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Structure-pharmacokinetic Relationships for Misonidazole Analogues in Mice

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Summary. *We have compared the mouse pharmacokinetics of six analogues of the hypoxic cell sensitizer misonidazole (MISO). The analogues were all uncharged and similar in redox potential, but widely different in octanol-water partition coefficient (range 0.026–1.5). Lipophilic analogues were cleared mainly by metabolism and non-linear elimination kinetics were seen at high doses. Hydrophilic analogues, including desmethylmisonidazole, SR-2508, and SR-2555, were removed principally by renal clearance exhibiting linear elimination kinetics. Lipophilic analogues were cleared more rapidly after hepatic microsomal enzyme induction by phenobarbitone, whereas the kinetics of hydrophilic analogues were unaffected. Low-dose clearance was similar for most of the analogues. But the hydrophilic SR-2555 was cleared twice as quickly as MISO, and the liphophilic Ro 07-0913 seven times faster than MISO. Plasma protein binding was low for all the analogues. The significance of these results for the predictive value of the mouse as a model for man is discussed.*

Introduction

Nitroimidazoles are of current interest in oncology because of their preferential activity against hypoxic cells. This activity includes selective cytotoxicity towards and radiosensitization of hypoxic cells [10] and preferential enhancement of the in vivo antitumour effect of several cytotoxic drugs [12, 24]. Misonidazole (1-[2-nitroimidazol-1-yl]-3-methoxypropan-2-ol; Ro 07-0582, Roche Laboratories, MISO) is undergoing extensive clinical trial as a radiosensitizer, but there is a need for a less neurotoxic derivative [e.g., 13, 14, 25, 27].

Radiosensitization and cytotoxicity towards hypoxic cells by nitroimidazoles in vitro are both primarily dependent on the one-electron reduction potential of the nitro group [1, 2]. Lipophilicity also has some effect on in vitro radiosensitization of bacterial [3] and mammalian cells [4]. In addition, lipophilicity is of great importance in vivo because of its influence on pharmacokinetics and toxicity [5–7, 29, 30, 32]. Analogues more hydrophilic than MISO, including its metabolite desmethylmisonidazole (1-[2-nitroimidazol-1-yl]-2,3-propandiol; Ro 05-9963, Roche Laboratories, DEMIS) may be potentially less neurotoxic. With decreasing lipophilicity they are progressively excluded from brain and peripheral nerve, but not from tumours, in both mice and dogs [6, 29–31]. In addition, the dog clears the hydrophilic analogues more rapidly than MISO [29, 30]. DEMIS is eliminated twice as fast as MISO in the dog [29] and also in man [15; N. M. Bleehen and P. Workman, unpublished work].

Recent work from this laboratory showed that comparative pharmacokinetic studies in mice can be complicated by non-linear kinetic behaviour [33]. MISO exhibits saturable non-linear elimination kinetics, whereas the more hydrophilic DEMIS exhibits linear kinetics. Thus, whereas at high doses DEMIS is cleared more rapidly, at low doses the clearance is similar and the mouse predicts poorly for behaviour in dog and man.

Because of the widespread use of mice for in vivo testing of radiosensitizers [e.g., 8, 9, 11, 22], we felt it was important to extend the previous work with MISO and DEMIS to establish structure-pharmacokinetic relationships for other neutral nitroimidazoles similar in reduction potential but differing in lipophilicity. We have described the effects of lipophilicity on tumour and brain penetration in mice [6].

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Here we report on the influence of lipophilicity on renal and metabolic clearance, pharmacokinetic dose-dependence, and the effects of hepatic microsomal enzyme induction with phenobarbitone.

Materials and Methods

Drugs. MISO and analogues designated with the prefix Ro- were provided by Roche Laboratories (Welwyn Garden City, Great Britain); those with the prefix SR- were synthesised by Dr. W. W. Lee (SRI International, Menlo Park, CA, USA). Table 1 shows the structures of the analogues studied, together with their molecular weights, one-electron reduction potentials at pH 7 (E_7^1), and octanol-water partition coefficients at pH 7.4 (PC). All are effectively (> 99%) un-ionised at this pH. The reduction potentials are very similar (−383 to −398 mV); but the partition coefficients vary from 1.5 for the most lipophilic (Ro 07-0269) to 0.026 for the most hydrophilic (SR-2555), with MISO intermediate (PC = 0.43).

Mice. Adult male BALB/c mice were obtained from the breeding colony at NIMR (Mill Hill, London, Great Britain) and from Olac (Southern) Ltd. (Bicester, Great Britain). Except for urinary excretion studies, they were housed in plastic cages on sawdust bedding prepared from soft white woods. They were fed PRD nuts (Labsure Animal Diets, Poole, Great Britain) and allowed water ad libitum. Contact with known microsomal-enzyme inducers, such as halogenated hydrocarbon insecticides, was avoided. Mice weighed 20–25 g. Bilateral nephrectomy and sham operations were carried out as described previously [9]; drugs were administered after recovery from sodium pentobarbitone anaesthesia

Drug Administration and Sample Collection. Drugs were dissolved in Hank's buffered salt solution (HBSS) and injected IP (10–40 ml/kg) or IV (10 ml/kg). Blood samples were collected from the tail or by cardiac puncture [31]. For 24 h urine collection groups of four to six mice were contained in a Urimax metabolism cage. All samples were stored at −20° C before analysis.

