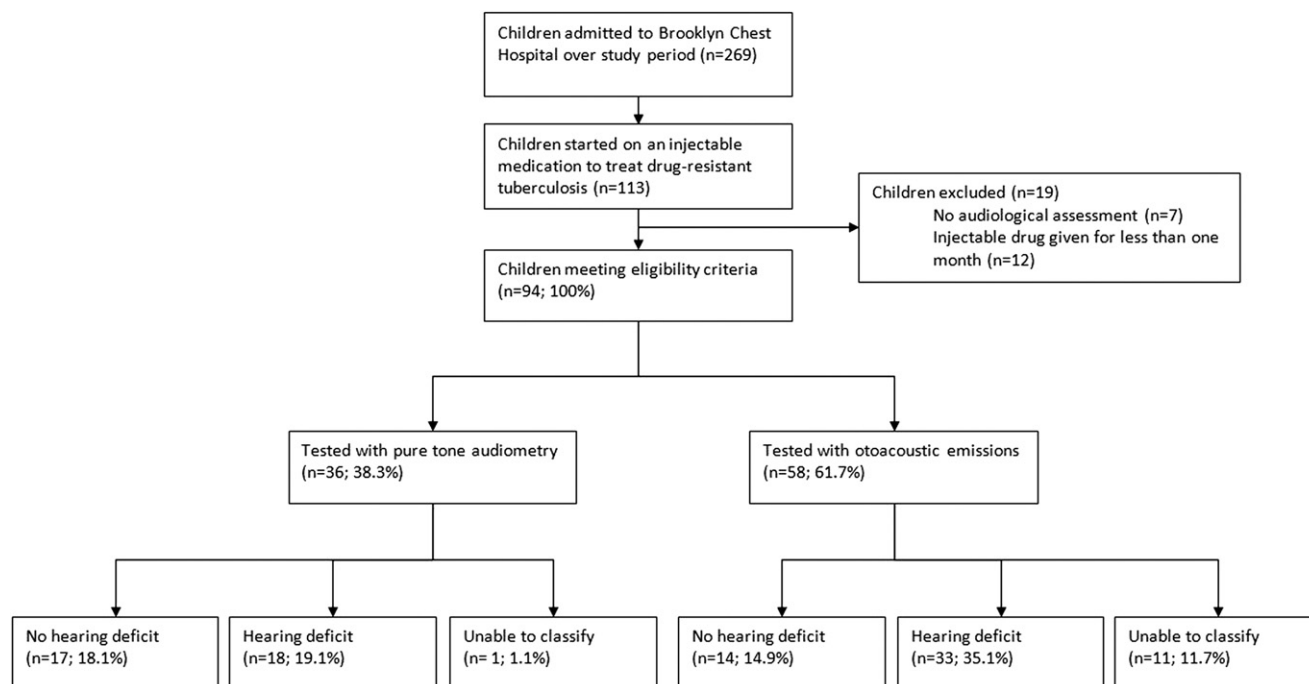
### Audiological testing

Thirty-six children were assessed using PTA and 58 assessed using OAEs. Hearing deficit is demonstrated in Fig. 1, and hearing loss in Fig. 2. When combining results of both PTA and OAE testing, 23 (24%) children had hearing loss and 27 (29%) had normal hearing. Forty-four (47%) children could not be classified using this approach. In 7 of the 11 children who had hearing loss determined by PTA (Table 3), hearing loss progressed even after the injectable medication was discontinued. In no child who had been shown to have hearing loss did his/her hearing improve on subsequent testing.

### Assessment of risk factors for hearing loss

A culture-confirmed diagnosis of TB (OR: 4.12; 95% CI: 1.13–15.0;  $p = 0.02$ ) was significantly associated with





**Figure 1** Hearing deficit in children treated for multidrug-resistant tuberculosis with second-line injectable drugs. Percentages calculated from the number of children meeting eligibility criteria ( $n = 94$ ).

hearing loss (Table 4). There was a trend towards the median duration of injectable antibiotic use being longer in children with hearing loss: (164 days; IQR: 119–184 vs. 123; IQR: 70–183;  $p = 0.07$ ).

## Discussion

We have demonstrated that both hearing deficit and hearing loss are common in children treated for MDR-TB. The association between hearing loss and culture-confirmed TB disease may reflect the extent or severity of disease and might suggest that treating clinicians are more likely to continue injectable drug use in children with extensive pathology. However, it is also possible that some aspect of having severe disease, such as changes in inflammation, altered immune response or differing drug absorption and elimination may contribute to hearing loss. Since we aimed to describe children with definitive hearing loss or normal hearing, we developed a classification system which precluded the accurate classification of a relatively large number of children. However, despite our conservative estimates, over half of the children had hearing deficit at the end of therapy and a quarter of children experienced hearing loss.

In addition to documenting the risk and degree of hearing loss in children treated for MDR-TB, our study highlights some of the challenges in the assessment of hearing in children, including the classification of hearing deficit and hearing loss. Hearing testing is partially subjective, requires relatively sophisticated equipment, trained staff and co-operative patients. Elements of the frequency (pitch), amplitude (volume), laterality (unilateral or bilateral) and aetiology (sensorineural, conductive or both) need to be considered; all of these factors need to

be monitored longitudinally and change classified. We adhered to the established ASHA criteria to classify whether hearing loss occurred between two PTA assessments. However, we developed a classification system to determine whether children in this study should be classified as having hearing loss or not. This lack of established existing criteria limits meaningful comparisons between different studies.

Several studies have documented the treatment of MDR-TB, mainly in adults; only a handful have systematically assessed hearing loss and analysed risk factors for ototoxicity. De Jager et al. found no association between clinical or treatment factors and the incidence of hearing loss.<sup>34</sup> Peloquin et al. assessed whether the size and frequency of dosage affected hearing loss and found no association, but demonstrated that older age and cumulative dose were associated with an increased risk.<sup>35</sup> Sturdy et al. found that impaired renal function, older age and the use of amikacin were associated with hearing loss in adults treated for MDR-TB.<sup>36</sup> A number of studies describe cohorts including small numbers of children but few have included those less than ten years of age. Only two previous paediatric studies examine the adverse effects of children on treatment for MDR-TB. The first describes 38 children treated in Peru; 30 underwent hearing assessments.<sup>8</sup> The testing methodology and classification was not specified; audiology testing was undertaken in children receiving an injectable for more than six months. Two children were found to have mild, high-frequency, conductive hearing loss. The second was carried out in Lesotho where six out of 19 (32%) children developed hearing loss.<sup>37</sup> Studies of short courses of aminoglycoside use in neonates<sup>38</sup> and children with cystic fibrosis<sup>39</sup> demonstrate limited toxicity but assessment of hearing loss in children receiving longer courses of aminoglycosides following liver transplantation



**Table 2** Demographic and treatment data in children treated for multidrug-resistant tuberculosis ( $n = 94$ ).

Characteristic	Number (% unless indicated otherwise)
Median age in months (IQR)	43 (20–110)
Male gender	45 (47.9)
Type of TB	
	Pulmonary
	64 (68.1)
	Extrapulmonary
	17 (18.1)
	Both extrapulmonary and pulmonary
	13 (13.8)
Site of extrapulmonary TB ( $n = 30$ )	
	Miliary
	1 (3.3)
	Pleural effusion
	2 (6.7)
	TB meningitis
	8 (26.7)
	Abdominal TB
	4 (13.3)
	Lymph node TB
	6 (20.0)
	Musculoskeletal TB
	9 (30.0)
Median weight in kg (IQR)	13.5 (10.1–21.2)
Median height/length in cm (IQR) ( $n = 90$ )	93 (78–121)
Median MUAC in cm (IQR; $n = 83$ )	15.3 (14–17)
Mean weight for age z-score (SD)	–1.48 (1.55)
Median BMI (IQR)	15.5 (14.5–17.3)
Certainty of TB diagnosis	
	Culture-confirmed
	52 (55.3)
	Presumed
	42 (44.7)
DST of child or source case if diagnosed presumptively	
	DS <sup>a</sup>
	1 (1.1)
	HMR <sup>a</sup>
	2 (2.1)
	RMR
	11 (11.7)
	MDR
	62 (66.0)
	Pre-XDR
	16 (17.0)
	XDR
	2 (2.1)
HIV-infected ( $n = 93$ )	28 (30.1)
On ART prior to TB diagnosis ( $n = 28$ )	20 (71.4)
Type of injectable drug given	
	Amikacin
	82 (87.2)
	Capreomycin
	9 (9.6)
	Streptomycin
	1 (1.1)
	Two or more injectables
	2 (2.1)
Mean dose of injectable drug (mg; SD)	320 (189)
Mean dose of injectable drug (mg/kg; SD)	19.4 (2.04)
Mean duration of injectable drug uses (days; SD)	136.2 (51.6)

IQR: inter-quartile range; TB: tuberculosis; MUAC: mid upper arm circumference; BMI: body mass index; DST: drug susceptibility testing; HIV: human immunodeficiency virus; ART: antiretroviral therapy; DS: drug-susceptible; HMR: isoniazid-monoresistant; RMR: rifampicin-monoresistant; MDR: multidrug-resistant; XDR: extensively drug-resistant; Pre-XDR-TB: MDR-TB with additional resistance to either a fluoroquinolone or an injectable medication but not both.

Confirmed diagnosis: *M. tuberculosis* isolated from child with resistance demonstrated.

Presumed diagnosis: child treated for MDR-TB due to a clinical diagnosis of TB and either contact with an MDR-TB source case or following failure of first-line therapy.

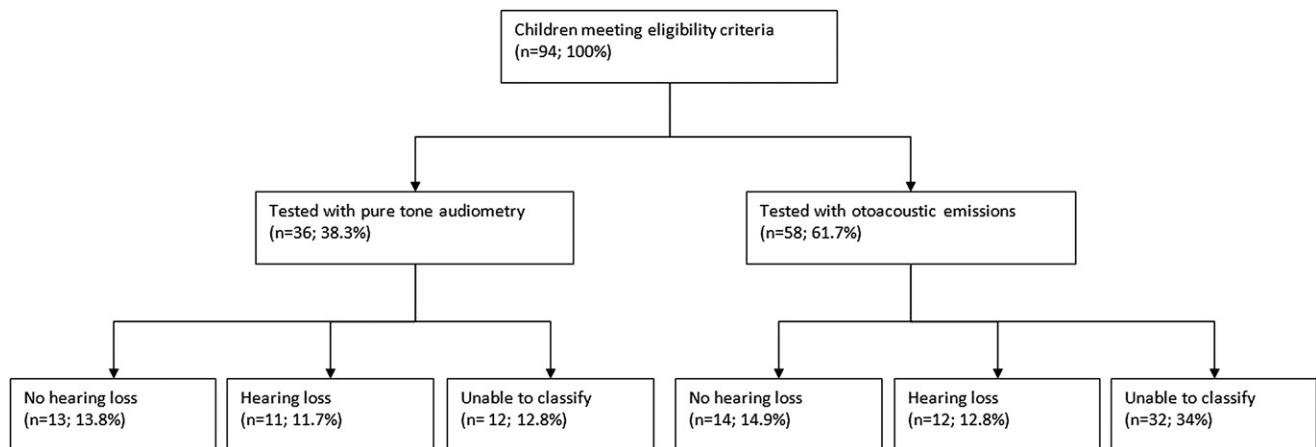
<sup>a</sup> These three children were started on treatment for MDR-TB due to contact with an MDR-TB source case but were subsequently found to have DS- or HMR-TB.

found hearing loss in 15 of 66 children evaluated, using a 35 dB loss at one frequency to define hearing loss.<sup>40</sup>

Hearing has particular relevance in children since they need hearing to develop skills and acquire language. The primary means of education is through oral teaching. Hearing loss during childhood can therefore have profound implications for development.<sup>17–19,41–44</sup> If ototoxicity is identified early, rapid intervention can be implemented.<sup>45,46</sup>

Our study has a number of strengths and limitations. We report the largest study to date documenting hearing loss in children treated for MDR-TB and assess risk factors for hearing loss using a robust classification system. We report on hearing loss resulting from care provided under routine,

programmatic conditions. The retrospective nature of the study limited systematic data collection; therefore some audiological assessments were missing, irregular or incomplete. Clinical parameters were determined from routine data and were incomplete in some instances. One particular example was the lack of information regarding previous TB treatment which may have influenced hearing loss. Our findings may not be representative of all children treated for MDR-TB since we report on children admitted to hospital. Also, as so few children were treated with drugs other than amikacin, it was not possible to assess the relative toxicity of different drugs. Finally, we were unable to classify and analyse a considerable number of children due to the rigorous classifications used and we did not have



**Figure 2** Hearing loss in children treated for drug-resistant tuberculosis with second-line injectable drugs ( $n = 94$ ).

pharmacokinetic data for children on the injectable drugs to correlate with toxicity.

