Cancer Chemother. Pharmacol. 5, 27–37 (1980)

Cancer Chemotherapy and Pharmacology

Dose-dependence and Related Studies on the Pharmacokinetics of Misonidazole and Desmethylmisonidazole in Mice

Paul Workman

MRC Clinical Oncology and Radiotherapeutics Unit, Hills Road, Cambridge CB2 2QH, England

Summary. *An understanding of lipophilicity and pharmacokinetics is important in developing alternative radiosensitizers to misonidazole (MIS). Analogues more hydrophilic that MIS, including its O-demethylated metabolite DEMIS (Ro 05-9963), appear promising candidates. In vivo testing is usually carried out in mice, and the present paper reports dose-dependence and related studies on the comparative kinetics of MIS and DEMIS in this species.*

The data are consistent with a model in which MIS is eliminated mainly by metabolism, including demethylation to DEMIS, and saturable (non-linear) kinetics are exhibited. Apparent $t^1/_2$ increased with dose. But DEMIS is eliminated mainly by renal clearance exhibiting first-order (linear) kinetics. Phenobarbitone reduced the $t^1/_2$ and toxicity of MIS but not of DEMIS. SKF 525A increased the $t^1/_2$ of MIS.

The following were not responsible for the non-linear kinetics of MIS: short-term enzyme induction by MIS; potent enzyme inhibition by the product DEMIS; decrease in body temperature by MIS; injection volume; and protein binding.

For both MIS and DEMIS peak blood concentrations were about 2-fold lower for IP than IV injection. The IP bioavailability of DEMIS (1.07 mmol/kg) was 100%, but that of MIS (1 mmol/kg) was 80%, suggesting some first-pass metabolism. Both MIS and DEMIS were absorbed more slowly and gave lower peak blood concentrations after IP injection in a large (40 ml/kg) as against a small (10 ml/kg) volume. Peak concentrations were lower for equimolar IP DEMIS, but this was less marked at lower doses.

With both MIS and DEMIS, tumour/blood and brain/blood ratios were slightly increased at higher doses. Some kinetic differences were also observed between male and female mice.

The above findings, particularly the major differences in elimination kinetics between MIS and DEMIS, should be considered in in vivo experiments with nitroimidazoles.

Introduction

The nitroimidazoles are selective radiosensitizers of hypoxic cells [1]. One of these, the 2-nitroimidazole misonidazole (1-[2-nitroimidazol-1-yl]-3-methoxypropan-2-ol; Ro 07-0582, Roche Laboratories; MIS), is undergoing extensive clinical trial. However, radiosensitization with MIS is likely to be sub-optimal because the risk of neurotoxicity limits the total dose to about 12 g/m^2 [8, 9, 15, 16]. Thus there is considerable interest in the development of alternative radiosensitizers with improved therapeutic ratios.

Hypoxic radiosensitization efficiency and aerobic cytotoxicity towards mammalian cells in vitro exhibit a marked dependence on reduction potential [2, 3]. In contrast, these properties are independent of lipophilicity over quite a wide range. However, lipophilicity does affect radiosensitization of hypoxic bacterial cells in vitro [4], and this is likely to be true for mammalian cells with extremely lipophilic and hydrophilic analogues.

Despite the undoubted value of in vitro studies for investigation of structure-activity relationships, there is now considerable evidence that radiosensitization and toxicity in vivo are strongly influenced by pharmacokinetic behaviour; this in turn is highly dependent on lipophilicity [20]. In vivo radiosensitization is a function of the overall tumour drug concentration during or just before radiosensitization [12; JM Brown and NY Yu, submitted for publica-

0344-5704/80/0005/0027/$ 02.20

tion]. In contrast, acute lethality in mice [19] and neurotoxicity in man [9] both appear to be related to tissue exposure, as measured by the area under the curve (AUC) of plasma drug concentration × time.

Recently, application of the lipophilicity concept has led to the selection of analogues more hydrophilic and potentially less neurotoxic than MIS [5, 17, 20]. Their advantages include rapid clearance and exclusion from nervous tissues, with no loss in tumour penetration. One of the hydrophilic analogues is the O-demethylated metabolite of MIS, desmethylmisonidazole (1-[2-nitroimidazol-1-yl]-2,3-propandiol; Ro 05-9963, Roche; DEMIS), which has now entered phase 1 clinical trial.

It is clear that pharmacokinetic studies should feature prominently in rational radiosensitizer development. Initial in vivo testing is usually carried out in mice [e.g., 6, 14]. However, as part of a detailed study of the structure-pharmacokinetic relationships of nitroimidazole radiosensitizers in this species, marked differences have been observed between closely related derivatives, and these have considerable implications for in vivo drug development. The present paper reports on the relationship between pharmacokinetic properties and dose for MIS and DEMIS in mice. Related experiments on the effects of route and volume of administration, body temperature, phenobarbitone, SKF 525A and mouse sex are also described.

Materials and Methods

Mice and Tumours

Adult BALB/c mice were obtained from the breeding colony at NIMR (Mill Hill, London) and from Olac (Southern) Limited (Bicester). Adult C3H/He mice were obtained from Olac. Except for urinary excretion studies, they were housed in plastic cages on sawdust bedding prepared from soft white woods (Usher Limited, London) and allowed PRD nuts (Labsure Animal Diets, Poole, Dorset) and water ad lib. Contact with known microsomal-enzyme inducers, such as halogenated hydrocarbon insecticides, was avoided. Mice weighed 20–30 g and unless otherwise stated BALB/c males were used.

EMT6/Ca/VJAC tumours were grown intradermally in the flank of BALB/c mice, as described previously [19]. Mice with tumours in the volume range 50–200 mm^3 were selected.

Drugs

Supplies of MIS, DEMIS and other nitroimidazoles were provided by Roche Laboratories (Welwyn Garden City). β-Diethylaminoethyldiphenylpropylacetate hydrochloride (proadifen hydrochloride; SKF 525A) was provided by Smith, Kline and French Laboratories Limited (Welwyn Garden City), and phenobarbitone (sodium salt) was obtained from BDH Laboratories (Poole).

Measurement of Body Temperature

Body temperatures were measured with a rectal thermistor probe connected to an externally calibrated electric thermometer (Light Laboratories, Brighton).

Determination of LD_{50}

Acute LD_{50} determinations were carried out as before [19]. MIS and DEMIS were injected IP in a volume of 40 or 80 ml Hank's balanced salt solution (HBSS) per kg body weight. Mice were observed for 7 days, but deaths occurred within 3. In some experiments mice were pretreated with phenobarbitone (100 mg/kg/day for 5 days in 10 ml 0.85% w/v saline per kg body weight IP) or saline alone. MIS and DEMIS were given 48 h later.