Nitroimidazole Analysis. Concentrations of drugs and metabolites were determined by isocratic reverse-phase high-performance liquid chromatography (HPLC) as described previously [34] with minor modifications [6].

Kinetic Parameters and Statistical Analysis. All drugs given IP showed monophasic elimination profiles at low doses, but dose-dependent kinetics were observed with some analogues at high doses. Apparent elimination half-life ($t_{1/2}$) was obtained from $t_{1/2} = \ln 2/k'$, where k' is the initial apparent elimination rate constant given by the slope of the ln blood concentration against time plot [33]. For linear kinetics $k' = k$, the true elimination rate constant. The two drugs given IV, SR-2508 and SR-2555, showed biphasic elimination profiles consistent with the two-compartment open model [23, 26]:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_t is the blood concentration at time t, the coefficients A and B are zero time intercepts on the ordinate (ln C), and α and β are hybrid rate constants for the distribution and elimination phases, respectively. Data exhibiting unambiguously two exponential phases were fitted to this equation by the method of residuals (also known as curve stripping, peeling, feathering, or back-projection) [20, 26]. Briefly, this involves fitting a single exponential equation to the elimination phase data, and a second exponential equation to the residuals of the distribution phase data. In all cases, lines of best fit, with standard errors, were calculated by least-squares linear regression analysis. For drugs given IP, the area under the

Table 1. Structures, physiochemical properties, and toxicities of MISO analogues

Compound	Structure R=	Molecular weight	E_7^1 (mV)	Partition coefficient (PC)	Acute LD_{50} (IP) (mmoles/kg)
Ro 07-0269	$CH_2CH(OH)CH_2Cl$	205	− 384[a]	1.5[a]	0.8[f]
Ro 07-0913	$CH_2CH(OH)CH_2OCH_2CH_3$	215	− 391[b]	1.27[b]	7.5[g]
Ro 07-0741	$CH_2CH(OH)CH_2F$	189	− 383[a]	0.41[a]–0.44[e]	3.2[g]–4.8[f]
Misonidazole (Ro 07-0582)	$CH_2CH(OH)CH_2OCH_3$	201	− 389[c]	0.43[c]	8.9[f]
Desmethylmisonidazole (Ro 05-9963)	$CH_2CH(OH)CH_2OH$	187	− 389[c]	0.11[c]–0.13[e]	16.6[f]
SR-2508	$CH_2CONHCH_2CH_2OH$	214	− 388[d]	0.046[e]	22.9[f]
SR-2555	$CH_2CON(CH_2OH)_2$	258	− 398[d]	0.026[e]	34.5[h]

[a] [2]
[b] E. D. Clarke and P. Wardman, personal communication
[c] [1]
[d] P. O'Neill and S. Ho, personal communication
[e] W. W. Lee, personal communication
[f] [6]
[g] J. M. Brown and P. Workman, unpublished work
[h] [7]

curve (AUC) from time 0 to the final time t was estimated by Simpson's rule. The remaining AUC from $t-\infty$ (which was small) was given by C_t/k. AUC values shown are for $AUC_{(0-\infty)}$ obtained by summing $AUC_{(0-t)}$ and $AUC_{(t-\infty)}$. For drugs given IV $AUC_{(0-\infty)}$ was calculated in a similar way or using the equation $AUC_{(0-\infty)} = A/\alpha + B/\beta$, the two methods giving identical results.

Systemic drug clearance (Cl_s) was estimated from the equation Cl_s) = Dose/$AUC_{0-\infty}$ [26]. For drugs given IP we have assumed complete absorption: this has been demonstrated for MISO and DEMIS [33], but for others the values given are Cl_s/F, where F is the fraction absorbed.

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

Plasma Protein Binding. Pooled plasma was obtained from the heparinised blood of healthly volunteers. In some experiments pooled mouse plasma was also used. Drug binding was determined by ultrafiltration with Ultra-free anticonvulsant drug filter units (Millipore). According to the manufacturer, the nominal 40,000 molecular weight cut-off retains > 99% of plasma albumin. Drugs were dissolved in plasma at a concentration of 30 μg/ml and incubated for 1 h at 37° C. Each sample (2 ml) was placed in the filter cup and ultrafiltered at room temperature by aspiration of the syringe plunger to provide a vacuum source. Approximately 0.2 ml ultrafiltrate was usually collected in 1 h. Drug concentrations in plasma and ultrafiltrate were determined by HPLC and the percentage bound was calculated from the difference. Parallel studies with the drugs dissolved in water showed that there was usually no measurable drug binding to the membrane.

Results

Effect of Dose and Lipophilicity on the Pharmacokinetics of Nitroimidazoles

With the exception of Ro 07-0269 pharmacokinetic studies were carried out at two doses, differing by at least a factor of five. The doses were chosen on the basis of previous studies with MISO and DEMIS [33]. Because of its greater toxicity Ro 07-0269 was studied at only one dose level. All doses were considerably below the acute LD_{50} (Table 1). Drugs were given IP except the most hydrophilic SR-2508 and SR-2555, which were given IV because of their slow absorption.