Despite these factors, we document that hearing loss occurs in at least a quarter of children treated with

a second-line injectables for MDR-TB. Clinicians should give careful consideration to the use of injectable medications. Children should be screened prior to beginning injectable medications with either PTA or OAE (dependent

**Table 3** Characteristics of children treated for multidrug-resistant tuberculosis with hearing loss determined using pure tone audiometry ( $n = 11$ ).

Age	Gender	HIV status	DST	Diagnosis	Treatment	Hearing loss
15 yr	Girl	Neg	RMR	Confirmed abdominal TB	2 months amikacin	Unilateral severe high frequency hearing loss at the first assessment carried out one month after the start of treatment. One month later bilateral severe high frequency hearing loss so injectable stopped. No further hearing loss.
10 yr	Girl	Pos	MDR	Confirmed PTB	5 ½ months amikacin	Normal hearing at baseline and at monthly intervals whilst on therapy. Moderately severe high frequency hearing loss detected two months after completing injectable treatment.
5 yr	Girl	Neg	MDR	Confirmed PTB	6 months amikacin	Normal hearing at baseline and throughout therapy. At the end of therapy found to have unilateral moderate high frequency hearing loss.
10 yr	Boy	Neg	MDR	Confirmed LN TB	6 months amikacin	Normal hearing at baseline and throughout therapy. At the end of therapy found to have moderately severe unilateral high frequency hearing loss. A further month later found to have bilateral moderately severe high frequency loss.
10 yr	Girl	Neg	MDR	Confirmed PTB	6 months amikacin	Normal hearing at baseline and throughout therapy. Two months after completing therapy found to have unilateral high frequency moderate loss.
12 yr	Boy	Pos	MDR	Confirmed PTB	8 months amikacin	Normal hearing at baseline. After four months found to have unilateral moderate high frequency loss, progressing to severe unilateral high frequency loss by the end of therapy and to bilateral high frequency loss, severe in one ear and moderate in the other by 4 months after finishing.
13 yr	Boy	Pos	MDR	Confirmed PTB	6 months amikacin	Normal hearing at baseline and monthly throughout treatment. At end of therapy found to have bilateral moderately severe high frequency loss.

(continued on next page)

Table 3 (continued)

Age	Gender	HIV status	DST	Diagnosis	Treatment	Hearing loss
8 yr	Girl	Pos	MDR	Confirmed PTB	2 ½ months amikacin	Normal hearing at first assessment one month after starting therapy. After two months found to have bilateral moderately severe high frequency loss. After stopping the injectable, hearing loss progressed to severe bilateral hearing loss affecting all frequencies. Hearing aid required.
3 yr	Boy	Neg	MDR	Confirmed LN TB	4 months amikacin	Normal hearing at baseline. Found to have moderate unilateral high frequency loss after four months so injectable stopped. No further tests carried out.
12 yr	Girl	Neg	MDR	Presumed PTB	5 months amikacin	Normal hearing at baseline and at the end of therapy. One month after completing injectable medications found to have moderate unilateral high frequency loss, progressing to bilateral high frequency loss (mild in one ear and moderately severe in the other) after a further month.
10 yr	Girl	Pos	MDR	Confirmed PTB	4 months amikacin	Found at first assessment (2 months after starting therapy) to have bilateral moderate high frequency loss. By 2 months after completing therapy high frequency loss progressed in one ear to severe.

HIV: human immunodeficiency virus; TB: tuberculosis; RMR: rifampicin-mono-resistant; MDR: multidrug-resistant; PTB: pulmonary TB; LN: lymph node.

on age) and should be screened at least every month. If it is possible without compromising MDR-TB treatment, injectable drugs stopped or switched if there are any audiological concerns. Monitoring for hearing should continue after the drug has been stopped and therapeutic drug monitoring should be considered. Certain inherited mitochondrial mutations have been shown to predispose patients to hearing loss<sup>47–50</sup> and there is evidence that aspirin and L-carnitine may offer some protection.<sup>51,52</sup> These require further investigation, as does the relationship between dose schedule, resulting drug serum concentration and toxicity. Pharmacokinetic data on the use of injectable drugs in

children treated for MDR-TB are limited and there are no pharmacodynamic data. New, safer and effective drugs for the treatment of MDR-TB are urgently needed in children.

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Table 4 Univariate assessment of risk factors of hearing loss in children treated for multidrug-resistant tuberculosis.

	Hearing loss (n = 23)	No hearing loss (n = 27)	Or (95% CI)	P-value
Age in months (IQR)	52 (28–132)	53 (25–120)		0.90
Male gender	9	11	0.94 (0.30–2.95)	0.91
EP involvement	9	6	2.25 (0.63–8.00)	0.20
WFA z-score	–1.07 (–2.29–0.32)	–0.82 (–2.34–0.33)		0.78
BMI in kg/m <sup>2</sup> (IQR)	15.9 (13.9–17.6)	16.1 (14.9–17.3)		0.48
MUAC in cm (IQR)	15.0 (14.0–17.0)	16.4 (14.5–18.1)		0.28
Culture-confirmed diagnosis of TB	17	11	4.12 (1.13–15.0)	0.02
HIV-infected	9	6	2.14 (0.60–7.63)	0.23
Amikacin use	21	24	0.76 (0.11–5.11)	0.78
mg/kg dose injectable (IQR)	19.6 (18.3–20.4)	19.4 (17.4–20.1)		0.30
Duration of injectable in days (IQR)	164 (119–184)	123 (70–183)		0.07
Pre-XDR or XDR-TB	4	5	0.93 (0.21–4.01)	0.92

EP: extrapulmonary; WFA: weight-for-age; BMI: body mass index; MUAC: mid upper arm circumference; HIV: human immunodeficiency virus; XDR: extensively drug-resistant; OR: odds ratio; CI: confidence interval.

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## Conflict of interest

None declared.

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## Population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in children with tuberculosis: *in silico* evaluation of currently recommended doses

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**Objectives:** To describe the population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in children and evaluate the adequacy of steady-state exposures.

**Patients and methods:** We used previously published data for 76 South African children with tuberculosis to describe the population pharmacokinetics of rifampicin, pyrazinamide and isoniazid. Monte Carlo simulations were used to predict steady-state exposures in children following doses in fixed-dose combination tablets in accordance with the revised guidelines. Reference exposures were derived from an ethnically similar adult population with tuberculosis taking currently recommended doses.

**Results:** The final models included allometric scaling of clearance and volume of distribution using body weight. Maturation was included for clearance of isoniazid and clearance and absorption transit time of rifampicin. For a 2-year-old child weighing 12.5 kg, the estimated typical oral clearances of rifampicin and pyrazinamide were 8.15 and 1.08 L/h, respectively. Isoniazid typical oral clearance (adjusted for bioavailability) was predicted to be 4.44, 11.6 and 14.6 L/h for slow, intermediate and fast acetylators, respectively. Higher oral clearance values in intermediate and fast acetylators also resulted from 23% lower bioavailability compared with slow acetylators.

**Conclusions:** Simulations based on our models suggest that with the new WHO dosing guidelines and utilizing available paediatric fixed-dose combinations, children will receive adequate rifampicin exposures when compared with adults, but with a larger degree of variability. However, pyrazinamide and isoniazid exposures in many children will be lower than in adults. Further studies are needed to confirm these findings in children administered the revised dosages and to optimize pragmatic approaches to dosing.

**Keywords:** pharmacometrics, anti-*Mycobacterium*, paediatrics, NONMEM, modelling and simulation

### Introduction

Tuberculosis is the most frequent infectious cause of death worldwide with high impact in developing countries.<sup>1</sup> In high-burden settings, children comprise 15%–20% of tuberculosis cases.<sup>2</sup> Young children (<5 years of age) and children with HIV infection are prone to rapid progression to tuberculosis disease following infection and frequently experience severe forms of tuberculosis,

including disseminated disease and meningitis.<sup>2</sup> Isoniazid and rifampicin are important for their bactericidal activity against metabolically active *Mycobacterium tuberculosis*. The sterilizing activities of pyrazinamide and rifampicin prevent the relapse of disease after treatment.<sup>3,4</sup> Isoniazid plays an important role in preventing the development of resistance to companion drugs such as rifampicin.<sup>5</sup> Dosages of rifampicin higher than those currently employed have been suggested to improve efficacy.<sup>6</sup>



Until recently, the daily dosages of first-line antituberculosis drugs recommended in children were derived from the adult dosage based on the assumption that the same dose per kg is appropriate across all ages of patients. Even though rifampicin, isoniazid and pyrazinamide have been available for many years, the limited pharmacokinetic information in children suggests that young children receiving adult-derived dosages have drug exposures lower than adults.<sup>7-9</sup> In children, factors such as maturation of metabolizing enzymes and transporters, body composition, organ function, nutritional status and the pathophysiology of severe forms of tuberculosis may contribute to changes in pharmacokinetics, drug response and toxicity.<sup>10</sup> Previous studies have reported reduced tuberculosis drug concentrations in adults with HIV infection,<sup>11</sup> but the effect of HIV infection on the pharmacokinetics of tuberculosis drugs has not been adequately evaluated in children. The WHO has recently recommended increased dosages of first-line antituberculosis drugs for children,<sup>12</sup> which are to be implemented using dispersible fixed-dose combination (FDC) tablets for paediatric use, manufactured according to newly recommended specifications, with each tablet containing 50 mg of isoniazid, 75 mg of rifampicin and 150 mg of pyrazinamide.<sup>13</sup>

In this paper, we describe the population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in non-linear mixed-effects models, using previously published data from children with tuberculosis aged 2 months to 11.3 years who were given a wide range of dosages.<sup>7,8,14,15</sup> In addition, we used our final models to predict the steady-state area under the concentration–time curve (AUC) and maximum plasma concentration ( $C_{max}$ ) in a paediatric population and compared them with the corresponding pharmacokinetic measures in ethnically similar adults with tuberculosis.

## Patients and methods

### Patient population

Combined data of 76 children obtained from previously published studies describing the plasma concentrations of rifampicin, pyrazinamide and

isoniazid in two cohorts of South African children with tuberculosis were used for analysis.<sup>7,8,14,15</sup> On the basis of analysis of genetic polymorphisms of the arylamine *N*-acetyltransferase 2 (NAT2) gene, the children were categorized as slow-, intermediate- or fast-acetylator genotypes for acetylation of isoniazid. The methods used in the classification of NAT2 genotypes have been previously described for Cohort 1<sup>9</sup> and Cohort 2.<sup>15</sup> The demographic and clinical characteristics of all the children are summarized in Table 1.

### Patient treatment and pharmacokinetic sampling

Daily doses of rifampicin and isoniazid were given for 6 months with pyrazinamide added for the first 2 months. Dispersible FDC tablets formulated for children were used<sup>7,8,14,15</sup> and details about the dosing are included in Table 1. In Cohort 1, the median daily doses of rifampicin, pyrazinamide and isoniazid approximated 10, 23 and 5 mg/kg, respectively. In Cohort 2, on the first pharmacokinetic occasion, the median daily doses were similar to Cohort 1: 10 mg/kg for rifampicin, 25 mg/kg for pyrazinamide and 5 mg/kg for isoniazid. For the second pharmacokinetic occasion, the doses were adjusted, so the median values increased: 15 mg/kg for rifampicin, 36 mg/kg for pyrazinamide and 10 mg/kg for isoniazid. For Cohort 1, blood sampling for pharmacokinetic analysis was conducted at 1 and 4 months after starting treatment. At each sampling occasion, venous blood was drawn at 0.75, 1.5, 3, 4 and 6 h post-dose. Cohort 2 underwent pharmacokinetic sampling  $\geq 2$  weeks after initiation of antituberculosis therapy and sampling was repeated 1 week later, with samples drawn pre-dose and at 0.5, 1.5, 3 and 5 h after the dose. Cohort 1 plasma concentrations of rifampicin, pyrazinamide and isoniazid were determined by liquid chromatography–tandem mass spectrometry (LC/MS/MS) as detailed previously.<sup>7,14,16</sup> The lower limit of quantification (LLOQ) for both rifampicin and isoniazid was 0.1 mg/L and for pyrazinamide was 0.2 mg/L. In Cohort 2, isoniazid and pyrazinamide plasma concentrations were determined by HPLC and UV detection,<sup>15</sup> whereas rifampicin was determined by LC/MS/MS.<sup>15</sup> The lower limit of detection (LLOD) in Cohort 2, under which no concentration was reported, was 0.25, 0.5 and 0.15 mg/L for rifampicin, pyrazinamide and isoniazid, respectively. Moreover, the assay could not guarantee 5% precision between the LLOD (0.75, 1.5 and 1 mg/L for rifampicin, pyrazinamide and isoniazid, respectively) and LLOQ values.