Pharmacokinetics

In most experiments MIS and DEMIS were injected IP in a volume of 40 ml/kg HBSS. Where indicated, injection volumes of 10 ml/kg were used for the IP route and also for IV injection in the tail vein. In some experiments mice were pretreated with saline or phenobarbitone (see above). In others mice received 50 mg SKF 525A/kg injected IP in 10 ml HBSS per kg body weight or HBSS alone, 1 h before MIS.

Blood samples were collected from the tail or by cardiac puncture [19]. In some experiments whole brain and whole tumour were also removed and immediately frozen in solid CO_2. All samples were stored at −20° C before analysis.

Urinary Excretion

Groups of four to six mice were contained in a Urimax metabolism cage and urine was collected for 24 h after drug administration.

Estimation of Nitroimidazoles

Concentrations of MIS and DEMIS in blood, urine and tissue homogenates were determined by reverse-phase high-performance liquid chromatography (HPLC) as described previously [21] with minor modifications. Concentrations of the glucuronides of MIS and DEMIS were determined by HPLC after hydrolysis with Glucurase (Sigma, Poole).

Estimation of Kinetic Parameters and Statistical Analysis

Apparent elimination half-life ($t^1/_2$) was calculated from the equation $t^1/_2 = 1n2/k'$, where k' is the initial apparent elimination rate constant given by the slope of ln blood concentration × time. Lines of best fit, with standard errors, were calculated by least-squares linear regression analysis. Under conditions where linear kinetics prevail $k' = k$, the true elimination rate constant.

AUC from time 0 to the final time t was estimated by Simpson's rule. The remaining AUC from t-∞ (which was small) was estimated from the equation $AUC_{(t-\infty)} = C_t/k$, where C_t is the

blood concentration at t. AUC values in the *Results* section are for $AUC_{(0-\infty)}$, obtained by summing $AUC_{(0-t)}$ and $AUC_{(t-\infty)}$.

Confidence limits and significance levels were calculated according to Student's *t*-distribution.

Results

Effect of Dose on the Pharmacokinetics of MIS and DEMIS

Figure 1 shows the effect of different doses of MIS, injected IP in 40 ml HBSS/kg, on the concentrations of MIS and its metabolite DEMIS in the blood of BALB/c mice. At lower doses (0.25–1 mmol/kg) the rates of MIS elimination were similar (Fig. 1A). But at higher doses (2.5 and 5 mmol/kg) the initial rate of elimination progressively decreased, becoming similar to that at lower doses only at later times, when the blood concentrations had fallen considerably. Doses > 5 mmol/kg were not studied as these approach the acute LD_{10}.

Figure 2 shows the initial apparent elimination $t^1/_2$ plotted against MIS dose for several independent experiments. This demonstrates both the increase in apparent $t^1/_2$ with dose and the greater between-experiment variability at higher doses.

Similar non-linear kinetics were seen in an experiment with C3H male mice. Apparent $t^1/_2$ values (with 95% confidence limits) were 0.63 (0.55–0.74) h at 0.5 mmol/kg and 2.14 (1.76–2.71) h at 5 mmol/kg ($P < 0.001$).

The effect of different injected doses of DEMIS on its blood concentrations are shown in Fig. 3. The rates of elimination were unchanged over the dose range 1.07–10.7 mmol/kg. Apparent $t^1/_2$ values were 0.70 (0.68–0.72) h, 0.73 (0.67–0.79) h and 0.72 (0.60–0.84) h at 1.07, 5.35 and 10.7 mmol/kg, respectively ($P > 0.1$). In a similar experiment in C3H male mice $t^1/_2$ values were 0.82 (0.69–1.01) h at 0.535 mmol/kg and 0.86 (0.78–0.96) h at 5.35 mmol/kg ($P > 0.1$).

Figure 4A illustrates the dependence of blood AUC on dose for MIS and DEMIS. The AUC for injected DEMIS shows a linear dependence on dose, whereas that for MIS exhibits a marked upward curvature at doses > 1 mmol/kg. At lower doses of MIS (< 2.5 mmol/kg) the AUC is similar to that for equimolar DEMIS, but at higher MIS doses the AUC is considerably greater.

In contrast to the above relationships for AUC, the peak concentration for MIS exhibits a linear dependence on dose, whereas that for injected DEMIS shows some downward curvature (Fig. 4B). Peak concentrations for DEMIS were lower than those for MIS at equimolar injected doses, particularly at high doses.

Figure 4C shows the relationship between the dose of MIS injected and the peak concentration of

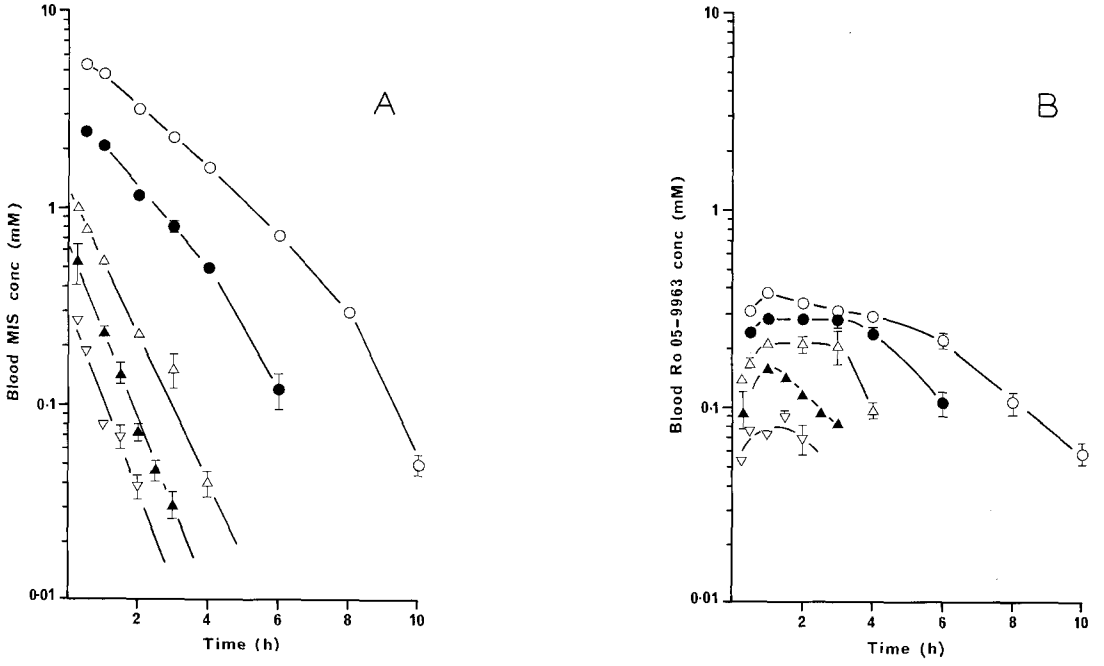

Fig. 1A and B. Effect of dose on the pharmacokinetics of MIS. **A** Blood MIS and **B** blood DEMIS after IP MIS at doses of 0.25 (▽), 0.5 (▲), 1 (△), 2.5 (●), and 5 (○) mmol/kg IP (± SE). Injection volume was constant (40 ml/kg)