Examples of the pharmacokinetic behaviour are shown in Fig. 1. and various kinetic parameters summarised in Table 2. At low doses given IP, elimination kinetics for Ro 07-0913, Ro 07-0741, Ro 07-0269, MISO and DEMIS were mono-exponential down to concentrations representing the lower limits of detection for the assays (about 0.01 mM). Nonetheless, as reported previously for MISO [33], the kinetics for high doses of Ro 07-0741 and Ro 07-0913 showed marked non-linearity. The apparent elimination $t_{1/2}$ values were longer and the clearance slower at the higher doses. This is particularly obvious with Ro 07-0913, which was cleared more than twelve times as rapidly at a dose of 0.5 mmoles/kg than at 5 mmoles/kg, as against a difference of about three-fold for MISO (Fig. 1 and Table 2). The difference in elimination $t_{1/2}$ for high- and low-dose Ro 07-0913 (53 and 11 min, respectively) was highly significant (t-test, $P < 0.001$). For Ro 07-0913, Ro 07-0741, and MISO, the rate of elimination at the high dose became similar to that at the lower dose only when the circulating blood concentrations approached the initial levels seen at the lower dose.

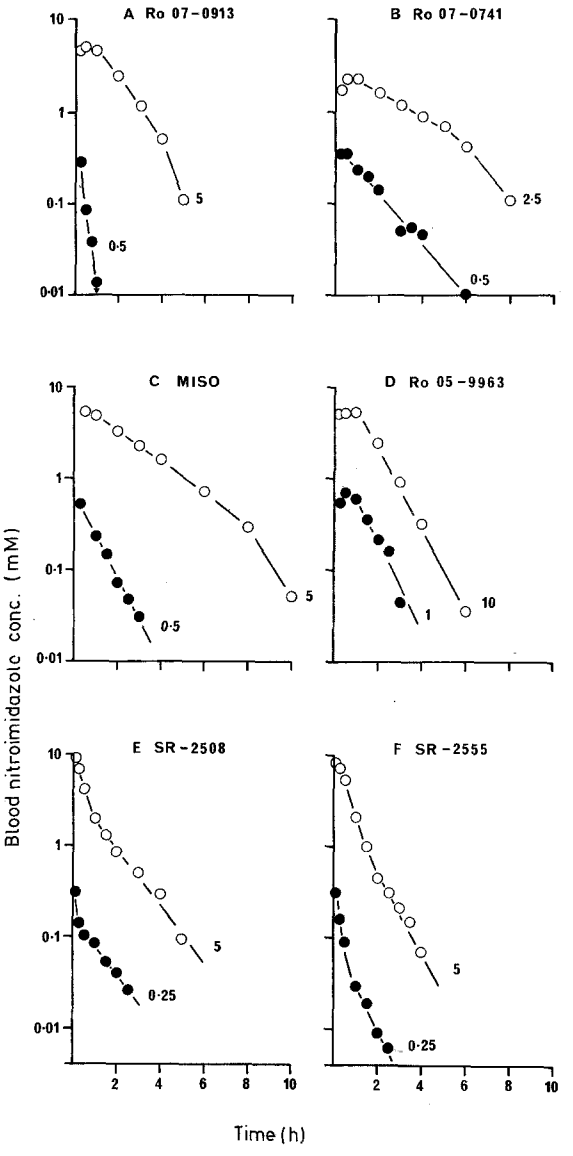

Fig. 1A–F. Pharmacokinetics of MISO analogues at different doses in BALB/c mice. Each drug was given as a single injection, Ro 07-0913 (**A**), Ro 07-0741 (**B**), MISO (**C**), and Ro 05-9963 (**D**) IP, and SR-2508 (**E**) and SR-2555 (**F**) IV. Each dose is given in mmoles/kg beside the corresponding data set. Data *points* are mean values, usually of five mice, but of a minimum of three

Table 2. Pharmacokinetic parameters for MISO analogues at different doses[a]

Drug	Dose (mmoles/kg)	Half-life[b] (h)	$AUC_{(0-\infty)}$ ($mM \cdot h$)	Clearance ($l \cdot kg^{-1} \cdot h^{-1}$)
Ro 07-0269	0.5	0.99 (0.90–1.10)	0.65	0.77
Ro 07-0913	5.0	0.88 (0.70–1.17)	10.80	0.46
	0.5	0.18 (0.15–0.22)	0.086	5.81
Ro 07-0741	2.5	2.42 (2.04–3.00)	8.22	0.30
	0.5	1.02 (0.92–1.14)	0.72	0.69
MISO	5.0	1.89 (1.82–1.97)	16.66	0.30
	0.5	0.66 (0.59–0.74)	0.60	0.83
DEMIS	10.7	0.72 (0.60–0.84)	11.20	0.96
	1.07	0.70 (0.68–0.72)	1.16	0.92
SR-2508	5.0	$t_{1/2}\alpha$ 0.20 (0.15–0.30) $t_{1/2}\beta$ 0.97 (0.91–1.04)	7.31	0.70
	0.25	$t_{1/2}\alpha$ ~0.11 $t_{1/2}\beta$ 0.98 (0.79–1.29)	0.21	0.99
SR-2555	5.0	$t_{1/2}\alpha$ 0.36 (0.31–0.42) $t_{1/2}\beta$ 0.81 (0.70–0.95)	6.68	0.75
	0.25	$t_{1/2}\alpha$ 0.16 (0.12–0.25) $t_{1/2}\beta$ 0.64 (0.57–0.73)	0.15	1.67

[a] Data shown are typical values from replicated experiments. A minimum of four time points were studied at each dose, and a minimum of three mice per time point