**Table 1.** Patient demographic and clinical characteristics

Covariate	Cohort 1 <sup>a</sup>	Cohort 2 <sup>a</sup>	Combined
Number of patients	56	20	76
Sex (males/females)	29/27	11/9	40/36
Genotype (S/I/F/M <sub>s</sub> )	20/24/8/4	8/4/8/0	28/28/16/4
HIV positive (males/females)	22 (12/10)	5 (2/3)	27 (14/13)
Age (years)	3.22 (0.598, 10.9)	1.10 (0.321, 1.99)	2.17 (0.417, 10.7)
Weight (kg)	12.5 (4.87, 26.9)	7.80 (4.12, 12.8)	10.5 (4.90, 21.8)
RIF dose 1st PK (mg/kg)	9.54 (6.70, 13.3)	10.1 (9.0, 10.5)	9.71 (7.07, 13.2)
RIF dose 2nd PK (mg/kg)	9.57 (5.46, 16.0)	15.4 (10.4, 15.8)	10.3 (6.37, 15.9)
PZA dose 1st PK (mg/kg)	22.7 (15.9, 26.7)	25.2 (22.5, 26.5)	24.6 (17.6, 26.5)
PZA dose 2nd PK (mg/kg)	22.2 (12.2, 26.3)	36.2 (33.4, 39.5)	33.7 (15.6, 39.1)
INH dose 1st PK (mg/kg)	4.92 (3.35, 12.8)	5.04 (4.50, 5.36)	5.03 (3.56, 12.1)
INH dose 2nd PK (mg/kg)	4.95 (2.36, 16.0)	10.2 (8.74, 10.8)	9.77 (2.73, 13.3)

S/I/F, slow/intermediate/fast arylamine *N*-acetyltransferase acetylators; M<sub>s</sub>, missing covariate data; RIF, rifampicin; PZA, pyrazinamide; INH, isoniazid; 1st PK and 2nd PK, first and second pharmacokinetic sampling occasion, respectively.

<sup>a</sup>Continuous variables reported as median (2.5th percentile, 97.5th percentile), while the size of each group is reported for categorical covariates.<sup>7,8,14,15</sup>

### Pharmacokinetic data analysis

The concentration–time data for each drug were analysed separately using a non-linear mixed-effects approach. The estimation of typical population pharmacokinetic parameters, along with their random inter-individual variability (IIV) and inter-occasional variability (IOV), was performed in NONMEM7 (Icon Development Solutions, Ellicott City, MD, USA)<sup>17</sup> using a first-order conditional estimation method with  $\epsilon$ – $\eta$  interaction (FOCE INTER).<sup>18</sup> For all three drugs (rifampicin, pyrazinamide and isoniazid), model development was carried out first by developing the structural and stochastic model, including allometric scaling, and then investigating covariate effects. Graphical diagnostics were created using Xpose 4.<sup>19</sup> In all models, allometric scaling was applied on the oral clearance (CL) and apparent volume of distribution in plasma ( $V_c$ ) according to Anderson and Holford.<sup>20</sup> As reference, the median body weight of 12.5 kg from Cohort 1 (Table 1) was used.

$$CL_i = CL_{\text{std}} \cdot (WT_i/12.5)^{0.75} \quad (1)$$

$$V_i = V_{\text{std}} \cdot (WT_i/12.5)^1 \quad (2)$$

$CL_i$  is the scaled typical value of CL for individual  $i$ ,  $CL_{\text{std}}$  is the typical CL for an individual of 12.5 kg and  $WT_i$  is the body weight of individual  $i$  in kg. A similar notation applies to  $V_i$ . In the case of a two-compartment model, allometric scaling was also applied on the inter-compartmental clearance (Q) (as in Equation 1) and on the apparent peripheral volume of distribution ( $V_p$ ) (as in Equation 2). After allometric scaling was included, the need for including maturation models following the approach previously described by Anderson and Holford<sup>21</sup> was evaluated. The maturation factor (MF) was calculated using Equation 3:

$$MF = 1/[1 + (PMA/TM_{50})^{-\text{Hill}}] \quad (3)$$

where PMA is age derived by adding 36 weeks to the post-natal age,<sup>22</sup> assuming no premature birth.  $TM_{50}$  is the age at which maturation reaches 50% of the final value and Hill is the coefficient that regulates the rate of onset of the maturation. Models with and without the Hill factor fixed to 1 were tested.

The structural models tested included one- and two-compartment models with first-order elimination. Different absorption models were explored, such as first-order absorption or a sequence of zero- and first-order absorption incorporating either lag times or transit compartment absorption.<sup>23,24</sup> The effect of a dose dependency on the pharmacokinetic parameters was also tested. For relative bioavailability (F), first-order absorption rate constant ( $k_a$ ), mean transit time (MTT) and CL of rifampicin (a substrate of polymorphic OATP1B1 transporter)<sup>25</sup> and isoniazid (a substrate of polymorphic metabolizing enzyme NAT2) the possibility of subpopulations having different parameter estimates was tested using mixture models. A log-normal model for IIV and IOV was used and shrinkage in the *post hoc* estimates was derived. Additive and/or proportional models for the residual unexplained variability were evaluated. In Cohort 1, the drug concentrations reported as below the LLOQ were 0.6%, 0% and 3.4% for rifampicin, pyrazinamide and isoniazid, respectively. In Cohort 2, the values below the LLOD were 19%, 5.5% and 17.1% for rifampicin, pyrazinamide and isoniazid, respectively. Additionally, 4.5%, 3% and 24.1% of the concentrations of rifampicin, pyrazinamide and isoniazid, respectively, were in the ‘low precision’ range of the assay, but above the LLOD. The larger proportion of low concentrations in Cohort 2 is partly due to the collection of a pre-dose sample. All the data below the LLOQ in Cohort 1 were handled by fixing the error structure to additive with size LLOQ/2, to include these samples in the fit while accounting for the lower precision. In Cohort 2, values below the LLOD were replaced with LLOD/2, discarding consecutive undetectable concentrations in a series. Moreover, for all LLOD values and the samples in the

‘low precision’ range of the assay, the error structure was fixed to additive and with size half of the threshold of low precision of the assay, once again to account for the larger uncertainty in these measurements.

The effect of age, sex and HIV infection was tested on F, CL,  $V_c$ ,  $V_p$ ,  $k_a$  and MTT. Arm muscle area (AMA) was tested as a predictor of CL,  $V_c$  and  $V_p$  while albumin and other nutritional status measures (weight-for-age, weight-for-height and height-for-age z scores) were tested on F, CL,  $V_c$  and  $V_p$ .

The AMA values were derived from percentiles from the US Health and Nutrition Examination Survey I from 1971 to 1974<sup>26</sup> and the z scores were derived from National Center for Health Statistics/CDC/WHO international reference standards.<sup>27</sup> Missing continuous covariate data were replaced with the individual information from the other dosing and sampling occasion, if available. Otherwise, median values from the cohort were used. Missing categorical covariates were replaced with the most common category, except for acetylator genotype. For the missing genotype data (four individuals), a mixture model was used. The likelihood of belonging to each acetylator status was fixed to the frequency observed in the subjects for whom the information was available.

Potential covariate relationships were identified by clinical and statistical significance. The covariate relationships were tested in the model by stepwise addition (forward step) using a difference in the NONMEM objective function value ( $\Delta\text{OFV}$ ) of  $\geq 3.84$  ( $P \leq 0.05$ ) as the cut-off for inclusion, followed by stepwise deletion (backward step) using  $\Delta\text{OFV}$  of  $\geq 6.83$  ( $P \leq 0.01$ ) as a requisite for covariate retention.

The effect of continuous covariates was parameterized as shown in Equation 4:

$$PAR = \theta_p \cdot [1 + \theta_{\text{cov}} \cdot (\text{COV} - \text{COV}_{\text{med}})] \quad (4)$$

where  $\theta_p$  is the parameter (PAR) estimate in a typical patient (a child with median covariate value of  $\text{COV}_{\text{med}}$ ), while  $\theta_{\text{cov}}$  is the fractional change in PAR with each unit change in the covariate (COV) from  $\text{COV}_{\text{med}}$ .

For categorical covariates, such as acetylator genotype, a separate typical value for the parameter under test was estimated in each group.

### Model evaluation

Model selection was based on the graphical assessment of conditional weighted residuals versus time, basic goodness-of-fit plots, changes in the OFV, precision of parameter estimates as provided by the covariance step (if successfully completed) and visual predictive checks (VPCs).<sup>28</sup>

### Simulations

Using Monte Carlo simulations, the final models were used to simulate the steady-state AUC and  $C_{\text{max}}$  for children using pragmatic weight bands (adhering as closely as possible to the revised guidelines in dose per unit weight)<sup>12</sup> using one to four undivided tablets of an FDC with the newly recommended specifications (i.e. 75/50/150 mg of rifampicin/isoniazid/pyrazinamide in each FDC tablet).<sup>13</sup> The AUC and  $C_{\text{max}}$  were predicted for children weighing 5.0–7.9, 8.0–11.9, 12.0–15.9 and 16.0–24.0 kg receiving respective daily doses of one, two, three and four FDC tablets. Individual weight and age values used in the simulations were additionally obtained from historical data of 246 South African children with tuberculosis; 72% were infected with HIV (2% with HIV status unknown) and 54% were males. Pooling these with the present data from the two cohorts (Table 1), we obtained a dataset of baseline values for 322 children. The pharmacokinetic models were applied (1000 repetitions) to this *in silico* population, using the WHO-recommended dosing guidelines, and the AUC and  $C_{\text{max}}$  values were collected for each weight band. To obtain reference values for comparison, previously published pharmacokinetic models<sup>24,29,30</sup> from an ethnically similar population of adult patients were used to simulate AUC and  $C_{\text{max}}$  ranges using the current

WHO-recommended dosing guidelines for adults.<sup>31</sup> The respective daily doses used for simulations in adults weighing 30.0–37.9, 38.0–54.9, 55.0–69.9 and  $\geq 70$  kg were: 300, 450, 600 and 750 mg for rifampicin; 800, 1200, 1600 and 2000 mg for pyrazinamide; and 150, 225, 300 and 375 mg for isoniazid.

## Results

### Rifampicin

A total of 629 concentration–time data points were available for 67 children.<sup>8,15</sup> The final pharmacokinetic model for rifampicin was a one-compartment model with transit compartments absorption<sup>23</sup> and first-order elimination. The parameter estimates of the final model are shown in Table 2. The absorption transit model was simplified by fixing  $k_a$  to the same value as the first-order transit rate constant ( $k_{tr}$ ), since the two were not significantly different. HIV status, age and albumin levels had no influence on the pharmacokinetics of rifampicin. Age maturation was supported for CL and MTT and resulted in a 23 point improvement in OFV, explaining 16% IIV in CL and 17% IOV in MTT (no IIV in MTT was present in the model).  $TM_{50}$  was estimated to be 58.2 weeks, which is 22.2 weeks (0.43 years) after birth for both CL and MTT. The proportional change of each parameter with post-natal age is shown in Figure 1. The final model included IIV, expressed as percentage coefficient of variation (%CV), in CL (33%) and  $V_c$  (43%), while IOV was significant for CL (25%), F

(48%) and MTT (40%). The residual error model had both additive (0.122 mg/L for Cohort 1 and 0.63 mg/L for Cohort 2) and proportional (23% for both cohorts) terms. The final pharmacokinetic model described the data well, as shown in the VPC (Figure 2). When scaled to a 70 kg individual, the typical values for CL and  $V_c$  were 29.7 L/h and 90.7 L, respectively.

### Pyrazinamide

A total of 518 concentration–time data points were available for 55 children.<sup>14,15</sup> The final pharmacokinetic model for pyrazinamide was a one-compartment distribution model with absorption transit compartments, first-order absorption and elimination. The final parameter estimates are shown in Table 3. No significant covariate relationships were supported by the data. IIV, expressed as %CV, was supported for CL (27%), while IOV was significant for CL (26%),  $k_a$  (86%), F (25%) and MTT (112%). The residual error model was proportional (10% for Cohort 1 and 6% for Cohort 2). The VPC for the final model is displayed in Figure 2, showing that the final model described the data well. The typical values of CL and  $V_c$  were 3.9 L/h and 54 L, respectively, when scaled to a 70 kg individual.

