Permeability of intestinal mucosa to crystalline and tabletted isoniazid (INH)

To the Editor: When administered orally, isoniazid (INH), which continues to form the basis of most first-line standard antituberculosis regimens, reaches peak plasma concentrations of 3 - 5 µg / ml within 1 - 2 hours after ingestion of usual doses.[1] Various tablet and slow-release matrix forms are available and these processed preparations are most often used clinically. However, various studies undertaken in our Department have shown crystalline INH to produce better blood levels within the first 2 hours after ingestion than the commonly used tabletted form.[2,3]

We previously investigated the suitability of using frozen / thawed human intestinal and vaginal mucosa for performing *in vitro* bioavailability / bioequivalence (BA / BE) studies for drug registration purposes.[4] From these studies it was clear that the above *in vitro* model showed promise, particularly for studying therapeutic agents with molecular weights < 500 Da.

This study aimed to further evaluate and validate the above *in vitro* methodology for BA / BE purposes.

Materials and methods

Intestinal mucosa. Clinically healthy small intestine specimens were obtained from 6 patients (2 males and 4 females), mean age 61 ± 10 standard deviations (SD) (range 47 - 72) years, following various surgical procedures at Tygerberg Hospital.

Specimens were transferred to our laboratory in transport fluid within 1 hour, snap-frozen in liquid nitrogen and stored at – 85°C.[4] After thawing, these specimens were used for experiments as earlier studies failed to show significant flux differences between fresh and frozen intestinal tissue.[4]

Only clinically healthy specimens were obtained and the study was approved by the local Ethics Committee.

Drugs. Crystalline isonicotinic acid hydrazide (isoniazid (INH)) (Fluka Chemie, Buchs, Switzerland) was made up to 100 mg / ml in PBS buffer (phosphate-buffered saline, pH 7.4). Twenty tablets (100 mg INH each) (Be-Tabs Pharmaceuticals, Roodepoort, South Africa) were weighed and pulverised in a mortar. An aliquot (274.4 mg) of the powder, representing an INH concentration of 100 mg, was suspended in 20 ml of PBS buffer. Centrifugation separated insoluble tablet components from the clear solution. Stability of the INH in PBS buffer was monitored, no degradation (recovery = 99.8 ± 0.4%, N = 6) being detected over the entire linear (r = 0.99998, N = 6) calibration range (1 - 20 µg / ml) over a period of 3 days.

Permeability experiments. Sections of thawed specimens were mounted in flow-through diffusion cells and permeation experiments performed as previously described.[4] Before experiments, specimens were equilibrated for 10 minutes with PBS at 20° C in the apparatus. Thereafter, PBS was removed from the donor compartment and replaced with either 1.5 ml of 5 µg / µl crystalline INH, or 5 µg / µl INH in PBS obtained from tablets. PBS was pumped through the receiving chambers (1.5 ml / h) and collected at 2-hour intervals for 24 hours.

HPLC analysis of permeants. Permeant-containing samples were derivatised with 1% (v / v) cinnamaldehyde in methanol and analysed by means of high-performance liquid chromatography (HPLC) using a Hewlett Packard 1090 L instrument equipped with a Partecil 5 C_8 250 mm column (inner diameter = 4.6 mm) as previously described.[5]

Calculation of flux values. Flux (J) values across membranes were calculated by means of the relationship J = Q / Ax t (µg / cm² / min), where Q = quantity of INH crossing membrane (µg), A = membrane area exposed (cm²) and t = time of exposure (minutes).

Steady-state kinetics. When no statistically significant differences ($p < 0.05$) (analysis of variance (ANOVA) and Duncan's multiple range test) between flux values were obtained over at least two consecutive time intervals, a steady state was assumed to have been reached for a particular mucosal specimen and permeant.

Statistical analysis. An unpaired *t*-test with Welch's correction was used to investigate possible differences between flux means at 2-hour intervals. A significance level of 5% was used for all tests and comparisons.