[b] 95% confidence limits in parentheses

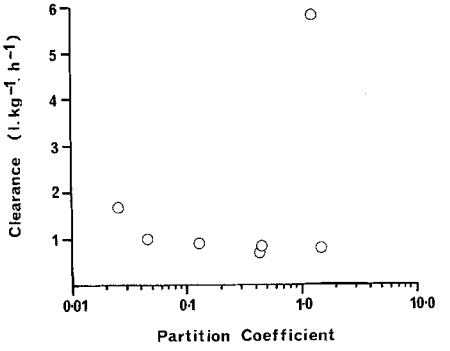

Fig. 2. Plot of clearance against partition coefficient for MISO analogues in BALB/c mice

We previously reported that DEMIS, less liphophilic than MISO, showed linear kinetics with similar clearance at low and high doses [33] (Fig. 1 and Table 2). It was therefore interesting to observe the effect of dose on SR-2508 and SR-2555 kinetics as these analogues are even more hydrophilic. Given IV, both exhibited bi-exponential elimination kinetics, indicating two-compartment open model characteristics (see *Methods* and Fig. 1). For both drugs the terminal elimination (β) phase $t_{1/2}$ was similar at low and high doses, in contrast to the lipophilic analogues (Table 2). In each case, however, the initial distribution phase $t_{1/2}$ was longer at the higher dose. For SR-2508 the overall clearance rate was only slightly slower at the high than at the low dose. However, with the more hydrophilic SR-2555 the α-phase made a larger contribution to the AUC and the difference in clearance at low and high doses was correspondingly greater (Table 2).

Because of the non-linear kinetics seen at higher doses with some analogues, comparisons between them should be made at low doses. Figure 2 shows a plot of low dose clearance against PC, and there is no clear relationship. MISO, Ro 07-0741, Ro 07-0269, DEMIS and SR-2508 all had similar clearance rates. SR-2555 was cleared about twice as rapidly as MISO. But the most striking difference is seen with Ro 07-0913, which was removed seven times faster than MISO.

Comparison of low-dose IV SR-2508 and SR-2555 (Table 2) shows that the initial distribution phase $t_{1/2}$ was longer for SR-2555 (22 min) than for the rather less hydrophilic SR-2508 (7 min); moreover, both values are longer than those observed for IV MISO and DEMIS (< 2 min) [33]. In contrast, the terminal elimination phase $t_{1/2}$ was shorter for SR-2555 (38 min) than for SR-2508 (59 min).

With the exception of SR-2508 and SR-2555 low doses of all drugs given IP gave equal blood concentrations to equimolar MISO (Fig. 1). With IV SR-2508 and SR-2555 peak concentrations were considerably higher than those for IV MISO and DEMIS [33]. But when given IP blood concentrations of SR-2508 and SR-2555 were considerably lower due to their slow absorption. The terminal $t_{1/2}$ was

Table 3. Urinary excretion of MISO analogues

Drug and dose	Urinary excretion products	% Injected dose
Ro 07-0269	Ro 07-0269	5
(0.5 mmoles/kg)	Ro 07-0269 glucuronide	15
	DEMIS	3
	DEMIS glucuronide	0.7
	Total	23.7
Ro 07-0913	Ro 07-0913	5
(5 mmoles/kg)	Ro 07-0913 glucuronide	5
	DEMIS	34
	DEMIS glucuronide	0
	Total	44
Ro 07-0741	Ro 07-0741	25
(2.5 mmoles/kg)	Ro 07-0741 glucuronide	3
	Total	28
MISO	MISO	10
(5 mmoles/kg)	MISO glucuronide	6
	DEMIS	11
	DEMIS glucuronide	0.5
	Total	27.5
MISO	MISO	4
(0.5 mmoles/kg)	MISO glucuronide	7
	DEMIS	20
	DEMIS glucuronide	2.5
	Total	33.5
DEMIS	DEMIS	63
(5.35 mmoles/kg)	DEMIS glucuronide	3
	Total	66
DEMIS	DEMIS	57
(0.535 mmoles/kg)	DEMIS glucuronide	8
	Total	65
SR-2508	SR-2508	89
(5 mmoles/kg)	Total	89
SR-2555	SR-2555	79
(0.5 mmoles/kg)	Total	79

[a] Values are means from at least two separate experiments

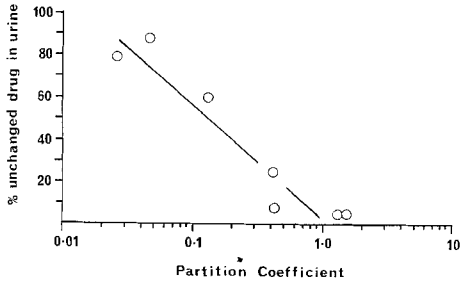

Fig. 3. Relationship between urinary excretion of unchanged drug and partition coefficient for MISO analogues in BALB/c mice

however similar for IP and IV routes. Following oral administration absorption was extremely poor (data not shown)

Effect of Liphophilicity on Metabolism and Urinary Excretion

Further information on the pharmacokinetics of MISO analogues is provided by urinary excretion data. Details of the urinary excretion products are shown in Table 3. To varying degrees MISO, Ro 07-0913, and Ro 07-0269 were metabolised to DEMIS, which appeared in the urine, and they were also excreted as glucuronides. Ro 07-0741 was not metabolised to DEMIS but was excreted in glucuronide form. The more hydrophilic DEMIS, SR-2508, and SR-2555 were excreted largely unchanged. For all compounds, but particularly the more liphophilic, a considerable amount remains unaccounted for. No additional metabolites were revealed by HPLC. Figure 3 shows the relationship between PC and the amount of drug excreted unchanged in the urine: urinary excretion of unchanged drug is reduced as lipophilicity is increased.