### Isoniazid

A total of 715 concentration–time data points were available for all 76 children.<sup>7,15</sup> A two-compartment distribution model with absorption transit compartments and first-order elimination best described the pharmacokinetics of isoniazid. The final parameter estimates are shown in Table 4. The NAT2 genotype was a significant covariate for CL and F. The OFV improved by 55.7 points when including the acetylator status, which explained 45% of the IIV in CL. Estimation of F for intermediate and fast acetylators (relative to slow acetylators) and accounting for age maturation of CL further improved the OFV by 11 and 16 points, respectively. The age maturation explained 6% IIV in CL. With respect to intermediate acetylators (CL = 8.94 L/h), slow acetylators had 50% lower CL (4.44 L/h), while fast acetylators had 26% higher CL (11.3 L/h). Also, F in intermediate and fast acetylators was estimated to be 77.2%, which is 23% lower than in slow acetylators. Thus, combining the genotype effect on CL and bioavailability, the value of CL/F is 4.44, 11.6 and 14.6 L/h for slow, intermediate and fast acetylators, respectively. The final model included IIV, expressed as %CV, in CL (25%) and IOV in  $k_a$  (61%), MTT (94%) and F (40%). The maturation of CL is represented in Figure 1 and the VPC for the final model is shown in Figure 2. When scaled to a 70 kg individual, the typical values of CL (not CL/F, so before adjusting for the effect of NAT2 genotype on F) were 16.2, 32.5 and 41.1 L/h for slow, intermediate and fast acetylators, respectively, and  $V_c$  and  $V_p$  were 61.6 and 28.2 L.

### Simulations

Using the final models and WHO's newly recommended higher dosages utilizing revised FDC recommendations, the predicted steady-state AUCs for all weight bands are shown in Figures 3–5 for rifampicin, pyrazinamide and isoniazid, respectively. Median rifampicin exposures were similar to those in adults, although wider variability was present in the simulated paediatric

**Table 2.** Parameter estimates of the final rifampicin pharmacokinetic model

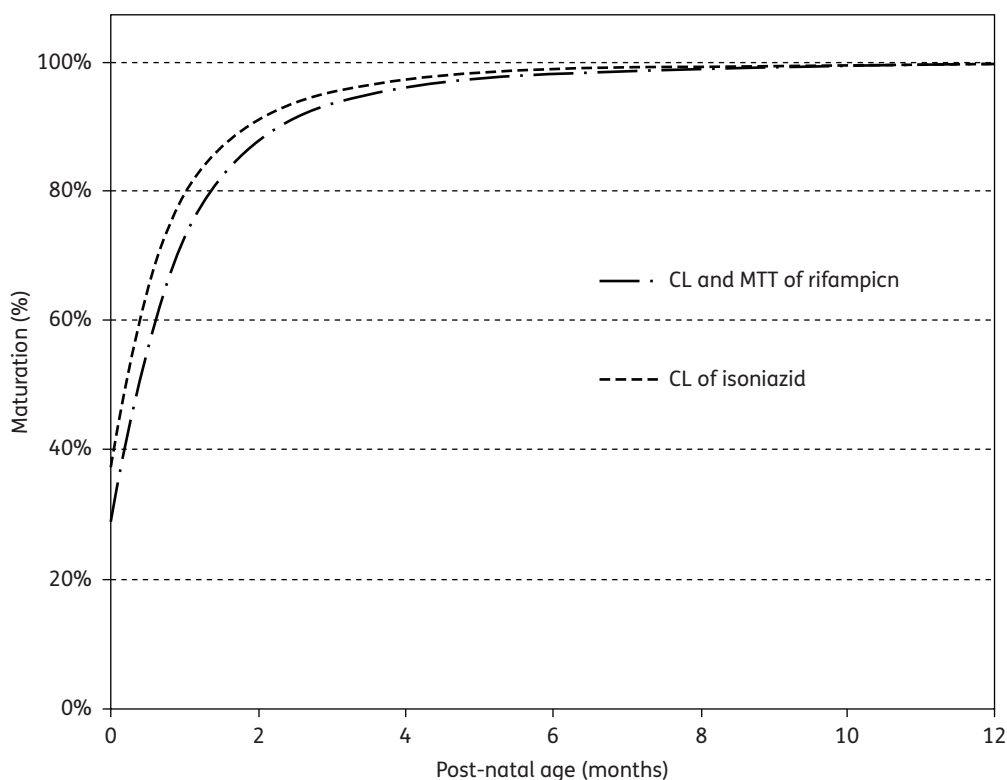
Parameter	Typical value [RSE (%)] <sup>a</sup>	IIV <sup>b</sup> [RSE (%)] <sup>a</sup>	IOV <sup>c</sup> [RSE (%)] <sup>a</sup>
CL <sub>std</sub> (L/h)	8.15 (9.00)	32.6 (27.7)	25.1 (20.0)
$V_{c, std}$ (L)	16.2 (10.2)	43.4 (19.8)	
MTT (h)	1.04 (6.10)		40.6 (8.60)
NN	8.04 (11.9)		
F (%)	1 (fixed)		48.1 (12.7)
$TM_{50}$ (weeks)	58.2 (9.00)		
Hill	2.21 (11.7)		
Additive error, Cohort 1 (mg/L)	0.122 (24.6)		
Additive error, Cohort 2 (mg/L)	0.630 (30.3)		
Proportional error (%)	23.4 (4.50)		

CL<sub>std</sub> and  $V_{c, std}$ , oral clearance and apparent volume of distribution, respectively (the values reported refer to a 12.5 kg child and for CL at full maturation); MTT, absorption mean transit time (value at full maturation); NN, number of transit compartments; F, relative bioavailability;  $TM_{50}$ , post-menstrual age at which 50% of clearance and mean transit time maturation is achieved; Hill, steepness of the maturation function.

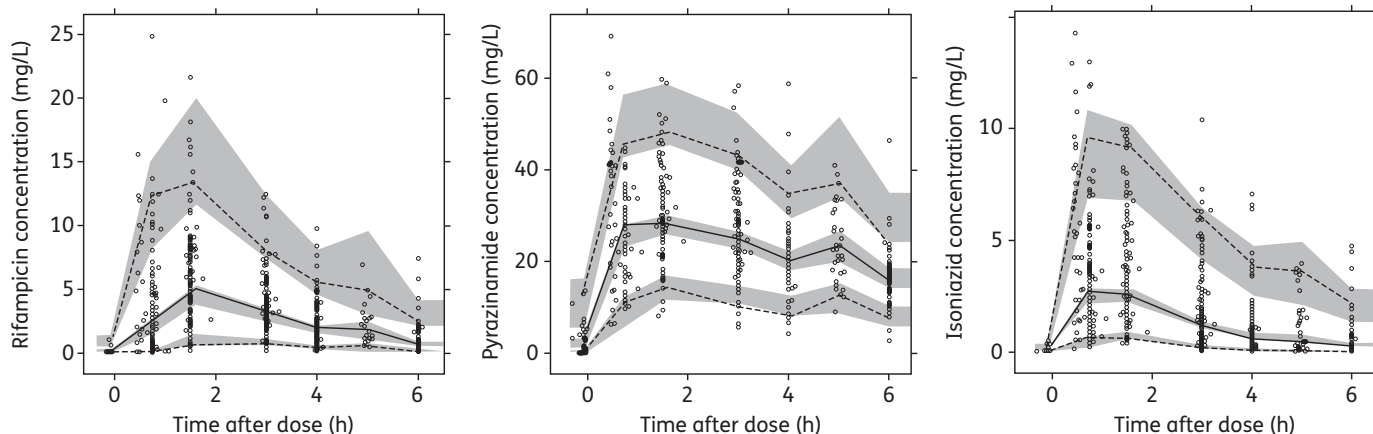
<sup>a</sup>RSE, relative standard error reported on the approximate standard deviation scale.

<sup>b</sup>IIV, inter-individual variability expressed as percentage coefficient of variation (%CV).

<sup>c</sup>IOV, inter-occasional variability expressed as percentage coefficient of variation (%CV).



**Figure 1.** Maturation of oral clearance (CL) and mean transit time (MTT) of rifampicin and CL of isoniazid in a typical patient with post-natal age. The plot is not adjusted for covariate effects or allometric scaling.



**Figure 2.** VPCs for the final models of rifampicin, pyrazinamide and isoniazid. The lower, middle and upper lines are the 5th percentile, median and 95th percentile of the observed data, respectively. The shaded areas are the 95% CIs for the 5th percentile, median and 95th percentile of the simulated data.

AUCs, as shown in Figure 3. Exposures after pyrazinamide and isoniazid were adequate, but younger children, particularly those in the lowest weight band, had lower exposures than those in the reference adult population and intermediate and fast acetylators had reduced isoniazid exposures compared with the majority of

adults. Our results also predicted that from 3 months to 2 years of age, the AUC decreased by 56% and 50% for rifampicin and isoniazid, respectively, but by only 18% for pyrazinamide [refer to Figures S1, S2 and S3 (available as Supplementary data at JAC Online), respectively].



**Table 3.** Parameter estimates of the final pyrazinamide pharmacokinetic model

Parameter	Typical value [RSE (%)] <sup>a</sup>	IIV <sup>b</sup> [RSE (%)] <sup>a</sup>	IOV <sup>c</sup> [RSE (%)] <sup>a</sup>
CL <sub>std</sub> (L/h)	1.08 (5.60)	27.1 (16.3)	25.5 (11.3)
V <sub>c, std</sub> (L)	9.64 (2.60)		
k <sub>a</sub> (h <sup>-1</sup> )	4.48 (6.10)		86.4 (14.6)
MTT (h)	0.10 (17.7)		112 (22.5)
NN	3.94 (8.00)		
F (%)	1 (fixed)		24.7 (8.40)
Proportional error, Cohort 1 (%)	10.0 (4.90)		
Proportional error, Cohort 2 (%)	5.53 (7.20)		

CL<sub>std</sub> and V<sub>c, std</sub>, oral clearance and apparent volume of distribution, respectively (the values reported refer to a 12.5 kg child); k<sub>a</sub>, first-order absorption rate constant; MTT, absorption mean transit time (value at full maturation); NN, number of transit compartments; F, relative bioavailability.

<sup>a</sup>RSE, relative standard error reported on the approximate standard deviation scale.

<sup>b</sup>IIV, inter-individual variability expressed as percentage coefficient of variation (%CV).

<sup>c</sup>IOV, inter-occasional variability expressed as percentage coefficient of variation (%CV).

## Discussion

We described the population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in South African children treated for tuberculosis. In addition, drug exposures resulting from the dosages recently recommended by the WHO were investigated. Our simulations predicted that, even with the increased dosages, smaller children would achieve relatively low exposures to pyrazinamide and isoniazid. Moreover, intermediate and fast acetylators (together comprising 61% of our population) may be underdosed with respect to isoniazid if exposures in an ethnically similar population of adults are considered therapeutic (Figure 5). It should be noted that the doses of pyrazinamide and isoniazid used for these predictions tended to be somewhat lower than the recommended dose per kg, especially in the youngest children. Our choice of weight bands was based on ongoing discussions about the optimal dose of the FDC by weight band, pragmatic considerations, such as the stability and equal distribution of active ingredients in each portion after breaking the FDC tablets, and concern that pharmacokinetic maturation could still be incomplete in young children.<sup>9,32,33</sup> Our proposed doses were optimized assuming that dividing the FDC tablets should be avoided if possible.

The typical paediatric parameter estimates for rifampicin CL/F and V<sub>c</sub>/F are in line with adult values reported in an ethnically similar population.<sup>24</sup> After adjusting for the difference in body size (the median weight of the adult population used for comparison was 50 kg), CL/F was 23.1 L/h in children versus 19.2 L/h in adults and V<sub>c</sub>/F was 64.8 versus 53.2 L, respectively. This suggests that pharmacokinetic differences between children and adults could mainly be explained by differences in body size (which

**Table 4.** Final parameter estimates for isoniazid pharmacokinetics

Parameter	Typical value [RSE (%)] <sup>a</sup>	IIV <sup>b</sup> [RSE (%)] <sup>a</sup>	IOV <sup>c</sup> [RSE (%)] <sup>a</sup>
CL <sub>std, sa</sub> (L/h) <sup>d</sup>	4.44 (11.6)	25.1 (12.3)	
CL <sub>std, ia</sub> (L/h) <sup>d</sup>	8.94 (13.1)	25.1 (12.3)	
CL <sub>std, fa</sub> (L/h) <sup>d</sup>	11.3 (14.8)	25.1 (12.3)	
V <sub>c, std</sub> (L) <sup>d</sup>	11.0 (10.2)		
k <sub>a</sub> (h <sup>-1</sup> )	2.47 (12.8)		61.6 (12.4)
MTT (h)	0.179 (10.9)		93.9 (17.9)
NN	4 (fixed)		
Q <sub>std</sub> (L/h) <sup>d</sup>	2.00 (26.3)		
V <sub>p, std</sub> (L) <sup>d</sup>	5.03 (33.4)		
F <sub>sa</sub>	1 (fixed)		39.7 (5.80)
F <sub>im/fa</sub>	0.772 (30.3)		39.7 (5.80)
TM <sub>50</sub> (weeks)	49.0 (13.5)		
Hill	2.19 (46.1)		
Proportional error, Cohort 1 (%)	20.6 (2.80)		
Proportional error, Cohort 2 (%)	7.00 (18.7)		

CL<sub>std, sa</sub>, CL<sub>std, ia</sub> and CL<sub>std, fa</sub>, oral clearance for slow, intermediate and fast acetylators, respectively (they refer to a child weighing 12.5 kg and at full maturation); V<sub>c, std</sub>, apparent volume of distribution in the central compartment for 12.5 kg child; k<sub>a</sub>, first-order absorption rate constant; MTT, absorption mean transit time; NN, number of hypothetical transit compartments; Q<sub>std</sub>, inter-compartmental clearance for 12.5 kg child; V<sub>p, std</sub>, volume of distribution in the peripheral compartment for 12.5 kg child; F<sub>sa</sub>, relative bioavailability of slow acetylators; F<sub>im/fa</sub>, relative bioavailability of intermediate and fast acetylators relative to slow acetylators; TM<sub>50</sub>, post-menstrual age at 50% of adult clearance; Hill, steepness of the maturation function.

