SAMJ

ARTICLES

The pharmacokinetic behaviour of hypoxoside taken orally by patients with lung cancer in a phase I trial

C. F. Albrecht, P. B. Kruger, B. J. Smit, M. Freestone, L. Gouws, R. Miller, P. P. van Jaarsveld

Objective. To study the pharmacokinetic behaviour of hypoxoside taken orally by 24 patients with lung cancer.

Design. Randomised open study with three single doses of 1 600, 2 400 and 3 200 mg standardised *Hypoxis* plant extract (200 mg capsules) and a multiple-dose study on the first 6 patients taking 4 capsules 3 times daily for 11 days.

Participants and setting. Patients with histologically proven squamous, large-cell or adenocarcinoma were hospitalised at the Radiation Oncology Ward, Karl Bremer Hospital, Bellville, W. Cape.

Methods. Blood was drawn at regular intervals up to 75 hours after single doses and the concentrations of metabolites of the aglucone of hypoxoside, rooperol, were measured with a high-performance liquid chromatography method. For the multiple-dose study blood was drawn before the first dose each day. Concentration-time relationships were analysed according to a conventional single open-compartment model and also by using the NONMEM digital computer programme.

Results. Neither hypoxoside nor rooperol appear in circulation. This is due to complete phase II biotransformation to diglucuronide, disulphate and mixed glucuronide-sulphate metabolites, of which the latter is the major component. Considerable interpatient variation in concentration-time relationships was found in the single-dose studies. It was due to an active enterohepatic recirculation in some patients and a distinct lag phase in others together with zero-order rate of formation of rooperol in the colon. Computer modelling indicated a single open-compartment model in which the mass of the patient did not influence volume of distribution and clearance because formation of the metabolites is dependent on the metabolising capacity of the patient. However, the elimination of the metabolites follows first-order kinetics with half-lives ranging from 50 hours for the major metabolite to 20 hours for the two minor metabolites. Multiple-dose studies also showed large interpatient variation.

Conclusion. In order to reach metabolite levels near 100 μg/ml, which have been shown to be tumouricidal after enzymatic deconjugation to rooperol, maintenance doses need to be individualised for each patient. For most patients, however, a daily dose of 2 400 mg was sufficient.

S Afr Med J 1995; **85:** 861-865.

Hypoxoside is the major diglucoside found in extracts of the corms of several *Hypoxis* species.[1-4] Historical and anecdotal cases of cancer cure by drinking extracts of *Hypoxis* led to a recognised claim for its value as an anticancer remedy.[5]

In vitro hypoxoside has been shown to be non-toxic to cancer cells at concentrations up to 100 μg/ml.[6,7] However, when it is hydrolysed to its aglucone, rooperol, by β-glucosidase, cytotoxicity is found at concentrations ranging from 2 to 10 μg/ml.[6,7] This action may be related to the formation of reactive semiquinones and quinones which are known to occur with dicatechols such as the closely related cytotoxic agent, nor-dihydroguaiaretic acid.[8]

In vivo hypoxoside has remarkable pharmacokinetic properties. After oral ingestion the parent drug is not found in the circulation. Only phase II metabolites (glucuronides and sulphates) of rooperol are found which are also non-toxic in tissue culture experiments.[4,6] Furthermore, a very distinct species difference is present in so far as the rooperol metabolites are found in the bile only of experimental animals such as the mouse, rat and dog, while they appear in the serum of man and the baboon.[4,6]

We obtained permission from our Ethics Committee and the South African Medicines Control Council to conduct a phase I pharmacokinetic and toxicity trial with 24 lung cancer patients for whom no alternative therapy was available, based on the arguments that: (*i*) the pharmacokinetic properties of hypoxoside preclude its use in rodent models for *in vivo* anticancer studies; (*ii*) several lung cancer cell lines are extremely sensitive to rooperol;[6] and (*iii*) rooperol is not a mutagen in the Ames test (to be published elsewhere).