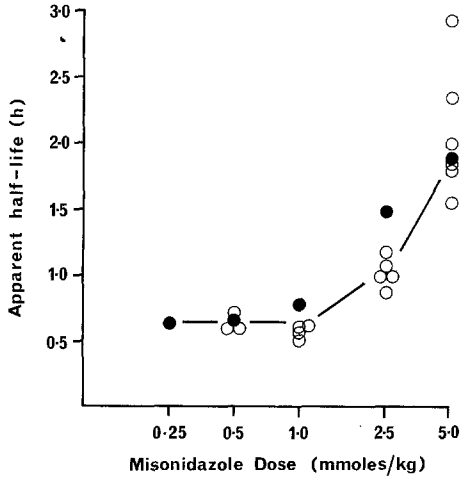

Fig. 2. Effect of dose on the apparent half-life of MIS. ●, data from Fig. 1; ○, data from other experiments. MIS given IP

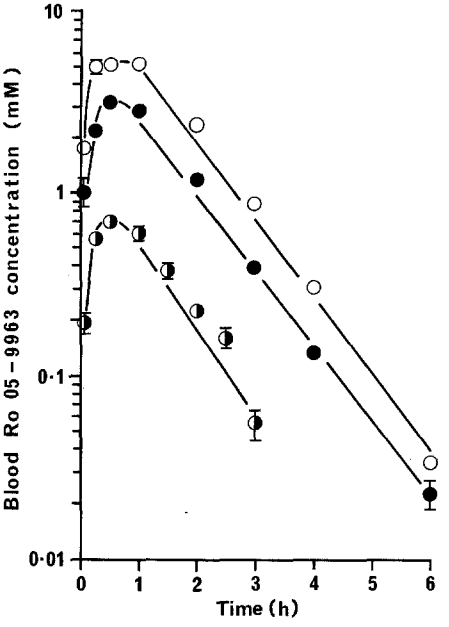

Fig. 3. Effect of dose on the pharmacokinetics of DEMIS. Blood DEMIS concentrations after 1.07 (◑), 5.35 (●) 10.7 (○) mmol/kg IP (± SE)

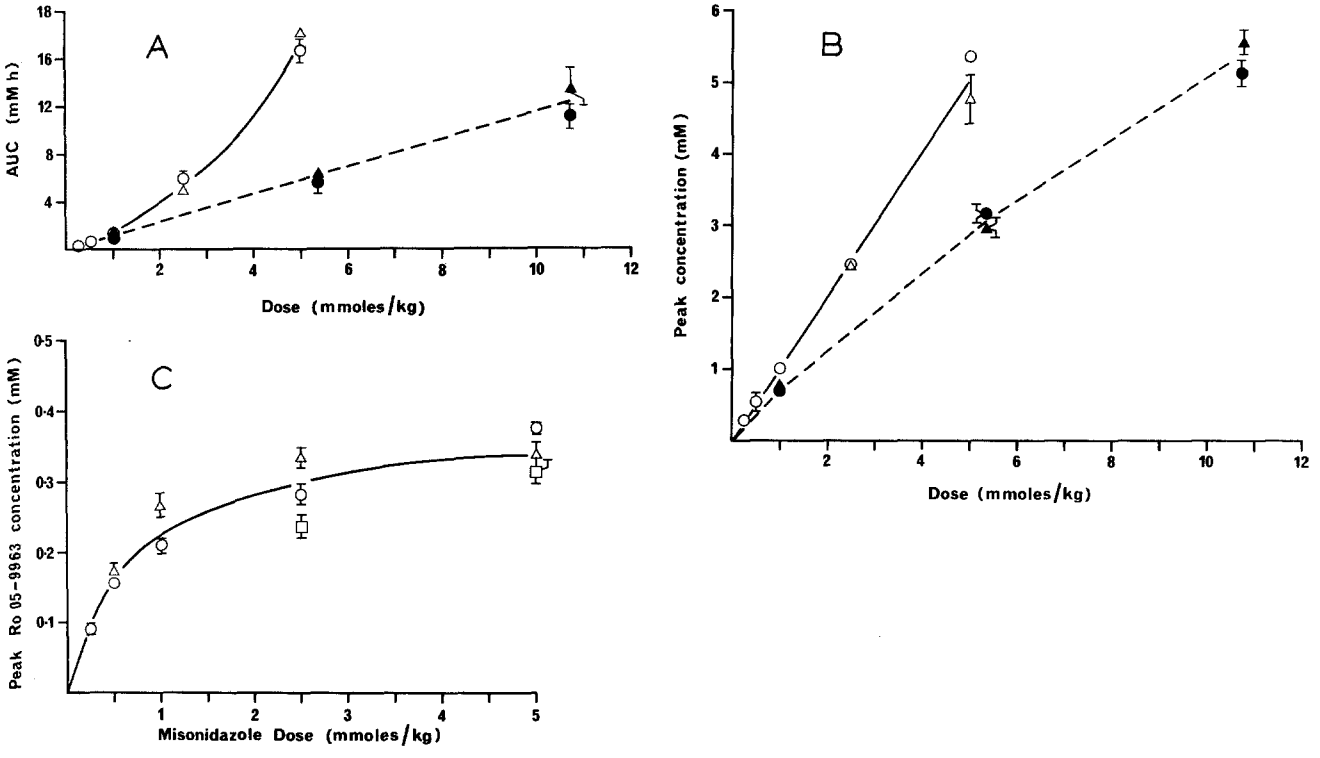

Fig. 4A–C. A Effect of dose on blood AUC. *Open symbols*, MIS; *closed symbols*, injected DEMIS (± SE); **B** Effect of dose on peak blood concentration. *Symbols* as for Fig. 4A (± SE); **C** Effect of MIS dose on DEMIS metabolite concentration (± SE). Drugs given IP in A–C

the demethylated metabolite DEMIS in the blood. The data were taken from Fig. 1B and similar experiments. The metabolite concentration clearly exhibits a non-linear dependence on MIS dose, indicating likely saturation of metabolising enzymes.