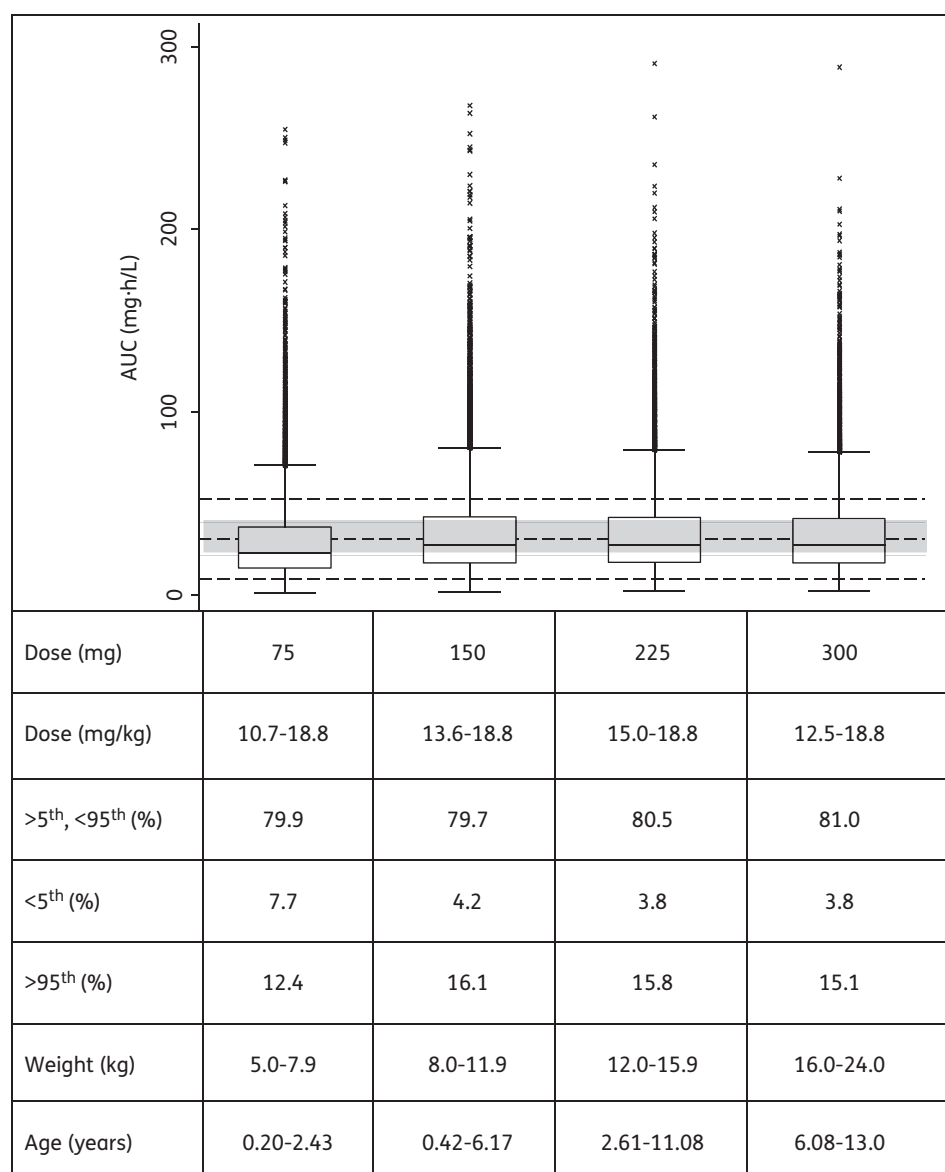
<sup>a</sup>RSE, relative standard error reported on the approximate standard deviation scale.

<sup>b</sup>IIV, inter-individual variability expressed as percentage coefficient of variation (%CV).

<sup>c</sup>IOV, inter-occasional variability expressed as percentage coefficient of variation (%CV).

<sup>d</sup>In order to obtain the values of CL/F, V<sub>c</sub>/F, Q/F and V<sub>p</sub>/F, the values in the table should be divided by the respective value of bioavailability, which changes according to acetylator status.

was accounted for by allometric scaling in our models) and enzyme maturation. Our data did not support non-linearity in the pharmacokinetics of rifampicin, even though the doses given ranged from ~5 to almost 18 mg/kg daily. Our results therefore suggest that at the doses used in the children studied, rifampicin may not display the dose-dependent non-linear pharmacokinetics in children that has been described in adults.<sup>34</sup> However, further studies, including sufficient numbers of children being given the increased dosages, would be needed to confirm this. Low rifampicin concentrations are associated with the development of acquired rifampicin monoresistance (ARR)<sup>35</sup> and a C<sub>max</sub> of 8 mg/L has been suggested for rifampicin,<sup>36</sup> although even higher concentrations are likely necessary for maximal bactericidal activity.<sup>6</sup> However, such concentrations are rarely achieved in children prescribed 8–12 mg/kg/day doses of rifampicin.<sup>8</sup> If the pharmacokinetics in an ethnically similar adult population<sup>24</sup>



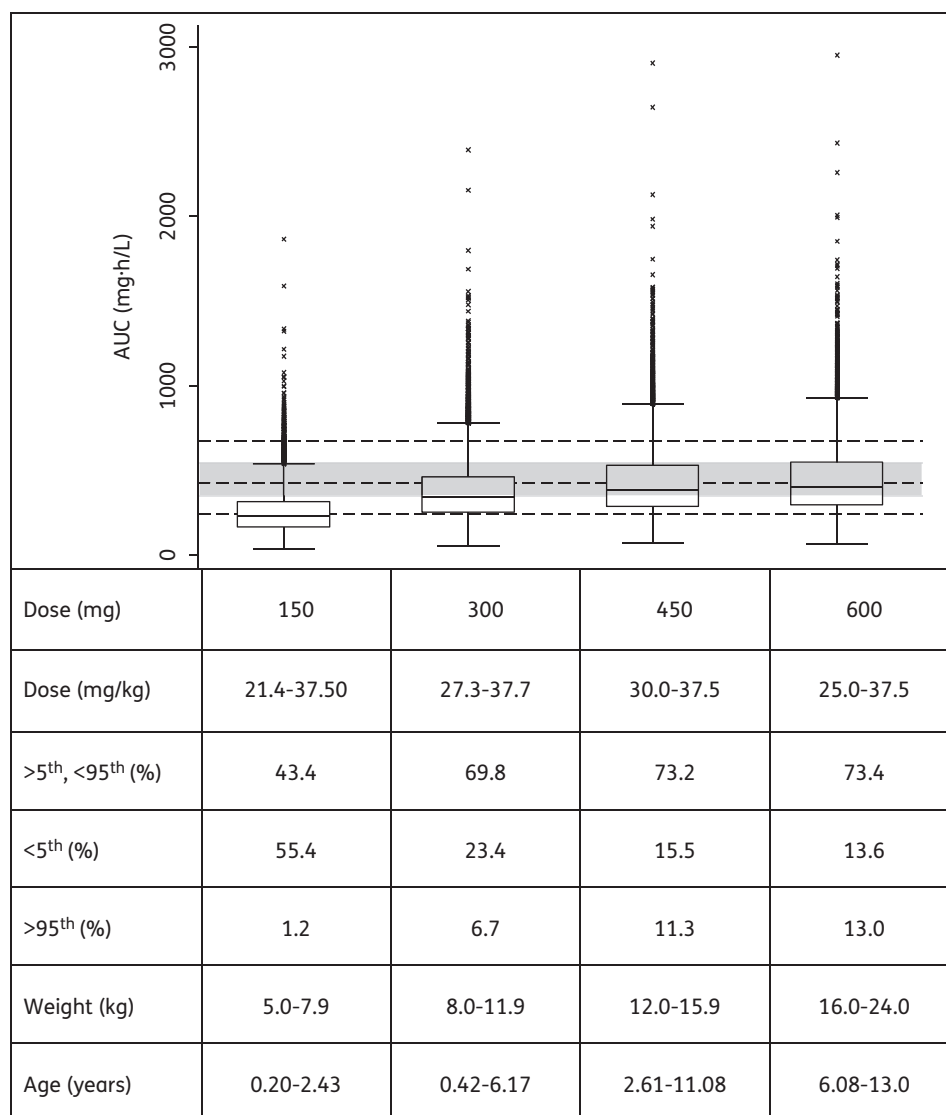
**Figure 3.** Box plot of simulated steady-state AUC of rifampicin across different age and weight bands, obtained adhering as closely as possible to the revised guidelines in dose per unit weight according to WHO 2010 dosing guidelines. Each box shows the 25th–75th percentile of simulated AUC and the symbol ‘x’ shows data falling outside 1.5 times the IQR (25th–75th). The lower, middle and upper broken lines are the derived 5th percentile (9.0 mg·h/L), median (30.7 mg·h/L) and 95th percentile (52.4 mg·h/L) of the adult AUC, respectively. The grey shaded area is the 25th–75th (21.9–39.5 mg·h/L) percentile of the adult AUC.

given the currently recommended doses is used for comparison, the majority of our children fall within the 5th–95th percentile range of exposures in the adult patients (Figure 3). The simulated adult exposures were similar to those found in a large pharmacokinetic study conducted in adults with pulmonary tuberculosis in Botswana.<sup>37</sup> Our simulations for children using the newly recommended WHO paediatric guidelines predicted more variable rifampicin exposures than those for the reference adult population (Figure 3). Moreover, both the predicted mean  $C_{max}$  using the revised paediatric guidelines (6.6 mg/L) and the current doses recommended by WHO in the adult reference population (4.8 mg/L) were lower than the proposed minimum  $C_{max}$  of

8 mg/L,<sup>36</sup> implying that even higher doses of rifampicin should be considered for both children and adults.