From the urine data Ro 07-0913 appears to be metabolised to DEMIS more extensively than MISO (Table 3). This was confirmed by the demonstration that DEMIS concentrations were considerably higher after Ro 07-0913 than after MISO, particularly with high doses (Fig. 4). Thus fast de-ethylation to DEMIS accounts for the rapid clearance of Ro 07-0913.

Effect of Microsomal Enzyme Induction on Pharmacokinetics

Previous studies showed that the clearance of MISO was much increased after phenobarbitone pretreatment through the induction of demethylation; in contrast the clearance of injected DEMIS was unchanged [33]. The effects of phenobarbitone pretreatment on the clearance of the MISO analogues is summarised in Table 4, and data for SR-2508 and Ro 07-0913 illustrated in Fig. 5. Microsomal enzyme induction increased the clearance (and correspondingly decreased the $t_{1/2}$ and AUC) of the more lipophilic analogues (Ro 07-0269, Ro 07-0913, Ro 07-0741, and MISO) but had no effect on the kinetics of those more hydrophilic (DEMIS, SR-2508 and SR-2555). With Ro 07-0913, as for MISO, initial concentrations of DEMIS metabolite were increased by 50%–100% after enzyme induction. But the low concentrations of DEMIS metabolite seen after Ro 07-0269 were unaffected by phenobarbitone.

Table 4. Effect of phenobarbitone pretreatment (100 mg · kg^{-1} · day^{-1} for 5 days) on the clearance of MISO analogues

Drug	Dose (mmoles/kg)	Partition coefficient	% Increase in clearance after phenobarbitone
Ro 07-0269	0.5	1.5	40
Ro 07-0913	5.0	1.27	46
Ro 07-0741	2.5	0.43	21
MISO	5.0	0.43	38
DEMIS	5.35	0.12	0
SR-2508	5.0	0.046	0
SR-2555	5.0	0.026	0

Effect of Nephrectomy on Pharmacokinetics

We have previously shown that with high dose MISO (5 mmoles/kg) drug clearance is reduced in mice which have undergone bilateral nephrectomy [9]. Figure 6 shows the effect of dose level administered on the pharmacokinetics of MISO and the more hydrophilic DEMIS in nephrectomised, sham-nephrectomised, and control mice. The sham operation had little effect. Although we confirmed the three- to four-fold increase in MISO $t_{1/2}$ at the high dose (5 mmoles/kg), this was not seen at the lower dose

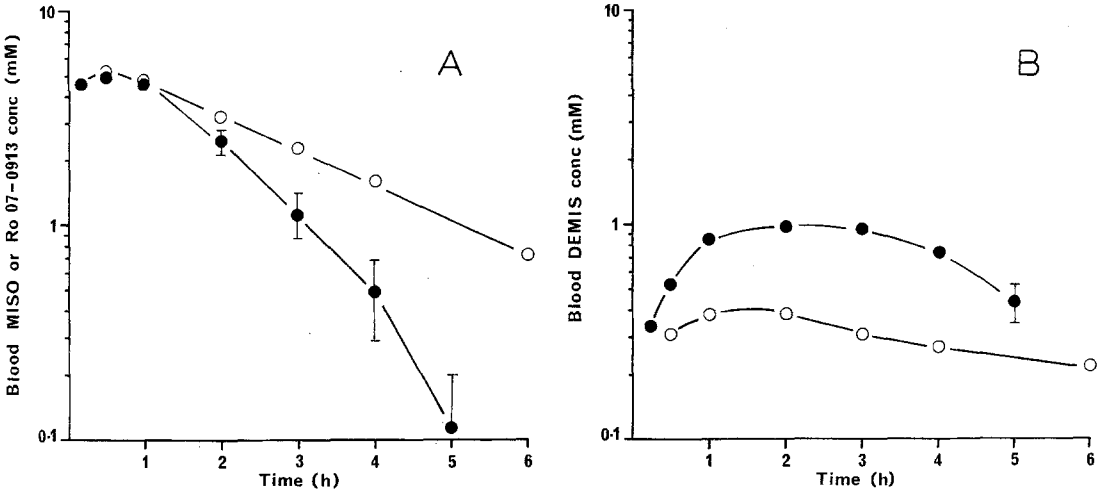

Fig. 4A and B. Comparative pharmacokinetics of Ro 07-0913 (●) and MISO (○) in BALB/c mice. **A** Parent drug, **B** DEMIS metabolite concentrations in blood after 5 mmoles/kg IP. *Error bars* show one standard error; five mice per *point*