Effect of Dose on the Urinary Excretion of MIS and DEMIS

The effects of dose on the urinary excretion of injected DEMIS and MIS are summarised in Table 1. With the large MIS dose a higher percentage was recovered unchanged in the urine and a lower percentage excreted as the metabolite DEMIS. The DEMIS/MIS ratio was 0.68 with the large dose and 1.38 for the small dose. These data also indicate non-linear kinetics and saturation of the demethylation pathway. Compared with MIS, a considerably greater proportion of injected DEMIS was recovered unchanged in the urine at both low and high doses. In contrast to MIS, the percentage of DEMIS recovered unchanged was independent of dose, indicating linear kinetics.

Effect of Phenobarbitone Pretreatment on the Pharmacokinetics of MIS and DEMIS

Figure 5 shows the effect of pretreatment with phenobarbitone (100 mg/kg/day IP for 5 days) on the blood concentrations of injected DEMIS

Table 1. Effect of dose on the 24-h urinary excretion of MIS and DEMIS. Drugs were injected IP

Drug and dose	Percent administered dose excreted in 24-h urine[b]						
	MIS			DEMIS			MIS + DEMIS + glucuronides
	Free	Glucuronide	Total	Free	Glucuronide	Total	
MIS[a] 5 mmol/kg	12, 8	6, 5	18, 13	12, 9	0.2 1	12, 10	30, 23
MIS 0.5 mmol/kg	4, 4	7, 6	11, 10	21, 18	4, 1	25, 19	36, 29
DEMIS 5.35 mmol/kg	–	–	–	65, 61	2, 4	67, 55	67, 55
DEMIS 0.535 mmol/kg	–	–	–	53, 60	11, 5	64, 55	64, 55

[a] Previously published data [19]
[b] Data are shown for two independent determinations

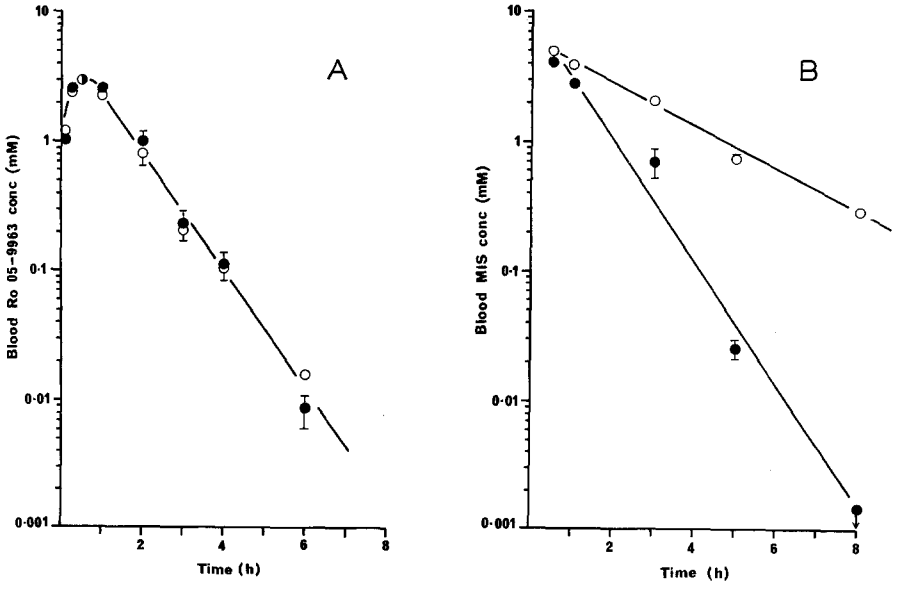

Fig. 5A and B. Effect of phenobarbitone on the blood concentrations of injected DEMIS (5.35 mmol/kg IP), (**A**), and MIS (5 mmol/kg IP) (**B**). ○, saline control; ●, phenobarbitone-pretreated (± SE)

(5.35 mmol/kg IP) compared with data previously published [19] for MIS (5 mmol/kg IP). Phenobarbitone had no effect on the kinetics of injected DEMIS (Fig. 5A). In the experiment shown, the $t^1/_2$ values obtained were 0.68 (0.64–0.74) h for saline pretreatment and 0.66 (0.62–0.72) h after phenobarbitone ($P > 0.1$). In contrast, the $t^1/_2$ for MIS was reduced by about 40% after phenobarbitone (Fig. 5B), and this was associated with an initial 1.5- to 2-fold increase in circulating DEMIS metabolite concentrations, indicating induction of demethylation [19].

Two experiments were also done with MIS given IV at the lower dose of 1 mmol/kg. The $t^1/_2$ was reduced by 10% and 37%, which compares with reductions of 36%–40% with high-dose MIS.

Effect of Phenobarbitone Pretreatment on the Acute Toxicity of MIS and DEMIS

The effect of phenobarbitone pretreatment on the acute LD_{50} of IP injected DEMIS is shown in Table 2 with earlier data [19] for MIS. Whereas the LD_{50} of MIS was increased by 23% ($P < 0.001$), phenobarbitone had no effect with DEMIS ($P > 0.1$). The control acute LD_{50} for DEMIS was more than twice that for MIS.

Effect of MIS Pretreatment on the Pharmacokinetics of MIS

Mice were injected IP with 5 mmol MIS/kg or HBSS vehicle; 12 h later both received 5 mmol MIS/kg IP. This MIS pretreatment had no effect on the kinetics for the second dose (Fig. 6). Thus MIS caused no enzyme induction over this short period.

Effect of Body Temperature on the Pharmacokinetics of MIS

High doses of MIS cause a considerable drop in mouse body temperature [19]. In these experiments blood concentrations of MIS and the metabolite DEMIS were measured after a dose of 5 mmol/kg to mice whose body temperatures were either allowed to fall or maintained at approximately 37°–38° C by controlled external heating with an anglepoise lamp (Fig. 7). Peak blood MIS (30 min) was 27% higher in the warm mice ($P < 0.001$), but otherwise the MIS concentrations were similar. The $t^1/_2$ values were 1.68 (1.53–1.87) h for the controls and 1.59 (1.46–1.74) h for the warm mice ($P > 0.1$). However, the metabolite DEMIS concentrations were rather higher between 1 and 3 h, and lower after 4 h, than in controls. The AUC values for MIS and DEMIS were respectively 6% and 11% higher in the warm mice.