In this report we document the pharmacokinetic properties of the metabolites when hypoxoside is taken orally by humans.

Departments of Pharmacology and Radiotherapy, University of Stellenbosch, Tygerberg, W. Cape

C. F. Albrecht, PH.D.

P. B. Kruger, PH.D.

B. J. Smit, M.MED.

L. Gouws, M.MED.

P. P. van Jaarsveld, PH.D.

Essential Sterolin Products, Halfway House, Gauteng

M. Freestone, R.N.

Department of Pharmacology, University of Durban-Westville, Durban

R. Miller, PH.D. (Present address: Division of Biopharmaceutics, Food and Drug Administration, Rockville, Md., USA)

Material and methods

Medication

Hypoxoside was supplied by Essential Sterolin Products in capsule form, each capsule containing 200 mg of standardised plant extract. Fig. 1 shows a high-performance liquid chromatogram of the contents of a capsule which illustrates the elution profile of hypoxoside as described by Kruger *et al.*[4] Routine HPLC analyses assured uniform quality of each batch of plant extract within the following limits: 50 - 55% hypoxoside, 8 - 12% β-sitosterol and 0,2 - 0,3% β-sitosterol glucoside. The remaining 33 - 42% of the extract, not detectable by the HPLC system, consisted largely of complex carbohydrates with traces of reducing sugars, amino acids, tannins and flavonoids. Tests for alkaloids were negative.

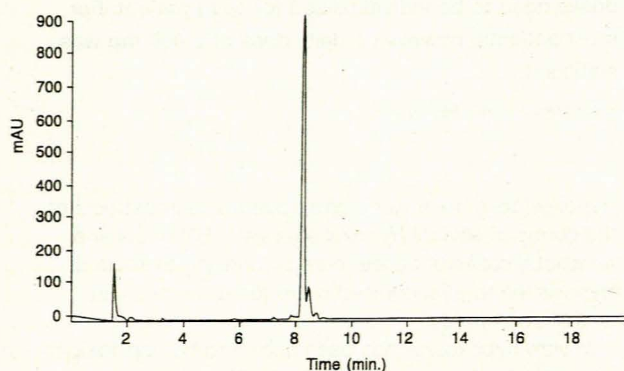

Fig. 1. HPLC of standardised *Hypoxis* extract. Hypoxoside with Rt = 8,2 minutes is the major component with traces of dehydroxy- and bis-dehydroxy-hypoxoside present (Rt = 8,5 and 8,7 minutes, respectively).

Patient eligibility

Patients had to be above 21 years of age with histologically proven squamous cell, large-cell or adenocarcinoma of the bronchus. Informed consent according to the Declaration of Helsinki was required from each patient. Exclusion criteria included impaired renal, hepatic or cardiac function, concomitant anticancer therapy, inadequate performance status (H2 or lower), and patients who, in the opinion of the investigator, had less than 3 months to live.

Study design

The approved protocol included a randomised, open, single-dose study with three dosage levels (8, 12 and 16 capsules) administered to three groups of the 24 patients. The first group of 6 patients who entered the trial also participated in a controlled multiple-dose study for 11 days.

Patients were hospitalised in the Radiation Oncology Ward, Karl Bremer Hospital, Bellville, W. Cape for the single- and multiple-dose studies. Upon admission the medical histories of all patients were recorded and they underwent thorough baseline physical examinations which included full neurological and cardiological examinations, ophthalmoscopy and audiometry as well as full haematological and biochemical investigations.

Analyses and pharmacokinetic calculations

Hypoxoside metabolite concentrations were measured by high-performance liquid chromatography (HPLC) according to the method of Kruger *et al.*[9] For pharmacokinetic calculations a single open-compartment model[10] was used initially to fit concentration-time data of the metabolites as they became available. The single-dose data were analysed after completion of the trial by using the digital computer programme NONMEM (version 2, level 1, double precision).[11] Estimates were obtained for: (*i*) the population means of pharmacokinetic parameters assuming a one- or two-compartment open model with first-order absorption; (*ii*) variance of the population means; (*iii*) variance of the residual random error; (*iv*) standard errors of the parameter estimates; (*v*) correlation matrix of the estimates; and (*vi*) value of the objective function, which is equal to minus twice the log-likelihood of the data.