Using the final pyrazinamide model and parameter estimates for CL/F and  $V_c/F$  scaled to the median weight of the adult population used for comparison (51.5 kg),<sup>30</sup> the children had similar CL/F (~3.1 versus 3.42 L/h) and higher  $V_c/F$  (39.7 versus 29.2 L) compared with the adults. The higher  $V_c/F$  in children could partly be due to malnutrition and severe forms of tuberculosis disease. When we targeted previously reported exposures in an ethnically similar adult population who received doses recommended by WHO, our simulation results predicted that children weighing 5.0–7.9 kg may be underdosed (Figure 4). As noted above,



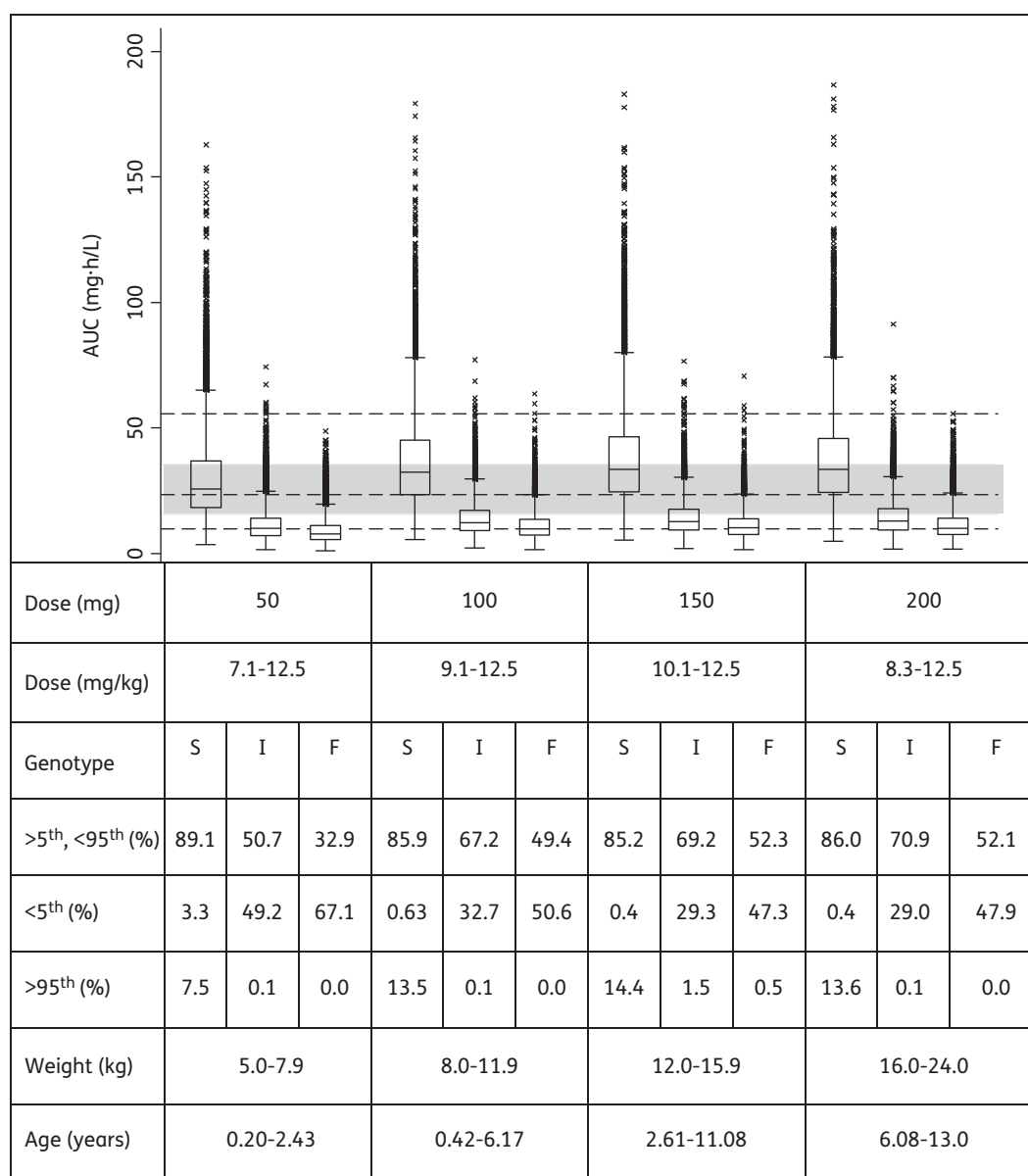


**Figure 4.** Box plot of simulated steady-state AUC of pyrazinamide across different age and weight bands, obtained adhering as closely as possible to the revised guidelines in dose per unit weight according to WHO 2010 dosing guidelines. Each box shows the 25th–75th percentile of simulated AUC and the symbol ‘x’ shows data falling outside 1.5 times the IQR (25th–75th). The lower, middle and upper broken lines are the derived 5th percentile (244.0 mg·h/L), median (427.0 mg·h/L) and 95th percentile (675.0 mg·h/L) of the adult AUC, respectively. The grey shaded area is the 25th–75th (346–547 mg·h/L) percentile of the adult AUC.

children in the lowest weight band tended to receive doses somewhat lower than the 30–40 mg/kg target proposed in the revised guidelines. Our simulations suggest that a 50 mg/kg dose for children in the lowest weight band of 5.0–7.9 kg would achieve the same median AUC as that in the adults. The pyrazinamide exposures we simulated for our reference adult population were similar to those in the large cohort in Botswana.<sup>37</sup> In addition, the majority of adults had simulated  $C_{max}$  within the suggested range of 20–50 mg/L.<sup>36</sup> Importantly, patients in the Botswana cohort with pyrazinamide  $C_{max}$  <35 mg/L had an increased risk of poor treatment outcome.

Isoniazid pharmacokinetics is highly dependent on the polymorphic NAT2, which is a major determinant of isoniazid plasma concentrations.<sup>38</sup> Targeting average exposures simulated for

ethnically similar adults following doses currently recommended by WHO, the new doses recommended by the WHO for children are adequate for slow acetylators, but insufficient for intermediate and fast acetylators (Figure 5). We have noted wide IIV in the systemic concentrations and AUC mostly because children were not dosed according to the NAT2 acetylator genotype. Hence, further studies are needed to adequately define the implications of IIV for safety and efficacy in children. Interestingly, children who were younger than 1 year old had higher AUC irrespective of their weight band. In addition, isoniazid CL showed maturation with age and this supports the notion that finer weight bands in conjunction with age should be considered to avoid overdosing very young children. The reduced bioavailability for intermediate and fast acetylators is most likely a result of



**Figure 5.** Box plot of simulated steady-state AUC of isoniazid in slow (S), intermediate (I) and fast (F) acetylators across different age and weight bands, obtained adhering as closely as possible to the revised guidelines in dose per unit weight according to WHO 2010 dosing guidelines. Each box shows the 25th–75th percentile of simulated AUC and the symbol ‘x’ shows data falling outside 1.5 times the IQR (25th–75th). The lower, middle and upper broken lines are the derived 5th percentile (9.8 mg·h/L), median (23.4 mg·h/L) and 95th percentile (55.6 mg·h/L) of the adult AUC, respectively. The grey shaded area is the 25th–75th (15.6–35.5 mg·h/L) percentile of the adult AUC.

increased pre-systemic metabolism, which also correlates with systemic clearance. The lower exposure in intermediate and fast acetylators may predispose to the development of ARR. In the CDC Study 22,<sup>39</sup> an association was found between low isoniazid plasma concentrations and the occurrence of relapse in patients on a regimen of once-weekly rifapentine and isoniazid, suggesting that isoniazid might have been ineffective in preventing the development of rifamycin resistance.

In all our models, we decided to scale clearance and volume with allometric scaling and this choice over using weight as a linear covariate was also supported by the data following general

criteria such as the NONMEM OFV. The use of allometrics to account for differences in pharmacokinetics due to body size is supported by both empirical observation and biological theory, as discussed in depth by Anderson and Holford.<sup>20,21</sup>

Although our children received similar formulations across the two cohorts, formulation is known to be an important determinant of bioavailability.<sup>40,41</sup> Other factors encountered when preparing suspensions and administering the drug are likely to play a role in young children who cannot swallow tablets. There is a need for studies to evaluate such sources of variability in paediatric drug exposure and to develop solutions to reduce it. Genetic diversity

is also an important consideration when extrapolating the results of pharmacokinetic studies. There is considerable geographical variation in the distribution of *NAT2* polymorphisms.<sup>42,43</sup> Moreover, recent studies have identified polymorphisms of *SLCO1B1* (which encodes the OATP1B1 transporter) occurring in relatively high frequencies in African populations, associated with substantially reduced rifampicin exposure.<sup>25,44</sup> Hence, pharmacogenetic studies in children may allow further optimization of dosing strategies.

Limitations of our model include the fact that the AMA values and z scores used to test anthropometric associations with the pharmacokinetic parameters were not derived specifically from an African population and thus they may not have correctly described the children in our study population. Finally, a limitation of our simulation approach is that it does not account for any non-linearity in the pharmacokinetics that may occur at the higher doses; hence, our predictions should be confirmed in pharmacokinetic studies in children dosed according to the revised guidelines.

In conclusion, our models describe the population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in children with tuberculosis. Simulations based on our models predict that the newly recommended weight band-based doses in WHO guidelines for children result in rifampicin exposures in our paediatric population that are similar to those in adults. However, when dosed in pragmatic weight bands, there is wide variability in drug exposure and pyrazinamide and isoniazid exposures in many children will be lower than those in an ethnically similar adult population. Hence, adjustment of the recommended doses may be warranted should the findings be confirmed in other populations.

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## Transparency declarations

None to declare.

## Supplementary data

Figures S1, S2 and S3 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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# Pharmacokinetics and Safety of Ofloxacin in Children with Drug-Resistant Tuberculosis

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**Ofloxacin is widely used for the treatment of multidrug-resistant tuberculosis (MDR-TB). Data on its pharmacokinetics and safety in children are limited. It is not known whether the current internationally recommended pediatric dosage of 15 to 20 mg/kg of body weight achieves exposures reached in adults with tuberculosis after a standard 800-mg dose (adult median area under the concentration-time curve from 0 to 24 h [AUC<sub>0-24</sub>], 103 μg · h/ml). We assessed the pharmacokinetics and safety of ofloxacin in children <15 years old routinely receiving ofloxacin for MDR-TB treatment or preventive therapy. Plasma samples were collected predose and at 1, 2, 4, 8, and either 6 or 11 h after a 20-mg/kg dose. Pharmacokinetic parameters were calculated using noncompartmental analysis. Children with MDR-TB disease underwent long-term safety monitoring. Of 85 children (median age, 3.4 years), 11 (13%) were HIV infected, and of 79 children with evaluable data, 14 (18%) were underweight. The ofloxacin mean (range) maximum concentration (C<sub>max</sub>), AUC<sub>0-8</sub>, and half-life were 8.97 μg/ml (2.47 to 14.4), 44.2 μg · h/ml (12.1 to 75.8), and 3.49 h (1.89 to 6.95), respectively. The mean AUC<sub>0-24</sub>, estimated in 72 participants, was 66.7 μg · h/ml (range, 18.8 to 120.7). In multivariable analysis, AUC<sub>0-24</sub> was increased by 1.46 μg · h/ml for each 1-kg increase in body weight (95% confidence interval [CI], 0.44 to 2.47; P = 0.006); no other assessed variable contributed to the model. No grade 3 or 4 events at least possibly attributed to ofloxacin were observed. Ofloxacin was safe and well tolerated in children with MDR-TB, but exposures were well below reported adult values, suggesting that dosage modification may be required to optimize MDR-TB treatment regimens in children.**

Globally, in 2013 there were an estimated 480,000 new cases of multidrug-resistant tuberculosis (MDR-TB), defined as *Mycobacterium tuberculosis* resistant to isoniazid (INH) and rifampin (RIF) (1). Precise incidence data in children are unavailable, but modeling estimates suggest that there were 33,000 new pediatric MDR-TB cases in 2010 (2). In addition, assuming an average of two child contacts for each adult MDR-TB source case (3), there may be as many as 900,000 children newly exposed to MDR-TB globally each year. Fluoroquinolones are a key component of existing regimens for treatment (4) and prevention (5) of MDR-TB in adults and children.

Ofloxacin, a fluoroquinolone, has potent activity against *M. tuberculosis* (6, 7) and has been routinely used in MDR-TB treatment. The current World Health Organization (WHO) recommended adult dose of ofloxacin for MDR-TB is 800 mg daily. Ofloxacin is not metabolized; rather, it is excreted unchanged in the urine (8). It is well absorbed after oral administration, and food intake does not affect its pharmacokinetics appreciably (9–12).

There are limited data on ofloxacin pharmacokinetics in children, particularly in children <5 years of age, to guide appropriate dose selection (11, 13). The WHO recommends a pediatric ofloxacin dose for MDR-TB of 15 to 20 mg/kg of body weight daily (14); however, it is unknown if this dose achieves exposures in children approximating those in adults after the recommended 800-mg dose. Concerns regarding arthropathy (15, 16) had initially limited the use of fluoroquinolones in children. Although safe in short courses (16–18), there are limited data on fluoroquinolone safety in children with long-term use (5, 19).

The more potent fluoroquinolones levofloxacin and moxifloxacin (20, 21) are beginning to replace ofloxacin for MDR-TB treatment. However, because of its low cost and widespread availability, ofloxacin is still used for MDR-TB in many settings, and optimizing its use in children remains important.

The objective of this study was to evaluate the pharmacokinetics and safety of ofloxacin among a large cohort of HIV-infected and uninfected children of representative ages who were routinely receiving ofloxacin for the prevention or treatment of MDR-TB.

## MATERIALS AND METHODS

**Study design.** This was a prospective observational pharmacokinetic study.

**Study setting.** The study took place in the Western Cape, South Africa, where in 2010 the TB notification rate was 954.1 cases per 100,000 population, and from 2009 to 2011 MDR-TB represented 7.1% of culture-

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confirmed cases in children <13 years old (22, 23). The diagnosis of MDR-TB was based on (i) culture of *M. tuberculosis* from sputum or other relevant specimens with drug susceptibility testing (DST) demonstrating resistance to INH and RIF, (ii) clinical and radiologic evidence of TB and contact with an MDR-TB source case, or (iii) failure of first-line TB treatment. Treatment for MDR-TB in children was provided independent of the study, according to local and international guidance, based on the DST of the child's isolate or the isolate of their most likely source case. Treatment included at least four drugs likely to be active given for at least 12 to 18 months (14, 24).