Table 2. Effect of phenobarbitone on the acute LD_{50} of MIS and DEMIS in BALB/c male mice

	Acute $LD_{50\,(7d)}$ (95% confidence limits) (mmol/kg)	
	Saline pretreatment	Phenobarbitone pretreatment
MIS[a]	7.66 (7.36– 7.96)	9.45 (8.81–10.10)*
DEMIS	18.13 (17.70–18.56)	18.18 (17.59–18.82)†

[a] From Ref. [19]
* $P < 0.001$ against saline control
† $P > 0.1$ against saline control
9–11 Different doses of each radiosensitiser were used, and 5–16 mice per group

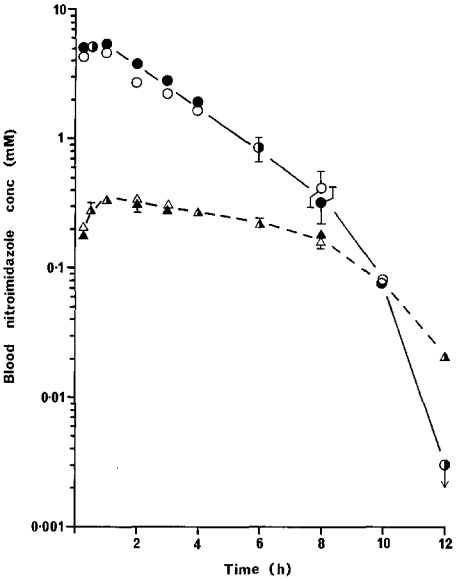

Fig. 6. Effect of 5 mmol MIS/kg IP on the pharmacokinetics of the same dose given 12 h later. Blood concentrations of MIS (○) and DEMIS (△) metabolite in HBSS controls; ●, MIS; ▲, DEMIS in MIS-pretreated group (± SE)

Effect of DEMIS on the Pharmacokinetics of MIS

To investigate the effects of DEMIS on the kinetics of MIS the drugs were injected simultaneously. Figure 8 shows data for 2.5 mmol MIS/kg IP alone or with 10.7 mmol DEMIS/kg. The MIS $t^1/_2$ increased by 33%, from 1.03 (0.93–1.15) h to 1.37 (1.23–1.55) h

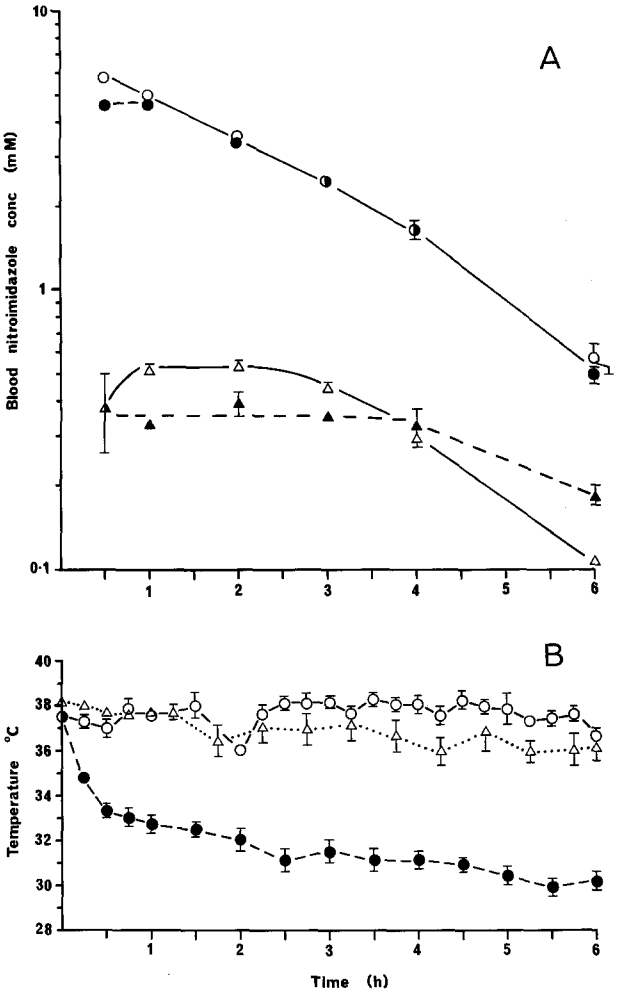

Fig. 7A and B. Effects of body temperature on the pharmacokinetics of MIS. **A** Blood concentration data. MIS (○) and DEMIS (△) metabolite in mice maintained at 37°–38° C; MIS (●) and DEMIS (▲) metabolite in controls (± SE). **B** Rectal temperature (± SE). ○, mice at 37°–38° C after MIS; ●, controls after MIS; △, control mice after HBSS. The MIS dose was 5 mmol/kg IP

($P < 0.001$). With the more comparable dose of 2.67 mmol DEMIS/kg, the MIS $t^1/_2$ increased by only 18% ($0.01 > P > 0.001$).

Effect of SKF 525A on the Pharmacokinetics of MIS

When mice received the mixed-function oxidase (MFO) inhibitor SKF 525A (50 mg/kg IP) 1 h before 2.5 mmol MIS/kg, the $t^1/_2$ for MIS increased by 17%, from 1.06 (0.98–1.15) h to 1.24 (1.16–1.33) h ($0.01 > P > 0.001$). In addition, blood concentrations of the metabolite DEMIS from 0.5–3 h were

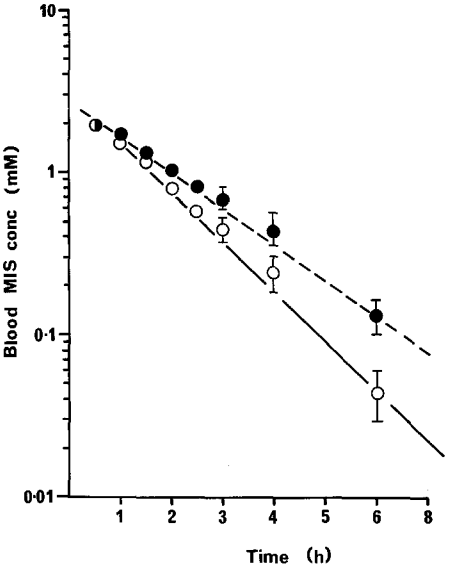

Fig. 8. Effect of injected DEMIS on the pharmacokinetics of MIS. Blood MIS concentrations after ○ 2.5 mmol/kg MIS IP alone; ●, 2.5 mmol MIS/kg plus 10.7 mmol DEMIS/kg IP (± SE)

reduced by 30%–40%, implicating inhibition of MIS demethylation. Similar results were obtained with 0.5 mmol MIS/kg.