Intersubject variability in clearance (Cl) and volume of distribution (V), as well as residual intrasubject error, were modelled with additive (homoscedastic) error models. As an example the one-compartment open-model parameters are depicted below; however, the same principle applies to the more complex models.

$$Cl_j = Cl + n_j^{Cl}$$
$$Vd_j = V + n_j^{Vd}$$
$$ka_j = ka + n_j^{ka}$$
$$C_{ij} = C_{Mij} + E_{ij},$$

where Cl_j, V_j and ka_j are the pharmacokinetic parameters for the individual as predicted by the regression model, Cl, V and ka are the population means of clearance, volume and absorption rate constant, n_j^{Cl}, n_j^{V} and n_j^{ka} are randomly distributed terms with zero mean and variances ω_{Cl}, ω_V and ω_{ka}, respectively, that distinguish the jth individual's Cl, V and ka from those predicted by the regression model, C_{ij} is the ith observed concentration for the jth individual, and C_{Mij} is the ith observed concentration predicted by the model for the jth individual. This value includes the contribution of the n_j^{Cl}, n_j^{Vd} and n_j^{ka} terms. ϵ_{ij} is the residual intrasubject error term, representing randomly distributed statistical errors with mean zero and variance σ^2.

The significance of various factors that influence the pharmacokinetic parameters was tested by noting the difference in the objective function values (DOBF) obtained from the evaluation of the full model and restricted model (one or more parameters fixed). The difference in the objective function values obtained for the general and restricted models is approximately chi-square-distributed with degrees of freedom equal to the number of fixed parameters in the restricted model.[12] A DOBF of 3,8 for 1 degree of freedom ($P < 0,05$) was considered statistically significant.

Results

Fig. 2 shows a high-performance liquid chromatogram of the serum of a patient taking hypoxoside orally. It was shown by

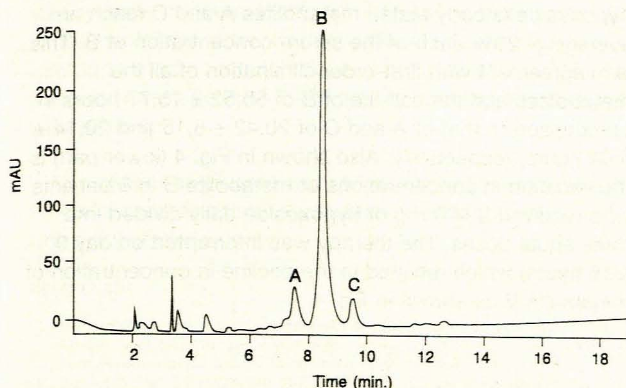

Fig. 2. HPLC of the metabolites of hypoxoside present in the serum of a patient. Metabolite A (Rt = 7,6 min) is the diglucuronide of rooperol; metabolite B (Rt = 8,4 min) the mixed glucuronide-sulphate and metabolite C (Rt = 9,6) the disulphate.[4]

Kruger *et al.*[4] that the major metabolite B with Rt = 8,4 minutes is a mixed glucuronide-sulphate conjugate of rooperol. The two minor metabolites A (Rt = 7,6 minutes) and C (Rt = 9,6 minutes) are the diglucuronide and disulphate conjugates, respectively.