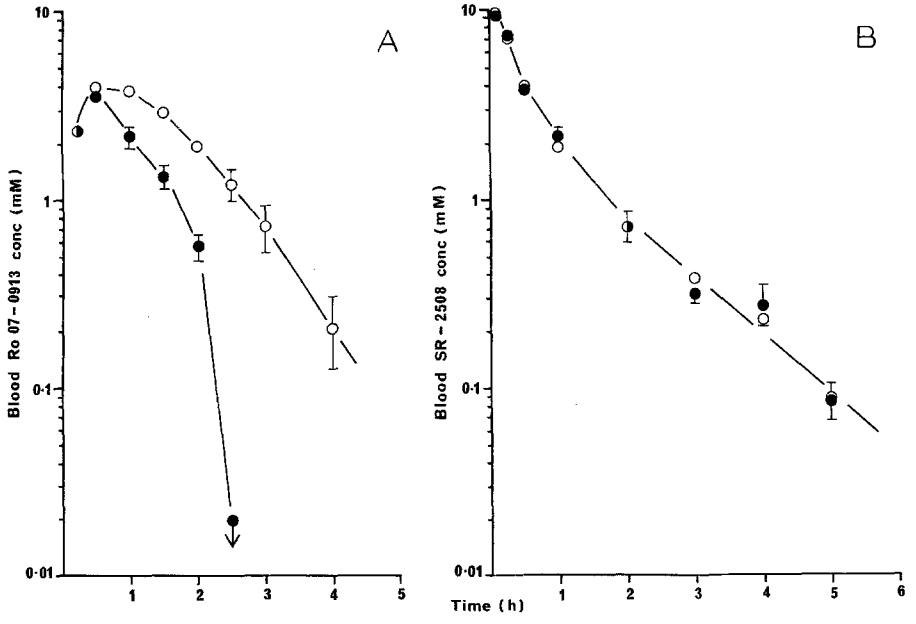

Fig. 5A and B. Effect of phenobarbitone pretreatment (100 mg · kg^{-1} · day^{-1} for 5 days) on the pharmakokinetics of Ro 07-0913 (**A**) and SR-2508 (**B**) in BALB/c mice. ○, saline pretreatment; ● phenobarbitone pretreatment. *Error bars* show one standard error; five mice per *point*

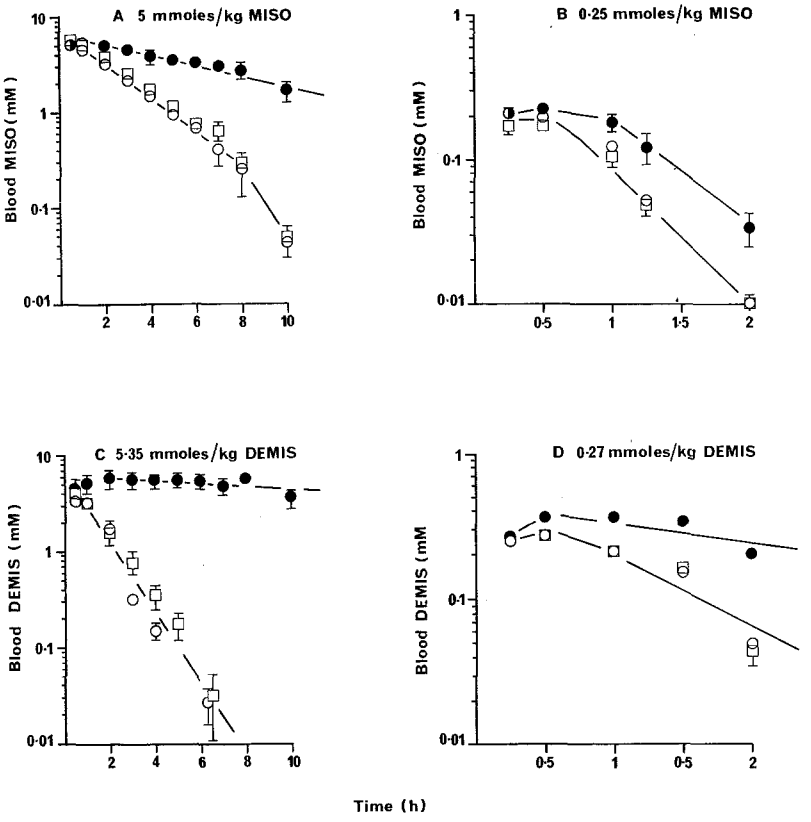

Fig. 6A–D. Effect of bilateral nephrectomy on the pharmacokinetics of MISO (**A** and **B**) and DEMIS (**C** and **D**) at different injected doses. ○, no operation; □ sham operation; ●, bilateral nephrectomy. *Error bars* show one standard error; three of five mice per *point*

Table 5. Effect of bilateral nephrectomy on the $t_{1/2}$ for MISO and DEMIS at different doses

Drug	Dose (mmoles/kg)	Half-life (SE limits)[a] (h)				Half-life ratio Nephrectomy $t_{1/2}$
		Control	Sham	Control and sham combined	Nephrectomy	Combined control $t_{1/2}$
MISO	5.00	1.46 (1.32–1.62)	1.74 (1.64–1.86)	1.59 (1.49–1.69)	5.78 (5.18–6.53)	3.6
MISO	0.250	0.35 (0.33–0.38)	0.36 (0.32–0.40)	0.35 (0.33–0.38)	0.40 (0.35–0.49)	1.1
DEMIS	5.350	0.80 (0.76–0.84)	0.90 (0.83–0.97)	0.86 (0.82–0.90)	15.9 (9.86–40.5)	18.5
DEMIS	0.268	0.62 (0.56–0.68)	0.56 (0.48–0.63)	0.58 (0.53–0.63)	2.00 (1.62–2.62)	3.4

[a] Three to five mice per time point

Table 6. Binding of MISO analogues to human plasma protein

Drug	Partition coefficient	% Bound ± SE
Benznidazole	8.2	58 ± 1.2 ($n = 5$)
Ro 07-0269	1.5	15 ± 3.4 ($n = 6$)
Ro 07-0913	1.27	12 ± 2.8 ($n = 6$)
Ro 07-0741	0.43	< 5
MISO	0.43	< 5
DEMIS	0.12	< 5
SR-2508	0.046	< 5
SR-2555	0.026	< 5

(0.25 mmoles/kg). The $t_{1/2}$ for DEMIS was lengthened at both doses, though less so at the lower dose. Furthermore, the prolongation was greater than with equivalent doses of MISO: at the high dose the increase in $t_{1/2}$ was almost 20-fold, and the 3-fold increase at the lower dose was similar to that seen with high-dose MISO (Fig. 6 and Table 5).