Effect of Route and Volume of Administration on the Pharmacokinetics of MIS and DEMIS

In these experiments MIS (1 mmol/kg) or DEMIS (1.07 mmol/kg) was injected IV or IP in a volume of 10 ml/kg or IP in a volume of 40 ml/kg. The data are shown in Fig. 9 and pertinent kinetic parameters in Table 3. With IV MIS and DEMIS, peak concentrations were seen at the earliest sampling time (2 min). Absorption after IP injection was slower from the larger injected volume, particularly for DEMIS, where this resulted in a delayed peak time. For both compounds peak blood concentrations for the IP route were about half those for the IV route, and also about 15% lower for the large as against the small volume. DEMIS concentrations following IP injection exceeded those for IV after 1 h, but this was not seen with MIS. The IP bioavailability (AUC IP/AUC IV) of DEMIS was 108% for the small volume and 109% for the large. With MIS the values were lower, at 80% and 74% for the small and large volumes, respectively. However, with the exception of large-volume MIS, no value was significantly different from 100% ($P > 0.1$). Apparent $t^1/_2$ was independent of route and volume ($P > 0.05$).

Table 3. Effect of route and volume of administration on the pharmacokinetics of MIS and DEMIS[a]

Drug and dose	Route and volume	Mean Peak conc. (±SE) (m*M*) [b,c]	Median Peak time (min)	Mean $t^1/_2$ (95% confidence limits) (h)	Mean AUC (±SE) (m*M* h)[b]
DEMIS (1.07 mmol/kg)	IV 10 ml/kg	2.03 ± 0.11	2	0.63 (0.55–0.71)	1.30 ± 0.08
	IP 10 ml/kg	0.96 ± 0.03	15	0.69 (0.62–0.76)	1.41 ± 0.08
	IP 40 ml/kg	0.82 ± 0.03*** †	30	0.74 (0.64–0.84)	1.42 ± 0.11
MIS (1 mmol/kg)	IV 10 ml/kg	2.44 ± 0.89	2	0.48 (0.40–0.56)	1.82 ± 0.16
	IP 10 ml/kg	1.19 ± 0.09	15	0.52 (0.47–0.57)	1.46 ± 0.10
	IP 40 ml/kg	1.03 ± 0.07*	15	0.54 (0.49–0.59)	1.35 ± 0.09**

[a] Data for two independent experiments for each group; ten mice per group
[b] IP values significantly different from corresponding IV values: * $0.05 > P > 0.02$; ** $0.02 > P > 0.01$; *** $P < 0.001$
[c] Large-volume IP values significantly different from corresponding small-volume values: † $0.01 > P > 0.001$

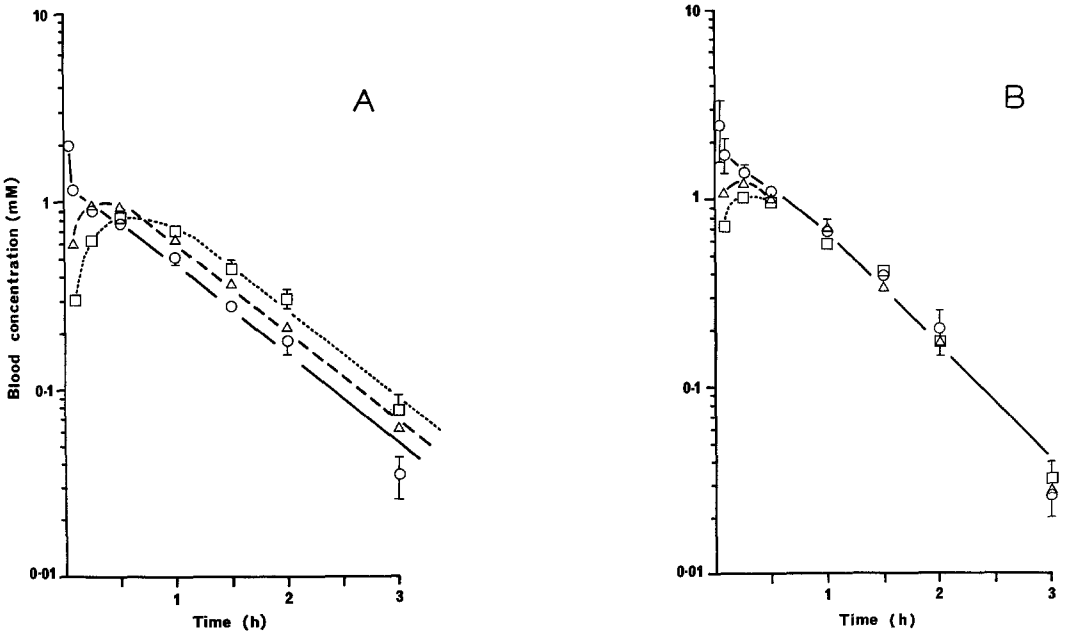

Fig. 9A and B. Effect of route and volume of administration on the pharmacokinetics of MIS and DEMIS. Blood concentrations after injected DEMIS (1.07 mmol/kg) (**A**) and MIS (1 mmol/kg) (**B**). O——O, IV in 10 ml/kg; Δ– – –Δ, IP in 10 ml/kg; □ · · · □, IP in 40 ml/kg

Sex Differences in MIS Pharmacokinetics

Previous experience suggested that MIS kinetics were more variable in female than in male BALB/c mice. For this reason two experiments were done to compare the sexes directly. The mean weights were 23 g for both sexes and the MIS dose 5 mmol/kg IP. In one experiment the peak concentration (± SE) was 4.60 ± 0.11 m*M* in males, compared with 7.87 ± 0.09 m*M* in females ($P < 0.001$). Concentrations remained proportionally greater in the females throughout and the AUC was 95% higher. However, the $t^1/_2$ values (2.00 h in the males and 2.11 h in the females) were similar ($P > 0.1$). Concentrations of the metabolite DEMIS were also generally higher in the females, and the AUC was 44% greater.

In the repeat experiment, peak MIS concentrations were again higher in females (4.94 ± 0.06 m*M*) than in males (4.30 ± 0.09 m*M*) ($0.01 > P > 0.001$), but the difference was much smaller. Concentrations of MIS at later times were similar, and AUC and $t^1/_2$ values for MIS were identical. However, the AUC for the metabolite DEMIS was 16% higher in females.