The concentration-time relationship of the major metabolite B, studied in groups of 7, 7 and 6 patients who received single doses of 1 600, 2 400 and 3 200 mg of standardised plant extract respectively, is shown in Fig. 3. It is clear that a relatively large interpatient variation exists. This is understood in terms of the fact that the concentration of metabolites in the serum obviously depends both on the rates of deconjugation of hypoxoside and on the conjugation of rooperol. Furthermore, an enterohepatic recirculation is evident in some patients where the initial rapid rise in concentration of metabolite B is followed by a drop and a further rise in concentration during the first 15 hours after ingestion. In some patients a distinct lag phase is present before appearance of metabolite B. Calculations of the average area under the curve (AUC) of the concentration-time relationships revealed that it is not linearly related to dose. If the average AUC of the 1 600 mg dose is taken as one, relative values of 0,86 and 1,55 are obtained for the 2 400 and 3 200 mg doses respectively. This implies that formation of rooperol, and possibly its absorption, is a zero order saturable process. Consequently escalating doses usually given in phase I trials in order to reach toxic blood levels were not applicable in this study.

The data shown in Fig. 3 were subjected to NONMEM analyses in a stepwise fashion to fit the following models:

A one-compartment open model (model 1) assuming that all patients have the same clearance (Cl), volume of distribution (V), rate of absorption (ka) and absorption lag time (Tlag). Differences are assumed to be due to random inter- and intrasubject variation. Iterative estimates of the fixed effects parameters with residual interindividual variance (ω) and residual intrasubject error variance (σ^2) are presented in Table I.

Model 2 is a two-compartment open model which assumes that all patients have the same Cl, ka, Tlag, volume of the central compartment (V_1), volume of the peripheral

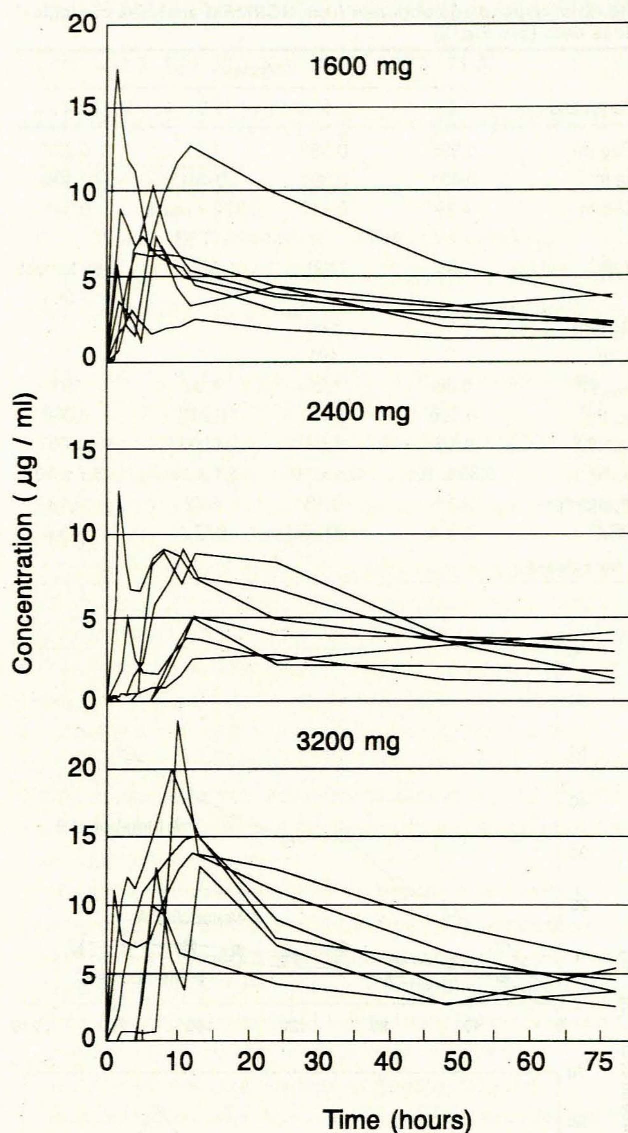

Fig. 3. Concentration-time relationships of the major metabolite (see Fig. 2) in the serum of three groups of patients who took single doses of 1 600, 2 400 and 3 200 mg of standardised plant extract respectively.

compartment (V_2) and intercompartmental clearance (Q_{23}). Differences are assumed to be due to random inter- and intrasubject variation. Results are also presented in Table I. In spite of the increased number of parameters the fit of the two-compartment model to the data is significantly worse than the one-compartment model, since the difference in objective function (OBJ) is 7,4. Model 1 is therefore considered appropriate in further model building.