In the study setting, child contacts of adult MDR-TB cases are referred to a specialty clinic for preventive therapy. Children <5 years of age and those HIV infected without evidence of TB were prescribed 6 months of a three-drug preventive therapy regimen: ofloxacin, ethambutol, and high-dose INH (5).

**Study population.** Children were recruited from a large provincial referral hospital (Tygerberg Children's Hospital) and two provincial TB hospitals (Brooklyn Hospital for Chest Diseases and Brewskloof Hospital). Children <15 years of age routinely started on ofloxacin for prevention or treatment of MDR-TB were eligible. Exclusion criteria were a weight of <5 kg or hemoglobin of <8.0 g/dl. Children treated for MDR-TB disease were followed longitudinally to assess safety and tolerability during treatment. The safety of this preventive therapy regimen has been previously documented; these children were followed independent of the study (5). Data from 23 children from a substudy of this cohort were previously published and are included in the present analysis (13).

**Data collection.** Children were categorized as receiving ofloxacin either for MDR-TB treatment or prevention. TB was categorized as confirmed, probable, or possible according to international consensus definitions (25) and as pulmonary, extrapulmonary, or both. HIV status was ascertained in all participants. Weight-for-age Z-score (WAZ) was calculated using the 1990 British growth reference (26).

All participants underwent intensive pharmacokinetic sampling 2 to 8 weeks after starting ofloxacin. Ofloxacin (200-mg tablets; Sanofi Aventis, Midrand, South Africa) at a dose of 15 to 20 mg/kg once daily was routinely prescribed. On pharmacokinetic sampling days, an exact 20 mg/kg dose of ofloxacin was weighed and administered by the study team after a minimum 4-h fast with a small amount of water. Medications were given either as whole tablets or were crushed and given by mouth or nasogastric tube, depending on what the child would tolerate. Crushed tablets were ground with a mortar and pestle, mixed with a small amount of water in a plastic cup along with any other crushed TB medications, and administered immediately. Any residue in the cup was rinsed 1 to 2 times with additional water and administered to the child. All other anti-TB medications in the regimen were given together with the ofloxacin. One hour after the TB medications, antiretrovirals were administered (if applicable) and a standard breakfast was offered. Samples for pharmacokinetic analysis were drawn predose and then at 1, 2, 4, 8, and either 6 or 11 h post-dose. Whole-blood samples were collected in EDTA-containing tubes and immediately centrifuged, and plasma was separated and frozen at  $-80^{\circ}\text{C}$ . Ofloxacin concentrations were determined by high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) using a previously described method validated over a range of 0.0781 to 20.0  $\mu\text{g}/\text{ml}$  (27).

Children receiving ofloxacin for MDR-TB treatment had clinical monitoring monthly for the first 6 months and then every 2 months thereafter and laboratory monitoring (potassium, creatinine, alanine aminotransferase [ALT], total bilirubin, thyroid functions) every 2 months. Adverse events were graded according to standardized Division of AIDS (National Institute of Allergy and Infectious Diseases) criteria (28) and were considered attributable to ofloxacin if they were (i) at least possibly drug related and (ii) thought by the investigator to be likely related to ofloxacin or if they were not otherwise attributed to another drug. The person-time of observation began at the initial study visit and ended at either the final study visit or the date of treatment completion; observa-

tion periods in which the child received an alternative fluoroquinolone for part of the time (treatment guidelines changed during the study period) were excluded from safety analyses.

**Statistical analysis.** Demographic and clinical characteristics were summarized using descriptive statistics. Pharmacokinetic measures were estimated using noncompartmental analysis (NCA). Observed maximum plasma drug concentration ( $C_{\text{max}}$ ) and time to  $C_{\text{max}}$  ( $T_{\text{max}}$ ) were recorded directly from the concentration-time data. The area under the concentration-time curve from 0 to 8 h ( $\text{AUC}_{0-8}$ ) was calculated using the linear trapezoidal rule. Oral clearance ( $\text{CL}/F$ ), half-life ( $t_{1/2}$ ), and  $\text{AUC}_{0-24}$  were estimated in patients with at least 3 concentration data points in the elimination phase, with the latter based on exponential extrapolation from the final three time-concentration data points. Fifteen predose drug concentrations below the limit of quantification (0.078  $\mu\text{g}/\text{ml}$ ) were set to zero in analyses.

The  $C_{\text{max}}$ ,  $\text{AUC}_{0-8}$ ,  $\text{AUC}_{0-24}$ , and  $t_{1/2}$  were compared by age group (0 to <2 years, 2 to <5 years, and  $\geq 5$  years), HIV status, nutritional status (WAZ,  $< -2$  versus  $\geq -2$ ), and administration method (crushed versus whole tablets). Using simple linear regression, the  $\text{AUC}_{0-24}$  and  $C_{\text{max}}$  were analyzed separately for associations with age, weight, height, HIV status, nutritional status, gender, ethnicity, disease status (receiving preventive therapy versus treatment for MDR-TB), and administration method. Covariates with a  $P$  of  $< 0.05$  in univariable analysis, and factors known to affect drug disposition (age and weight) were included in multivariable models. We also assessed whether body surface area (BSA) (29) or lean body mass (LBM) (30) were better predictors than weight and height.

All analyses were performed using Stata 12.1 SE software (StataCorp, College Station, TX).

**Ethical considerations.** Written informed consent was obtained from the parents or legal guardian, and informed assent was collected from all children  $\geq 7$  years of age. Ethical approval was provided by the Health Research Ethics Committees of the Faculty of Medicine and Health Sciences of Stellenbosch University and the Faculty of Health Sciences of the University of Cape Town.

## RESULTS

**Baseline characteristics.** Eighty-five children were included (Table 1). All age groups were well represented. The median age was 3.4 years (interquartile range [IQR], 1.9 to 5.2 years). Eleven (13%) participants were HIV infected. Fourteen of the 79 patients with evaluable data (18%) were underweight for age (WAZ,  $< -2$ ) and 11 of these children were HIV infected (79%). Overall, 72 of 85 (85%) received crushed tablets on the day of pharmacokinetic sampling (97% of those <5 years old and 41% of those  $\geq 5$  years old).

**Pharmacokinetics and determinants of drug exposures.** With a dose of 20 mg/kg, the mean  $\text{AUC}_{0-8}$  ( $n = 85$ ) was 44.2  $\mu\text{g} \cdot \text{h}/\text{ml}$  and  $\text{AUC}_{0-24}$  ( $n = 72$ ) was 66.7  $\mu\text{g} \cdot \text{h}/\text{ml}$ ; other summary measures with reported adult values for comparison are shown in Table 2. Pharmacokinetic values by age group, HIV status, WAZ category, and type of administration are presented in Table 3. Half-life was shorter in the youngest children, and there was a trend toward a higher  $C_{\text{max}}$  in children receiving crushed tablets. In simple linear regression, no variables assessed were significantly associated with  $C_{\text{max}}$ , and only weight was significantly associated with  $\text{AUC}_{0-24}$ . In multivariable analysis,  $C_{\text{max}}$  was reduced by 0.44  $\mu\text{g}/\text{ml}$  for each 1-year increase in age (95% confidence interval [CI],  $-0.74$  to  $-0.13$ ;  $P = 0.005$ ) and was increased by 0.13  $\mu\text{g}/\text{ml}$  for each 1-kg increase in body weight (95% CI, 0.10 to 0.24;  $P = 0.029$ ). In multivariable analysis,  $\text{AUC}_{0-24}$  was increased by 1.46  $\mu\text{g} \cdot \text{h}/\text{ml}$  for each 1-kg increase in body weight (95% CI, 0.44 to 2.47;  $P = 0.006$ ). Controlling for age and weight, no other assessed



**TABLE 1** Baseline demographic and clinical characteristics of children receiving ofloxacin for treatment or prevention of drug-resistant tuberculosis

Characteristic <sup>a</sup>	No. (%) with MDR-TB disease (n = 55)	No. (%) receiving MDR-TB preventive therapy (n = 30)
Age at enrollment		
0 to <2 yr	16 (29.1)	8 (26.7)
2 to <5 yr	17 (30.9)	22 (73.3)
5 to <15 yr	22 (40.0)	0 (0.00)
Male sex	32 (58.2)	15 (50.0)
Certainty of TB diagnosis		
Bacteriological confirmation	20 (36.4)	
Probable TB	32 (58.2)	
Suspected TB	3 (5.5)	
TB disease type (n = 55)		
PTB only	40 (72.7)	
EPTB only	5 (9.1)	
PTB and EPTB	10 (18.2)	
HIV infected	11 (20.0)	0 (0.0)
Weight-for-age Z-score < -2.0 (n = 79) <sup>b</sup>	11 (22.5)	3 (10.0)
Height-for-age Z-score < -2.0 (n = 81) <sup>c</sup>	19 (35.9)	4 (14.3)
Weight-for-length Z-score < -2.0 (n = 60) <sup>c</sup>	2 (6.3)	1 (3.6)

<sup>a</sup> PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.

<sup>b</sup> Weight-for-age Z-scores only available for patients aged <10 years.

<sup>c</sup> The sample size is <85 due to missing data.

variables contributed to these models. Neither LBM nor BSA improved the model fit over weight.

**Safety.** Forty-six children contributed a total of 23.8 years of observation time on ofloxacin to the safety assessment, with a median time per child of 4.9 months (IQR, 1.2 to 10.2 months) (Table 4). Adverse events were mostly mild in severity; vomiting and pruritus were the most frequent. Most adverse events were not attributed to ofloxacin but represented known toxicities related to companion MDR-TB drugs. The only grade 3 or 4 events were two episodes of asymptomatic ALT elevation due to confirmed acute hepatitis A, which resolved without complication after brief interruptions of some TB medications while awaiting the hepatitis A results.

## DISCUSSION

Ofloxacin given at the WHO-recommended dose of 20 mg/kg to children was safe and well-tolerated, but exposures in this substantial pediatric cohort were considerably lower than those achieved in adults taking the standard MDR-TB treatment dose of 800 mg daily.

Although ofloxacin has been widely used for treatment and prevention of MDR-TB in children, the appropriate dosage has not been established. Indeed, only one other study evaluating the pharmacokinetics of ofloxacin in children has been conducted to our knowledge. In a study in Vietnam, 17 children (aged 5 to 17 years) with typhoid fever received a single oral dose of 7.5 mg/kg of ofloxacin (11). A  $C_{\max}$  of 5.73  $\mu\text{g/ml}$  and an  $\text{AUC}_{0-12}$  of 26.5  $\mu\text{g/ml}$  were achieved (11). The  $C_{\max}$  (8.97  $\mu\text{g/ml}$ ) and  $\text{AUC}_{0-8}$

**TABLE 2** Summary statistics for ofloxacin pharmacokinetic measures in children receiving treatment or prevention for multidrug-resistant tuberculosis<sup>a</sup>

Parameter <sup>b</sup>	No. of children	Values for children in the present study	Values for adults with TB given an 800-mg ofloxacin dose <sup>c</sup>
$C_{\max}$ ( $\mu\text{g/ml}$ )	85	8.97 (2.47–14.4)	10.5 (8.0–14.3)
$T_{\max}$ (h)	85	2.0 (1.0–4.0)	1.03 (0.5–6)
$t_{1/2}$ (h)	72	3.49 (1.89–6.95)	7.34 (3.53–28.3)
CL/F (liter/h/kg)	72	0.31 (0.11–1.06)	0.12 (0.02–0.32)
V (liter/kg)	72	1.45 (0.86–6.49)	1.28 (0.78–2.83)
$\text{AUC}_{0-8}$ ( $\mu\text{g} \cdot \text{h/ml}$ )	85	44.2 (12.1–75.8)	
$\text{AUC}_{0-24}$ ( $\mu\text{g} \cdot \text{h/ml}$ )	72	66.7 (18.8–120.7)	103 (48–755)

<sup>a</sup> All values are presented as means (ranges), except for  $T_{\max}$ , CL/F, and V, which are presented as medians (ranges); adult values are all reported as medians (ranges).

<sup>b</sup>  $C_{\max}$ , maximum serum concentration;  $T_{\max}$ , time to maximum serum concentration;  $t_{1/2}$ , half-life; CL, clearance; F, fraction absorbed; V, volume of distribution;  $\text{AUC}_{0-8}$ , area under the concentration time curve from 0–8 h;  $\text{AUC}_{0-24}$ , area under the concentration time curve from 0–24 h.