Table 4. Effect of dose on concentrations of MIS and DEMIS in blood, brain, and EMT6 tumour, 1.5 h after IP injection[a]

	Concentration (± SE)			Tumour/blood (%)[b]	Brain/blood (%)[b]
	Blood μmol/ml (m*M*)	Tumour μmol/g	Brain μmol/g		
MIS 5 mmol/kg	3.18 ± 0.10	2.70 ± 0.06	2.65 ± 0.09	87 ± 5	85 ± 3
MIS 0.5 mmol/kg	0.080 ± 0.01	0.055 ± 0.003	0.051 ± 0.005	77 ± 7	72 ± 8
DEMIS 5.35 mmol/kg	1.45 ± 0.10	1.00 ± 0.04	0.45 ± 0.01	73 ± 3	36 ± 2
DEMIS 0.535 mmol/kg	0.105 ± 0.01	0.050 ± 0.003	0.027 ± 0.002	51 ± 4**	29 ± 3*

[a] N = 22–23 per group
[b] Significant differences between low and high doses for the same drug: * $0.05 > P > 0.02$; ** $P < 0.001$

Effect of Dose on Brain and Tumour Concentrations of MIS and DEMIS

Drug concentrations were determined in blood, brain and EMT6 tumour (mean volume ~ 100 mm^3) 1.5 h after IP MIS (0.5 or 5 mmol/kg) or DEMIS (0.535 or 5.35 mmol/kg). For both drugs, but particularly for DEMIS, the tissue/blood ratios tended to be lower at the small than at the large dose (Table 4). As noted previously [5, 19], the brain/blood ratios were lower for DEMIS than for MIS, and this was true at both high and low doses.

Discussion

The experiments concerning the effects of dose on pharmacokinetics demonstrate major differences between MIS and DEMIS. The blood and urine data showed clearly that injected DEMIS is eliminated predominantly by renal clearance exhibiting first-order kinetics, with no evidence of saturable non-linear behaviour. In contrast, MIS is eliminated mainly by metabolism, including demethylation to DEMIS, and non-linear kinetics were apparent at higher doses. Computer simulation has confirmed that the MIS data are consistent with a model in which the metabolic clearance exhibits Michaelis-Menten kinetics while the less predominant renal clearance is first-order [JV Watson, personal communication].

The disparity appears to result from differences in lipophilicity. Renal clearance predominates for the more hydrophilic DEMIS because of slower reabsorption across the lipoid membranes of the kidney tubules. MIS, which is reabsorbed more rapidly, requires metabolism to more polar metabolites. This model is supported by studies in progress with analogues spanning a wider range of lipophilicity.

Because of this fundamental difference, the apparent $t^1/_2$ of MIS increased with dose whereas that of DEMIS did not, and the dependence of blood AUC on dose showed a marked upward curvature for MIS but was linear for DEMIS. On the other hand, the relationship between dose and peak blood concentration was linear for MIS but showed a slight downward curvature for DEMIS, probably because of slower absorption of DEMIS from the peritoneal cavity (see later).

Several alternative causes for the non-linear kinetics of MIS were ruled out. The following did not contribute in a major way: (1) short-term enzyme induction by MIS; (2) enzyme inhibition by the product DEMIS; (3) decrease in body temperature; and (4) injection volume. Possible binding effects can also be eliminated, as neither MIS nor DEMIS is appreciably protein-bound [21]. The decrease in $t^1/_2$ after induction of liver MFO by phenobarbitone and the lack of effect on DEMIS kinetics are also consistent with the proposed model, as are the increase in MIS $t^1/_2$ and reduced DEMIS metabolite levels with the MFO inhibitor SKF 525A.

The dependence of MIS $t^1/_2$ on injected dose considerably outweighs such variables as sex and strain and differences in analytical methodology, and accounts for marked disparities in published values [7, 10, 11, 13, 14, 19].

Previous studies with MIS in man [e.g., 8, 22] and dog [18] failed to reveal non-linear kinetics. This is because the MIS doses were not more than 1 mmol/kg (200 mg/kg), the threshold above which major deviation from linearity is observed (Fig. 1). Blood concentrations must exceed 1 m*M* to saturate metabolising enzymes. This can be achieved in mice because of their comparative resistance to high milligram-per-kilogram doses.

The application of these findings lies not in their direct clinical significance, but in their relevance to in vivo development of new radiosensitizers. We have argued that more rapid clearance than MIS is one desirable property [17]. DEMIS is cleared twice as fast as MIS in dog [17] and man (S Dische et al., personal communication). But in the mouse the result

depends on the administered dose. At high doses DEMIS is cleared more rapidly than MIS, but at lower doses if anything the reverse is true. Both extremes of dose are used for in vivo testing. To obtain genuine values for $t^1/_2$ drugs must be compared where linear kinetics prevail, i.e., at doses below saturation level. Under these conditions the mouse predicts poorly for the comparative clearance of MIS and DEMIS in dog and man. Studies are in progress to assess the predictive value over a wider range of lipophilicity.

The route and volume of administration experiments are also pertinent to in vivo testing. For DEMIS IP bioavailability was complete, regardless of injection volume. The IP bioavailability of MIS was a little lower (74%–80%), suggesting some first-pass metabolism. For both MIS and DEMIS peak concentrations were considerably higher for the IV route, as was seen with IV versus oral dosage in the dog [17, 18]. In addition, absorption was slower and peak concentrations lower when the drugs were injected IP in a large volume of dilute solution than in a smaller volume of concentrated solution. High-dose IP DEMIS gave 2-fold lower peak blood levels than MIS. But this was not seen after slowing of the elimination of DEMIS by nephrectomy [7], and here it was shown that the discrepancy is reduced at lower doses. The disparities are caused by differences in the relative rates of absorption and elimination, and are best avoided by using IV injection where possible.

Some differences were noted in MIS kinetics between male and female mice of equal weight. Higher peak concentrations and greater between-experiment variation was seen with females. Higher plasma levels have also been observed in heavier mice (J Denekamp et al., personal communication).

Tumour and brain penetration are clearly of importance for the therapeutic ratio of radiosensitizers [5]. Of particular concern is that the advantages shown by analogues more hydrophilic than MIS, i.e., rapid clearance and exclusion from nervous tissues, should not be offset by insufficient tumour penetration. Previous studies in mice, with high doses, showed that several hydrophilic analogues, including DEMIS, penetrated tumours with equal efficiency to MIS, although a lower limit of partition coefficient was indicated [5]. The present studies show that for MIS, and particularly DEMIS, the tumour/blood and brain/blood ratios were slightly lower for small than for large doses. But, more important, the *differential* penetration into tumour and brain shown by DEMIS but not MIS was seen at both high and low doses. Thus for MIS the *tumour/brain* ratios were 1.02 (high dose) and 1.07 (low dose), compared with the more favourable values of 2.22 (high dose) and 1.85 (low dose) for DEMIS. Similar results have been obtained with spontaneous tumours in dogs [17].

We have previously reported that EMT6 tumour/blood ratios for MIS are tumour size-dependent, large tumours ($> 350\ mm^3$) having lower ratios than 'small' ones ($< 170\ mm^3$) [11]. The present studies were carried out with small tumours ($\sim 100\ mm^3$).