Model 3 in Table I incorporates mass as a function of clearance. The increase in the objective function of 78 indicates that it is not appropriate to express clearance proportional to mass for this metabolite. Model 4 includes mass in volume of distribution which resulted in an increase of 49,4 in the objective function and is therefore not appropriate.

Table I. Pharmacokinetic parameters of the major metabolite (B in Fig. 2) of hypoxoside obtained from NONMEM analyses of single-dose data (see Fig. 3)

	Model			
Parameter	1	2	3	4
Tlag (h)	0,995	0,586	1,31	0,207
ka (h^{-1})	0,401	0,301	0,401	0,104
Cl (l/h)	4,39	0,317	0,372 x mass + 1,83	0,341
V_1 (l)	308	258	300	3,98 x mass + 394
Q_{23} (l/h)	*	8,66	*	*
V_2 (l)	*	491	*	*
ω_{Tlag} $(h)^2$	5,38	7,26	4,85	191
ω_{ka} $(h)^2$	0,306	0,181	0,219	0,308
ω_{Cl} $(l/h)^2$	1,55	1,04	0,00024	0,0087
ω_{V1} $(l)^2$	6,39 x 10^4	6,5 x 10^4	5,1 x 10^4	3,7 x 10^4
σ^2 $(\mu g/ml)^2$	3,11	3,16	4,72	4,18
OBJ	794,4	801,8	872,4	843,8

* Not included.

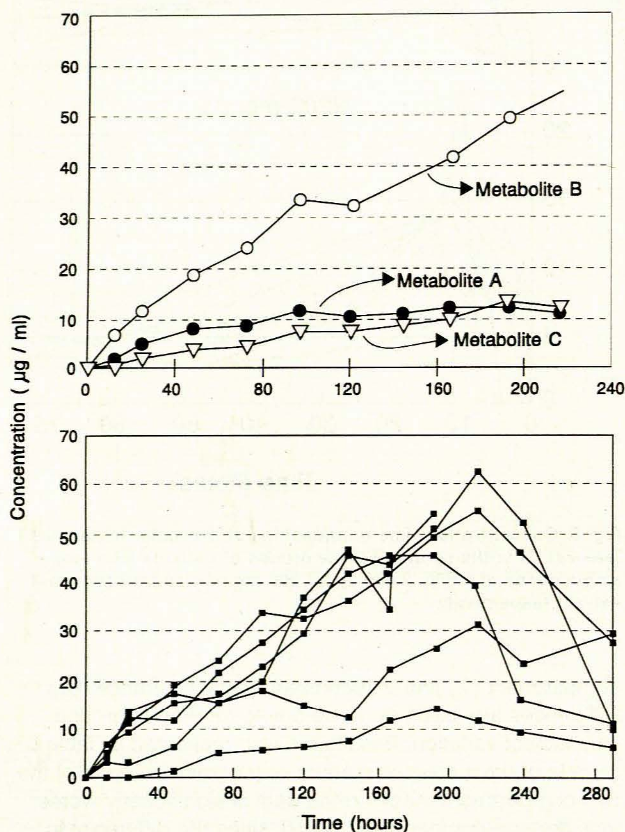

Fig. 4. Concentration-time relationships of metabolites in the serum of patients who ingested 2 400 mg of standardised plant extract daily. Three equal doses were taken and blood was drawn before the first dose each day. The upper part shows the concentrations of each of the three metabolites A, B and C (see Fig. 2) in one patient while the lower part shows the concentrations of metabolite B in 6 patients. The therapy was stopped after 216 hours in 5 patients and after 192 hours in 1 patient. The latter patient was not available to follow the decrease in concentration of metabolite B with time.