In similar experiments with SR-2508, nephrectomy prolonged the elimination $t_{1/2}$ following IP injection of 5 mmoles/kg from 0.8–15 h [6]. We have confirmed this approximately 20-fold increase with

IV injections of both 5 and 2 mmoles SR-2508/kg (data not shown).

Effect of Lipophilicity on Plasma Protein Binding

Comparative pharmacokinetics may be affected by different binding of analogues to plasma proteins. Data for binding of the MISO analogues to pooled human plasma, as determined by ultrafiltration, are shown in Table 6. Benznidazole (Ro 07-1051) was included with each batch of analyses as a standard. This compound is considerably more lipophilic than the others (PC = 8.2) [2] and showed the highest binding. The value of 58% is slightly higher than that obtained for human plasma (44%) [21] and bovine serum albumin (46%; E. D. Clarke and P. Wardman, unpublished work, cited in [28]) by means of equilibrium dialysis. The next most lipophilic, Ro 07-0269 and Ro 07-0913, were also bound, but less extensively, whereas the remainder showed no measurable binding. In comparative experiments we found that binding of Ro 07-1051 was somewhat lower for both BALB/c (38%) and C3H (39%) mouse plasma than for human plasma (57%).

Discussion

We have compared the systemic pharmacokinetics of the nitroimidazole hypoxic cell sensitizer MISO and six fairly closely related analogues in mice. These analogues are all uncharged at physiological pH and have very similar electron affinities (E_7^1), but they differ in octanol-water PC over a 60-fold range. The study was designed on the basis of a previous detailed comparison of MISO and DEMIS [33], which suggested that lipophilicity would be a predominant factor in determining the pharmacokinetics of MISO analogues.

Plasma protein binding was found to be of minor significance for the analogues in the present work. But the high degree of binding seen with benznidazole (PC = 8.2) indicates that this may be a problem with the more lipophilic compounds. Preliminary studies suggest that this is indeed the case (P. Workman, unpublished work; E. D. Clarke and P. Wardman, unpublished work, cited in [28]).

Previous studies have shown that high doses of MISO and metronidazole produce a considerable decrease in mouse body temperature [18, 19, 33]. We found that high doses of the lipophilic analogues MISO, Ro 07-0913, and Ro 07-0741 caused mice to become torpid and hypothermic; e.g., MISO or Ro 07-0913 at 5 mmoles/kg reduced temperatures to about 30° C (data not shown). In contrast, DEMIS at 10 mmoles/kg decreased temperature by only 2° C, and the most hydrophilic, SR-2508 and SR-2555, had no effect. None of the analogues affected temperature at low doses (0.25–0.5 mmoles/kg). We have previously shown that the pharmacokinetics of high-dose (5 mmoles/kg) MISO in mice are identical whether the mice are maintained at 37–38° C or allowed to become hypothermic [33]. For this reason, together with the fact that normal body temperatures are not usually maintained in therapeutic experiments with the analogues, no attempt was made to maintain body temperature in the experiments presented here. The experience with MISO [33] suggests that hypothermia is unlikely to have a major effect on gross pharmacokinetics.

Peak blood concentrations of all the analogues studied, with the exception of SR-2508 and SR-2555, were generally similar for equimolar doses injected IP. Rauth et al. [22] reported plasma concentrations of MISO, Ro 07-0269, Ro 07-0741, Ro 07-0913, and other analogues (determined by polarography) 60–100 min after IP injection, and the results are similar to ours after correction for dose and non-linear kinetics. Peak concentrations were lower for the more hydrophilic analogues injected IP [6], but SR-2508 and SR-2555 gave higher peaks than MISO when given IV.

Analogues as liphophilic and more liphophilic than MISO (PC = 0.43) exhibited dose-dependent elimination kinetics: clearance was slower and apparent elimination half-lives longer at high doses. This behaviour was most marked with the lipophilic analogue Ro 07-0913 (PC = 1.27), which was cleared 13 times faster at the low dose than at the high dose. With analogues more hydrophilic than MISO the elimination $t_{1/2}$ was not dose-dependent; but with SR-2508 and SR-2555, which were given IV, the distribution phase $t_{1/2}$ was somewhat longer at the higher dose.

A good correlation was observed between PC and urinary excretion, the amount of unchanged drug excreted in the urine decreasing with increasing lipophilicity. We have also shown that induction of hepatic microsomal enzymes with phenobarbitone results in faster clearance of MISO and the more lipophilic analogues, but has no effect with those that are more hydrophilic (DEMIS, SR-2508, and SR-2555). In addition, bilateral nephrectomy prolongs the elimination $t_{1/2}$ of the hydrophilic analogues considerably more than that of MISO. By varying the dose of MISO and DEMIS it was shown that the prolongation of $t_{1/2}$ was greater at the higher dose and virtually absent with low dose MISO.