Measurement of gross tumour concentration does not discriminate between intracellular and extracellular sites. However, radiosensitization assays with single large doses have shown that DEMIS (JM Brown and NY Yu, submitted for publication] and some more hydrophilic analogues (JM Brown et al., personal communication] do reach critical targets in the hypoxic cells of transplantable mouse tumours. Studies with multiple low doses will be important and remain to be done.

The way in which lipophilicity affects the relationship between dose and the pharmacokinetic behaviour of radiosensitizers will have an important influence on their comparative toxicities. Thus, whereas the fractionation of DEMIS doses will not markedly affect the total AUC, the AUC for a single large dose of MIS will considerably exceed that for the same total dose given in several small fractions. We have reported previously that the acute LD_{50} for IP MIS is about half that for DEMIS [7], and that the value for MIS is increased by phenobarbitone [19]. The present studies confirmed these findings and, in addition, showed that neither the kinetics nor the acute LD_{50} of DEMIS was altered by phenobarbitone. This supports the view that the mechanism of protection against MIS toxicity involves the reduction brought about in AUC by phenobarbitone.

The possibility of major disparities in pharmacokinetics between closely related analogues, such as those shown here for MIS and DEMIS, should be considered in the design and interpretation of in vivo experiments with radiosensitizers. In particular, factors influencing peak concentration and AUC are likely to affect radiosensitization and toxicity, respectively. Measurement of drug concentrations is therefore essential for rational in vivo testing. We are now extending the present studies with a large series of MIS analogues to determine structure-pharmacokinetic relationships for this series.

Acknowledgements. I thank Professor N. M. Bleehen and Mr. L. S. Freedman for their advice, Mrs. Jane Donaldson and Dr. Nancy Smith for excellent technical assistance, and Miss Paula Rayner for help with some of the animal experiments. I also thank Dr. Carey E. Smithen of Roche Products Limited for supplies of nitroimidazoles and Smith, Kline and French Laboratories Limited for the SKF 525A.

References

1 Adams GE (1978) Hypoxic cell sensitisers for radiotherapy. Int J Radiat Oncol Biol Phys 4: 135

2 Adams GE, Clarke ED, Flockhart IR, Jacobs RS, Sehmi DS, Stratfort IJ, Wardman P, Watts ME (1979) Structure–activity relationships in the development of hypoxic cell radiosensitisers. I. Sensitisation efficiency. Int J Radiat Biol 35: 133

3 Adams GE, Clarke ED, Gray P, Jacobs RS, Stratford IJ, Wardman P, Watts ME (1979) Structure–activity relationships in the development of hypoxic cell radiosensitisers. II. Cytotoxicity and therapeutic ratio. Int J Radiat Biol 35: 151

4 Anderson RF, Patel KB (1979) Effect of lipophilicity on radiosensitisation of hypoxic cells in vitro. Br J Cancer 39: 705

5 Brown JM, Workman P (1980) Partition coefficient as a guide to the development of radiosensitisers which are less toxic than misonidazole. Radiat Res

6 Brown JM, Yu NY, Cory MJ, Bicknell RD, Taylor DL (1978) In vivo evaluation of the radiosensitising and cytotoxic properties of newly synthesised electron-affinic drugs. Br J Cancer [Suppl III] 37: 206

7 Brown JM, Yu NY, Workman P (1979) Pharmacokinetic considerations in testing hypoxic cell radiosensitisers in mouse tumours. Br J Cancer 39: 310

8 Dische S, Saunders MI, Lee ME, Adams GE, Flockhart IR (1977) Clinical testing of the radiosensitiser Ro 07-0582: Experiments with multiple doses. Br J Cancer 35: 567

9 Dische S, Saunders MI, Flockhart IR, Lee ME, Anderson P (1979) Misonidazole – a drug for trial in radiotherapy and oncology. Int J Radiat Oncol Biol Phys 5: 851

10 Flockhart IR, Large P, Troup D, Malcom SL, Marten TR (1978) Pharmacokinetic and metabolic studies of the hypoxic cell radiosensitiser misonidazole. Xenobiotica 8: 97

11 Honess DJ, Workman P, Morgan JE, Bleehen NM (1980) Effects of local hyperthermia on the pharmacokinetics of misonidazole in the anaesthetised mouse. Br J Cancer

12 McNally NJ, Denekamp J, Sheldon PW, Flockhart IR, Stewart FA (1978) Radiosensitisation by misonidazole (Ro 07-0582). The importance of timing and tumour concentration of sensitiser. Radiat Res 73: 568

13 Pedersen JE, Smith MR, Bugden RD, Peckham MJ (1979) Distribution and tumour cytotoxicity of the radiosensitiser misonidazole (Ro 07-0582) in C57 mice. Br J Cancer 39: 429

14 Rauth AM, Chin J, Marchow L, Paciga J (1978) Testing of hypoxic cell radiosensitisers in vivo. Br J Cancer [Suppl III] 37: 202

15 Urtasun RC, Chapman JD, Feldstein ML, Band RP, Rabin HR, Wilson AF, Marynowski B, Starreveld E, Shnitka T (1978) Peripheral neuropathy related to misonidazole: Incidence and pathology. Br J Cancer [Suppl III] 37: 271

16 Wasserman TH, Phillips TL, Johnson RJ, Gomer CJ, Lawrence GA, Sadee W, Marques RA, Levin VA, Van Raalte G (1979) Initial United States clinical and pharmacological evaluation of misonidazole (Ro 07-0582), an hypoxic cell radiosensitiser. Int J Radiat Oncol Biol Phys 5: 775

17 White RAS, Workman P (1980) Pharmacokinetic and tumour-penetration properties of the hypoxic cell radiosensitiser desmethylmisonidazole (Ro 05-9963) in dogs. Br J Cancer 41: 268

18 White RAS, Workman P, Freedman LS, Owen LN, Bleehen NM (1979) The pharmacokinetics of misonidazole in the dog. Eur J Cancer 15: 1233

19 Workman P (1979) Effects of pretreatment with phenobarbitone and phenytoin on the pharmacokinetics and toxicity of misonidazole in mice. Br J Cancer 40: 335

20 Workman P (1980) Pharmacokinetics of hypoxic cell radiosensitisers. A review. Cancer Clinical Trials

21 Workman P, Little CJ, Marten TR, Dale AD, Ruane RJ, Flockhart IR, Bleehen NM (1978) Estimation of the hypoxic cell sensitiser misonidazole and its O-demethylated metabolite in biological materials by reversed-phase high-performance liquid chromatography. J Chromatogr 147: 507

22 Workman P, Wiltshire CR, Plowman PN, Bleehen NM (1978) Monitoring salivary misonidazole in man. Br J Cancer 38: 709

Received May 2/Accepted June 19, 1980