Fig. 4 (upper part) shows that with multiple dosing of hypoxoside (steady state), metabolites A and C reach an average of 20% each of the serum concentration of B. This is in agreement with first-order elimination of all the metabolites and the half-life of B of 50,52 ± 15,77 hours in comparison to that of A and C of 20,42 ± 6,15 and 20,14 ± 5,04 hours, respectively. Also shown in Fig. 4 (lower part) is the variation in concentrations of metabolite B in 6 patients who received 2 400 mg of hypoxoside daily divided into three equal doses. The therapy was interrupted on day 9 (216 hours) which resulted in the decline in concentration of metabolite B as shown in Fig. 4.

Discussion

When the pharmacodynamic and pharmacokinetic properties of hypoxoside were investigated by us[4,6,7] it became clear that its possible application as a prodrug in cancer therapy needed to be investigated further. Part of the phase I study undertaken was to assess the pharmacokinetic behaviour of the phase II metabolites since no hypoxoside or rooperol appear in the blood. Only the phase II metabolites can be measured, of which the mixed glucuronide-sulphate is the major component. The diglucuronide and disulphate metabolites appear in the blood at steady state as minor metabolites (approximately 20% of the major metabolite) because their half-lives are only 20 hours in comparison with the 50 hours of the major metabolite.

It was possible to fit the concentration-time relationship of the major metabolite to the simplest model, i.e. a single open-compartment model which provided reasonable estimates of the different pharmacokinetic parameters (Table I, model 1). It must be kept in mind that for calculation of clearance and volume of distribution a bio-availability factor is needed which can only be determined after intravenous administration of the compounds in question. The finding that there is no relationship between clearance, volume of distribution and mass of the patient (Table I, models 3 and 4), however, justifies the indirect method of analysing the data. It is also understandable that mass should not play a significant role since the extent of conversion of hypoxoside to rooperol, and in turn, rooperol to phase II metabolites, is obviously more dependent on the metabolising activity of the gastro-intestinal system (particularly the colon) and the liver of the patient rather than the mass of the patient. The rather large interpatient variation observed both with single-dose and multiple-dose therapy (Figs 3 and 4) is probably also allied to the metabolising capacity of the patient. This includes a lag phase for appearance of the metabolites in the blood which has a coefficient of variation of 225% (Table I). Some patients also clearly showed active enterohepatic recirculation of the metabolites shortly after hypoxoside was ingested (Fig. 3).

Because of the relatively large interpatient variation, and the zero-order formation of metabolites but predictable first-order elimination kinetics, the long-term maintenance doses of patients were adjusted in the toxicity study when necessary during hospital visits according to their combined

metabolite serum concentrations in order to maintain a steady-state concentration near 100 µg/ml. Based on *in vitro* experiments[6] the latter was considered adequate for formation of tumouricidal rooperol concentrations. For most patients a daily dose of 2 400 mg (4 capsules 3 times a day) was sufficient.

This study was initiated and sponsored by Essential Sterolin Products according to an agreement entered into with the University of Stellenbosch.