<sup>c</sup> n = 11 (10).

(44.1  $\mu\text{g} \cdot \text{h/ml}$ ) in our study are lower than would be expected with a 2.5 $\times$  higher dose given that ofloxacin exposures should be dose proportional in the dosing range tested (8, 10). It is unclear if this is because of differences in the study population or drug formulation used, but our findings underline the importance of not relying on a single study conducted in one geographic location to inform global dosing recommendations in children.

The differing  $\text{AUC}_{0-8}$  and  $\text{AUC}_{0-24}$  trends by age in univariable analysis may be due to the fact that the proportion of the total daily AUC that is captured in the first 8 h after dosing is greater in younger children (data not shown) due to more rapid absorption and clearance compared to older children. Children with slower absorption and elimination, and most likely a higher AUC, would be more likely to be excluded from our estimates of  $\text{AUC}_{0-24}$ . Indeed,  $\text{AUC}_{0-24}$  was not estimated in a higher proportion of older children (Table 3), suggesting we may have underestimated the  $\text{AUC}_{0-24}$  in children  $\geq 5$  years old. The differences in  $t_{1/2}$  by age in univariable analysis and association of  $\text{AUC}_{0-24}$  with weight are consistent with the principle of allometric scaling, in which smaller body size is associated with more rapid clearance.

Our large sample allowed us to evaluate covariate effects on the pharmacokinetics of ofloxacin. In multivariable analysis, age and weight were associated with  $\text{AUC}_{0-8}$  and  $C_{\max}$ , and weight was associated with  $\text{AUC}_{0-24}$ . HIV and undernutrition are frequent concomitant conditions among children with MDR-TB and have been associated with failure to culture convert at 2 months and death (31). HIV infection may affect concentrations of some TB medications (32); however, we did not observe any significant effect of HIV infection on ofloxacin pharmacokinetics. This is consistent with the available adult literature (9, 10). Undernutrition also did not have a clinically significant impact on ofloxacin pharmacokinetics. These data suggest that worse outcomes among children with HIV coinfection or undernutrition are not likely due to reduced concentrations of ofloxacin, the key bactericidal drug in the regimen.

The lack of child-friendly formulations of second-line TB medications is a major challenge for MDR-TB treatment in children, and the impact of formulation manipulation, such as the crushing or breaking of adult tablets, has not been evaluated fully.

**TABLE 3** Pharmacokinetic measures for ofloxacin (20 mg/kg) in children receiving treatment or prevention for multidrug-resistant tuberculosis, by age, HIV status, nutritional status, and administration method<sup>a</sup>

Parameter	No. of children	$C_{\max}$ ( $\mu\text{g}/\text{ml}$ )	$P$ value	$\text{AUC}_{0-8}$ ( $\mu\text{g} \cdot \text{h}/\text{ml}$ )	$P$ value	No. of children	$\text{AUC}_{0-24}$ ( $\mu\text{g} \cdot \text{h}/\text{ml}$ )	$P$ value	$t_{1/2}$ (h)	$P$ value
<b>Age group</b>										
0 to <2 yr	24	10.43 (1.96)		45.9 (8.8)		23	63.9 (15.3)		3.01 (0.53)	
2 to <5 yr	39	8.52 (2.37)		43.8 (12.0)		35	66.5 (20.9)		3.52 (0.75)	
$\geq 5$ yr	22	8.18 (2.01)	<0.001	43.1 (8.9)	0.632	14	71.7 (17.8)	0.473	4.18 (1.22)	<0.001
<b>HIV status</b>										
HIV infected	11	8.42 (1.51)		42.5 (9.0)		9	63.4 (16.4)		3.35 (0.59)	
Not HIV infected	74	9.05 (2.44)	0.404	44.4 (10.6)	0.560	63	67.1 (19.0)	0.579	3.51 (0.93)	0.614
<b>WAZ</b>										
< -2.0	18	8.94 (2.35)		42.7 (11.4)		15	61.0 (20.1)		3.06 (0.49)	
$\geq -2.0$	67	8.98 (2.35)	0.953	44.6 (10.1)	0.498	57	68.2 (18.1)	0.190	3.60 (0.94)	0.004
<b>Administration</b>										
Whole	11	7.87 (1.67)		42.2 (10.6)		8	72.4 (23.4)		4.32 (1.45)	
Crushed	72	9.16 (2.40)	0.089	44.6 (10.4)	0.481	62	66.1 (18.1)	0.375	3.39 (0.76)	0.114

<sup>a</sup> HIV status, nutritional status, and administration method comparisons were generated using  $t$  tests; age group comparisons were generated using one-way analyses of variance (ANOVAs); all values are presented as means (standard deviations). A total of 85 children participated in the study.

Many children in our study were unable to swallow whole ofloxacin tablets and took them crushed. In univariable analysis, there was a trend toward a higher  $C_{\max}$  with crushed tablets; however, crushing did not contribute to the multivariable model, which included age and weight. The associations of  $C_{\max}$  with age and weight described here are somewhat unexpected and should be interpreted cautiously, as crushing was highly associated with younger age and less so with weight, and it may have been difficult to separate these effects in the model. There was no association between crushing and  $\text{AUC}_{0-8}$  or  $\text{AUC}_{0-24}$ . Although this does not replace a formal assessment of relative bioavailability of crushed versus whole tablets, it suggests that crushing tablets does not negatively impact drug exposures and crushing may, in fact, increase the rate or magnitude of absorption.

When efficacy of a TB drug has been established in adults, efficacy studies may not be required in children, but studies characterizing a drug's pharmacokinetics and safety in children are essential. This allows the selection of dosages that achieve concentrations associated with treatment success in adults (9, 10). In a study of ofloxacin pharmacokinetics after an 800-mg dose (median dose, 14.5 mg/kg) in adults with MDR-TB at two sites in South Africa, estimated pharmacokinetic parameters were a  $t_{1/2}$  of 7.8 h and a  $C_{\max}$  of 8.8 to 10.4 mg/liter (9). In a U.S. study, 11 adults with TB (median age, 42 years [range, 22 to 57 years]; median weight, 64 kg [range, 50 to 86 kg]; 3 HIV infected) underwent intensive pharmacokinetic sampling on ofloxacin at steady state with a median dose of 800 mg (range, 600 to 1,200 mg). Assays were performed using high-performance liquid chromatography, and data were analyzed using population pharmacokinetic modeling (Table 2). Using simulations based on their population model generated from these data and from an additional group in this study having sparse pharmacokinetic sampling, estimated pharmacokinetic parameters after an 800-mg once-daily dose were an  $\text{AUC}$  of 100.7  $\mu\text{g} \cdot \text{h}/\text{ml}$  and a  $C_{\max}$  of 9.35  $\mu\text{g}/\text{ml}$  (10). The  $C_{\max}$  in our children was only slightly below these reported adult values, although the children received a higher milligram per kilogram dose (20 mg/kg) compared to the adults (9). However, the

estimated  $\text{AUC}_{0-24}$  in our children of 66.7  $\mu\text{g} \cdot \text{h}/\text{ml}$  was far below the adult value (103  $\mu\text{g} \cdot \text{h}/\text{ml}$ ) (10). This is likely related to the more rapid clearance of ofloxacin in children; calculated  $t_{1/2}$  in children in our study was 3.5 h compared to 7 to 8 h in the adult studies. That currently recommended dosages of ofloxacin result in AUCs in children well below those of adult targets has important implications for MDR-TB treatment and prevention, particularly given the fluoroquinolones' high bactericidal activity (33) and their key role in current treatment regimens (34). The AUC is believed to be the most important pharmacodynamic measure for the fluoroquinolones against *M. tuberculosis* (35). As our data were derived in an optimal setting with an exact 20-mg/kg dose, drug exposures with unsupervised dosages closer to the lower end of the recommended range (15 to 20 mg/kg) may be even lower. Although additional studies corroborating the findings in our study would be useful, it may not be prudent to wait on such studies before reevaluating pediatric dosing. Population pharmacokinetic modeling can be used to predict dosages most likely to achieve adult targets; this information is urgently needed, and such an analysis is planned from this cohort. Higher dosages should be introduced carefully, though, to assess their safety and tolerability, particularly given that the  $C_{\max}$  may exceed the  $C_{\max}$  in adults receiving 800 mg daily.

Ofloxacin was generally safe and well tolerated. The overall person-time of observation for adverse events was more limited than expected, as many children were switched from ofloxacin to levofloxacin or moxifloxacin during their treatment, following a national treatment guideline change mid-study. Adverse effects were of low grade, and there were no ofloxacin-related grade 3 or 4 events. There was no evidence of arthralgia or arthropathy in our cohort. Subtle arthralgia may not have been reported, but it is unlikely clinically significant arthralgia or arthritis would have been missed. There were two reports of insomnia attributable to ofloxacin, a well-described adverse effect of this medication (5, 36). Anecdotally, we have seen self-limited, mild insomnia and nightmares attributable to ofloxacin not infrequently; our data may underestimate the

incidence, as many children were admitted to hospital wards early in their treatment and sleep disturbance may be less noticeable by ward staff than by parents. Our safety assessment is limited by the lack of a control group and by the difficulty in attributing adverse effects to individual drugs in a multidrug regimen that typically includes ethambutol, pyrazinamide, amikacin, ethionamide, terizidone, and high-dose INH. Our approach was, however, conservative and more likely to have overestimated ofloxacin-related adverse effects. These data add to a growing body of evidence that the fluoroquinolones are safe in children, including in long-term use (37). The lack of adverse effects may be related to the relatively low exposures, and safety should continue to be monitored closely if dosages are increased.

Treatment outcomes in this cohort were generally good and will be reported elsewhere; however, one child had documented acquisition of ofloxacin resistance during treatment. This HIV-infected child had a complicated course with large recurrent tuberculous brain abscesses requiring the use of multiple immunosuppressant medications and was treated with multiple fluoroquinolones prior to resistance development, making it difficult to ascribe the resistance acquisition solely to ofloxacin concentrations. Additionally, this child's ofloxacin  $C_{max}$  and AUC were each above the median. Despite generally good outcomes, optimized dosing of the fluoroquinolones in children remains an important priority and may potentially improve outcomes further and facilitate the use of shorter, injectable sparing MDR-TB treatment regimens.

In conclusion, in this large cohort of children receiving ofloxacin, exposures were lower than those in adults. Although ofloxacin is being phased out of MDR-TB treatment regimens in favor of more potent fluoroquinolones, it is still used in many places and it is likely underdosed in children. That ofloxacin was safe and well tolerated is reassuring, particularly if higher dosages that will be needed to reach adult reference exposure targets are to be evaluated. A better understanding of the pharmacokinetics and safety profiles of all second-line anti-TB drugs is essential to ensure the provision of appropriate drugs at appropriate dosages to children with MDR-TB to optimize treatment outcomes.

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We declare no conflicts of interest.

**TABLE 4** Adverse events in children treated for multidrug-resistant tuberculosis with ofloxacin<sup>a</sup>

Adverse event	Adverse event by grade				Total no. of events	Event rate (per person-yr)	Adverse effects possibly, probably, or definitely attributed to ofloxacin by grade				Total no. of events	Event rate (per person-yr)
	No. of patients with event	Grade 1	Grade 2	Grade 3			Grade 4	No. of patients with event	Grade 1	Grade 2		
Arthralgia	1	1	0	0	1	0.042	0	0	0	0	0	0
Arthritis	0	0	0	0	0	0	0	0	0	0	0	0
Pain other than traumatic injury	10	10	2	0	12	0.504	1	1	0	0	1	0.042
Headache	7	5	3	0	8	0.336	1	1	0	0	1	0.042
Neurosensory alteration	0	0	0	0	0	0	0	0	0	0	0	0
Insomnia	2	0	2	0	2	0.084	2	0	2	0	2	0.084
Fatigue/malaise	3	3	0	0	3	0.126	1	0	0	0	1	0.042
Nausea	8	9	0	0	9	0.378	2	2	0	0	2	0.084
Vomiting	12	16	1	0	17	0.714	3	4	0	0	4	0.168
Anorexia	4	3	1	0	4	0.168	0	0	0	0	0	0
Cutaneous reaction	4	5	0	0	5	0.210	1	1	0	0	1	0.042
Pruritus	12	13	0	0	13	0.546	4	4	0	0	4	0.168
Acute systemic allergic reaction	0	0	0	0	0	0	0	0	0	0	0	0
ALT	8	5	1	1	1	0.336	5	4	1	0	5	0.210
Bilirubin	0	0	0	0	0	0	0	0	0	0	0	0

<sup>a</sup> Forty-six patients were followed for a median time of 149.5 days (IQR, 36 to 308 days); total number of person-years was equal to 23.80.



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