Results

Overall mean flux values for crystalline and tabletted INH across frozen / thawed intestinal mucosa versus time are shown in Fig. 1. Steady-state flux conditions were reached after

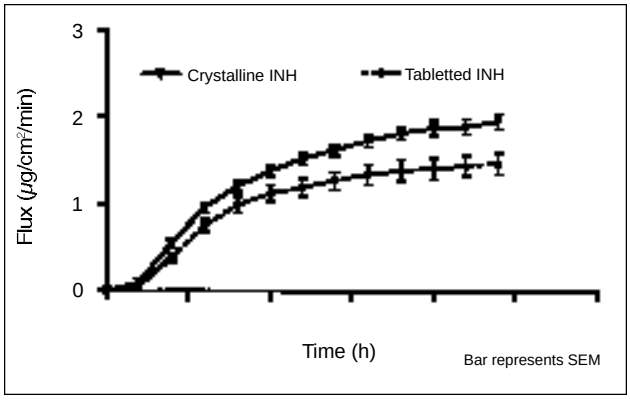

Fig. 1. Overall mean flux values for crystalline and tabletted INH across frozen/thawed human intestinal tissue.

approximately 8 hours. Flux rates of crystalline INH across intestinal mucosa tended to be higher than those for tabletted INH during the entire experiment, but were only significantly different ($p < 0.05$) after 10 hours.

Discussion

We recently demonstrated that a continuous flow-through mucosal perfusion system with intestinal mucosa showed promise as an *in vitro* method for determining the permeability of agents from the gastrointestinal tract for drug registration purposes.[4] However, simultaneously, we observed that intestinal mucosa used in the above system was not very permeable to molecules with weights (M_w) > 500 Da. We therefore suggested that other mucosae, e.g. vaginal mucosa, might have to be considered as substitutes if large M_w agents are to be compared for BA/BE. Akin to other hydrophilic compounds, INH (M_w = 137.14 Da) probably permeates the intestinal mucosa via intercellular routes, the mucosal membrane in conjunction with the epithelial tight junctions providing the rate-limiting barrier.[6]

Although there was a tendency for flux rates of crystalline INH to be higher than those of the tabletted form, these differences were only significantly different after 10 hours (Fig. 1). A possible explanation is that the presence of the soluble exipients in the tablets may alter the permeability properties of INH across this barrier. However, it is clear that the intestinal mucosal barrier appears to discriminate between the diffusion rates of what is the same molecule with and without exipients. In conclusion, we have shown that intestinal mucosa has barrier properties which may be useful to assess BA/BE properties of therapeutically active compounds *in vitro*. Further studies using the above system are therefore indicated.

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Clinically silent renal tumour producing erythropoietin

To the Editor: Asymptomatic patients may be referred for a haematology opinion when the routine blood count reveals elevation in haemoglobin level, haematocrit or red cell count.[1] There is an all too cavalier approach to this abnormality, which is not only an independent risk factor for arterial or venous thromboembolic disease[2] but may signal unsuspected underlying cardiac or pulmonary pathology.[3] Infrequently these findings may draw attention to an otherwise silent myeloproliferative syndrome in the form of primary proliferative polycythaemia or polycythaemia rubra vera of old.[4] Occasionally, and of major clinical importance, is inappropriate erythropoietin production by tumours, including kidney tumours.[5]

A systematic approach to this problem is essential.[1] The first step is to distinguish spurious from absolute erythrocytosis by simultaneous determination of red cell mass and plasma volume[6] since the latter may be independently reduced in a number of situations, including cigarette smoking.[7] In the majority of cases the most common cause is found in the lungs with desaturation that enhances normal erythropoietin production via a renal sensing mechanism. Less frequently encountered is right-to-left shunting at the level of the heart or the great vessels. Conversely, increases in neutrophil and platelet count signal autonomous haematopoiesis in chronic myeloproliferative syndromes. Less frequent, but of major importance, is the need to recognise individuals with ectopic production of erythropoietin; of these the classic example remains cerebellar haemangioblastoma.[1] More common causes are found in renal cell carcinoma,[5] uterine fibroids,[8] and haemangiomas of the liver[9] and lung.[10]

A case is reported here to illustrate the occurrence of symptomatic expansion of red cell mass as a reminder to search carefully for otherwise silent tumours in this clinical context.

A 54-year-old woman was referred by her primary care physician with a haemoglobin of 183 g/l, a packed cell volume of 55%, and normal platelet and white cell count. Her history dated back 6 weeks. While at a health farm she had had routine blood tests done primarily to define her endocrine status as a basis for postmenopausal hormone replacement therapy. Seventy-five per cent thyroidectomy had been carried out 3 years previously for a benign lesion. She was on 100 µg of