REFERENCES

1. Marini-Bettolo GB, Patamia M, Nicoletti M, Galeffi C, Messana I. Research on African medicinal plants: II. Hypoxoside, a new glycoside of unknown structure from *Hypoxis obtusa* Busch. *Tetrahedron* 1982; **38:** 1683-1687.
2. Drewes S, Hall AJ, Learmonth RA, Upfold UJ. Isolation of hypoxoside from *Hypoxis rooperi* and synthesis of (E)-1,5-Bis(3',4'-dimethoxyphenyl)pent-4-en-1-yne. *Phytochemistry* 1984; **23:** 1313-1316.
3. Drewes SE, Emslie ND, Hemingway M. Synthesis of three phenolic pent-1-en-4-ynes. *Synthetic Commun* 1990; **20:** 1671-1679.
4. Kruger PB, Albrecht C, Liebenberg RW, Van Jaarsveld PP. Studies on hypoxoside and rooperol analogues from *Hypoxis rooperi* and *H. latifolia* and their biotransformation in man by using high-performance liquid chromatography with in-line sorption enrichment and diode array detection. *J Chromatogr* 1994; **662:** 71-78.
5. Drewes SE, Liebenberg RW. Extracts of plants of the family of *Hypoxidaceae* in the treatment of cancer. US Pat. No. 4652636, 1987.
6. Albrecht CF, Theron EJ, Kruger PB. Morphological characterisation of the cell-growth inhibitory activity of rooperol and pharmacokinetic aspects of hypoxoside as an oral prodrug for cancer therapy. *S Afr Med J* 1995; **85:** 853-860 (this issue).
7. Theron E, Albrecht C, Kruger P, Jenkins K, Van der Merwe MJ. Beta-glucosidase activity in fetal calf serum renders the plant glucoside, hypoxoside, cytotoxic towards BL-6 mouse melanoma cells. *In Vitro Cell Dev Biol* 1994; **30A:** 115-119.
8. Van der Merwe MJ, Jenkins K, Theron E, Van der Walt BJ. Interaction of the dicatechols rooperol and nordihydroguaiaretic acid with oxidative systems in the human blood. *Biochem Pharmacol* 1993; **45:** 303-311.
9. Kruger PB, Albrecht CF, Van Jaarsveld PP. Use of guanidine hydrochloride and ammonium sulphate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high performance liquid chromatography. *J Chromatogr* 1993; **612:** 191-198.
10. Koch HP, Ritschel WA. *Synopsis der Biopharmazie und Pharmakokinetik.* Landsberg: Ecomed, 1986.
11. Beal SL, Sheiner LB. *Users Guides.* San Francisco, Calif.: NONMEM Project Group, University of California at San Francisco, 1988.
12. Sheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. *J Pharmacokinet Biopharm* 1977; **5:** 455-479.

Accepted 18 May 1995.

A phase I trial of hypoxoside as an oral prodrug for cancer therapy — absence of toxicity

B. J. Smit, C. F. Albrecht, R. W. Liebenberg, P. B. Kruger, M. Freestone, L. Gouws, E. Theron, P. J. D. Bouic, S. Etsebeth, P. P. van Jaarsveld

Objective. To assess the toxicity of hypoxoside taken orally by 24 patients with lung cancer.

Design. Open study with patients taking 1 200 - 3 200 mg standardised *Hypoxis* plant extract (200 mg capsules) per day divided in 3 doses in order to maintain metabolite blood levels near 100 µg/ml.

Participants and setting. Patients with histologically proven squamous, large-cell or adenocarcinoma were hospitalised initially at the radiation oncology ward, Karl Bremer Hospital, Bellville, W. Cape. Thereafter they returned every 2 weeks for full clinical examinations.

Methods. Routine biochemical and haematological measurements were done. Patients underwent regular full clinical examinations including radiographs and computed tomography scanning according to the discretion of the principal investigator.

Results. Nineteen patients on hypoxoside therapy survived for an average of 4 months with progression of their primary tumours and metastases, while 5 survived for more than a year. One of them survived for 5 years and histological examination of the primary lesion showed absence of cancer. No toxic effects, in clinical examinations or biochemical or haematological measurements, were found that could be ascribed to the ingestion of hypoxoside. Only one occasion of possible drug intolerance, with anxiety, nausea, vomiting and diarrhoea, was noted.

Departments of Radiotherapy and Pharmacology, University of Stellenbosch, Tygerberg, W. Cape

B. J. Smit, M.MED.

C. F. Albrecht, PH.D.

P. B. Kruger, PH.D.

L. Gouws, M.MED.

E. Theron, M.SC.

P. J. D. Bouic, PH.D.

S. Etsebeth, M.SC.

P. P. van Jaarsveld, PH.D.

Essential Sterolin Products, Halfway House, Gauteng

R. W. Liebenberg, B.COMM.

M. Freestone, R.N.