The above results are all consistent with the simple model proposed previously [33], in which the

lipophilic analogues are cleared mainly by inducible hepatic microsomal metabolism and exhibit dose-dependent elimination kinetics as a result of saturation of metabolising enzymes by the high drug concentrations achieved with large doses. For MISO and Ro 07-0913 the saturable metabolic degradations include dealkylation to DEMIS, to which Ro 07-0269 is also converted to a smaller extent. The demonstration of non-linear kinetics for Ro 07-0741, which is not metabolised to DEMIS, shows that other metabolic degradations, possibly nitroreduction, can also become saturated at high drug concentrations. Such reactions may be shared by other lipophilic analogues, including MISO, since considerable proportions remain unaccounted for. In contrast to the lipophilic analogues, those more hydrophilic than MISO are less subject to reabsorption in the kidney tubules, do not require metabolism, and are excreted largely unchanged in the urine.

The differences in the dependence of pharmacokinetic behaviour on dose for the various analogues emphasises the importance of performing kinetic studies at doses spanning the therapeutic and toxic range. Here the range studied (usually 0.5–5 mmoles/kg) is similar to that used for toxicity and efficacy testing in mice. The marked non-linear kinetics of the lipophilic analogues makes the results of high-dose acute toxicity testing particularly difficult to interpret.

The lipophilicity approach to the development of improved MISO analogues is based on the concept that toxicity is related to AUC in critical normal tissues, whereas radiosensitization is a function of concentration in the hypoxic cells during irradiation [5, 6]. Since the mouse is used for most in vivo developmental work with radiosensitizers, it is important that this species should predict fairly well for the pharmacokinetic behaviour in man. Table 7 summarises clearance and urinary excretion data for MISO and the three hydrophilic analogues DEMIS, SR-2508, and SR-2555 in mouse, dog, and man. For each of these analogues the extent of urinary excretion is similar regardless of species (data are not available for SR-2508 and SR-2555 in man), and the trend towards increasing urinary excretion with decreasing lipophilicity is maintained. Because of problems associated with high drug doses, particularly dose-dependent pharmacokinetics but also physiological effects such as hypothermia, torpor, and reduced heart rate [18, 19, 33], comparisons of clearances in the mouse should be made at low doses where linear kinetics hold true, as is the case with the doses used in the dog and in man. Chapman and co-workers [11] observed differences in the pharmacokinetics of nitroheterocyclic compounds at low doses in mice, but these differed in electron affinity as well as lipophilicity. For the analogues in Table 7 low-dose clearance rates in the mouse are fairly similar, except for SR-2555, which is removed about twice as fast as the others. In the dog there is a clearer trend towards faster elimination with decreasing liphophilicity, and the order is similar to that for MISO and DEMIS in man. The predictive value of the mouse and dog will be tested further if SR-2508 and SR-2555 enter phase 1 trial in man.

It is clear that lipophilic analogues are eliminated by mainly hepatic metabolism, whereas hydrophilic analogues are removed by the kidney, and this fundamental difference is likely to have important clinical consequences. In particular, factors influencing liver function (e.g., enzyme induction) will alter the clearance of the more lipophilic analogues, including MISO, whereas the elimination of the hydrophilic analogues will be governed largely by renal function.

To obtain the maximum benefit from the hydrophilic analogues they must be given IV to avoid their poor absorption. In agreement with results obtained in dogs [30] we found hat following IV injection the distribution phase half-lives in the mouse were considerably larger for SR-2508 and SR-2555 compared to MISO and DEMIS. This is consistent with the slower diffusion out of the central compartment expected for the more hydrophilic analogues.

Table 7. Comparative pharmacokinetic properties of MISO analogues in mouse, dog, and man

Drug	Partition coefficient	Clearance ($l \cdot kg^{-1} \cdot h^{-1}$)			% Unchanged drug in urine		
		Mouse[a]	Dog[b]	Man[g]	Mouse[a]	Dog[b]	Man[g]
MISO	0.43	0.83	0.09	0.036[c]	7; 11[e]	6	7[f]
DEMIS	0.12	0.92	0.19	0.093[d]	60	75	53
SR-2508	0.046	0.99	0.21	ND	89	89	ND
SR-2555	0.026	1.67	0.26	ND	79	100	ND

ND, not determined

[a] Present work; [b] IV route [30]; [c] [35]

[d] N. M. Bleehen and P. Workman, unpublished work; [e] [16]; [f] [17]; [g] Oral route

The hydrophilic analogues of MISO are of current interest because of their good tumour penetration and exclusion from nervous tissues in mice and dogs [6, 29–31], as well as their faster clearance in dogs [29, 30] and, with DEMIS, in man [15; N. M. Bleehen and P. Workman, unpublished work]. DEMIS and SR-2508 show equal radiosensitization to MISO in mouse tumours in vivo, whereas SR-2555 and analogues more hydrophilic are rather less effective indicating exclusion from hypoxic tumour cells at very low partition coefficients [5, 7]. DEMIS is now in phase 1 clinical trial and SR-2508 is undergoing preclinical toxicity evaluation.

The present studies show that Ro 07-0913, more lipophilic than MISO, is cleared much faster in mice. This compound shows similar penetration into tumour and brain tissue [6] and equal radiosensitization to MISO in mice [22]. Recent studies also suggest that in vitro radiosensitization of bacterial cells increases at high partition coefficients [3]. We are currently investigating the pharmacokinetic and radiosensitizing properties of analogues even more lipophilic than Ro 07-0913 and potentially superior to MISO.

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