Europ. J. Cancer Vol. 15, pp. 1233–1242
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0014-2964/79/1001-1233 $02.00/0

The Pharmacokinetics of Misonidazole in the Dog*

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Abstract—*The hypoxic cell radiosensitising drug misonidazole, [1-(2-nitroimidazol-1-yl)-3-methoxypropan-2-ol, Ro 07-0582] was administered to dogs at dose levels of 50–200 mg/kg on four consecutive weekly occasions by oral and intravenous routes.*

High-performance liquid chromatography was used to monitor the subsequent plasma concentrations of misonidazole and its 0-demethylated metabolite [1-(2-nitroimidazol-1-yl)-2,3-propandiol, Ro 05-9963]. Both misonidazole and Ro 05-9963 were also detected in the urine in the free and glucuronide-conjugated forms.

The detailed pharmacokinetics of misonidazole in the dog are presented and some observations are made on the toxicity of the drug in this species.

We conclude in the light of these pharmacokinetic data that the dog may prove to be a better model for the study of misonidazole than those presently used in the laboratory, particularly the rodent species.

INTRODUCTION

MISONIDAZOLE [1-(2-nitroimidazol-1-yl)-3-methoxypropan-2-ol; Ro 07-0582] is one of the most effective hypoxic cell radiosensitising drugs yet discovered and has now entered experimental clinical practice. Most laboratory studies have been carried out in the mouse and rat but there are problems associated with the relatively short biological half-life of the drug in these species as compared with that in man[1]. We have examined the pharmacokinetics of misonidazole in the dog to assess the suitability of this species for further radiosensitising studies and as an overall bridging model between rodents and man. The dog also has a high incidence of spontaneous tumours and a subsequent clinical trial of misonidazole perhaps may be planned.

Present veterinary radiotherapy protocols are dictated by the need for drug-induced restraint (often general anaesthesia) of the patient during radiotherapy. This has led to use of weekly fractions rather than smaller daily fractions; we have therefore studied misonidazole given in four consecutive weekly doses of up to 200 mg/kg. Both oral and intravenous routes of administration were studied since the latter is more suited to veterinary work and can provide more valuable pharmacokinetic data, whilst the former route is that currently used in human trials.

MATERIALS AND METHODS

Animals

The eight male dogs used in this study were either Labrador or Collie cross-bred and varied in size between 8 and 28 kg. All were adult (i.e., >12 months), and paired as far as possible as regards breed type and size. The condition of the animals, as judged by clinical examination and routine haematological and biochemical parameters, was monitored before and during the administration of the drug.

Misonidazole was provided by Roche Products Ltd., (Welwyn Garden City). It was prepared as a 5% solution for intravenous injection by gentle warming in 0.9% sodium chloride solution. For oral administration it was packed into gelatin capsules size No. 0, each capsule containing approximately 0.4 g of misonidazole.

Dose levels of 50, 100, 150 and 200 mg/kg were selected for the study, one dog in each matched pair receiving the drug orally and the other intravenously. The same dose level

Accepted 5 January 1979.
*We wish to acknowledge the financial support of the MRC and CRC.
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and route of administration was used for each dog because we wished to study the pharmacokinetic and toxic effects of four consecutive weekly doses. The two dogs receiving misonidazole at 50 mg/kg were also used in a further study 2 weeks later using dose levels of 200 mg/kg for two consecutive weeks. The dogs were deprived of food overnight and the misonidazole administered in the early morning. They were then restrained in large metabolism cages for the course of the study. The right cephalic vein was used for intravenous administration and this site was avoided for subsequent blood sampling.

Heparinised blood samples were collected at 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 30 and 36 hr from dogs given the drug orally whilst those dogs receiving the drug intravenously were sampled at 5min., 30 min., 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$ and 3 hr and then as for the oral regime. Urine was collected over the periods 0–24 hr, 24–48 hr and 48–72 hr. Both plasma and urine were stored at −20°C until assayed.

Plasma and urine concentrations of misonidazole and its *O*-demethylated metabolite [1-(2-nitroimidazol-1-yl)-2,3-propandiol, Ro 05-9963] were measured by reversed-phase high-performance liquid chromatography (HPLC) using the method described by Workman *et al.* [2]. Urinary concentrations of the *O*-glucuronide derivatives of misonidazole and Ro 05-9963 were determined as follows. For complete hydrolysis of the *O*-glucuronide forms urine samples were incubated in the dark at 37°C for 24 hr with an equal volume of Glucurase (Sigma Chemical Co.) (Glucurase is a solution of bovine liver β-glucuronidase buffered at pH 5; activity = 5000 Sigma Units/ml.) The samples were then analysed by HPLC and the concentrations of the *O*-glucuronide derivatives obtained from the difference in concentration between digested and undigested urine.

The statistical assessment of trends in pharmacokinetic parameters with consecutive doses was based on a two-way analysis of variance assuming no interaction terms. From such an analysis a *t*-statistic with 9 d.f. was calculated and compared with standard tables of the *t*-distribution. The resultant probability levels are reported in the text. The other statistical methods used are reported in the results section.

RESULTS

Misonidazole pharmacokinetic parameters

Figure 1 shows a typical plasma time-course, plotted on linear co-ordinates, for oral and intravenous misonidazole at the 150 mg/kg dose level. Similar patterns were seen at other doses. Figure 2 shows the plasma misonidazole concentration plotted on a logarithmic scale against time on a linear scale; data are shown for the various intravenous dose levels and are for the first occasion on which the drug was given. Similar patterns were observed with subsequent doses.

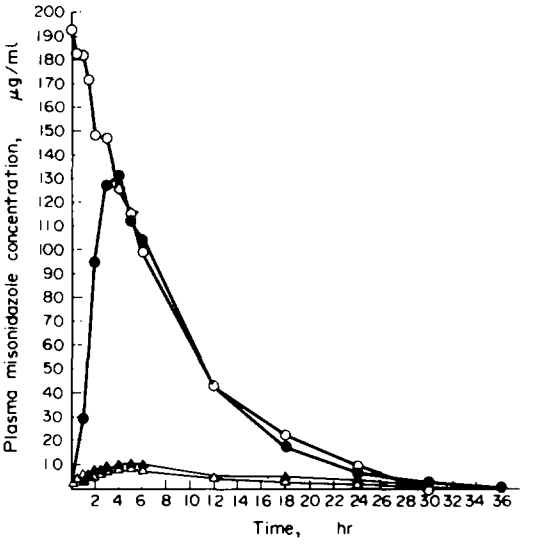

Fig. 1. Comparison of plasma misonidazole and Ro 05-9963 concentrations after intravenous oral administration of misonidazole at 150 mg/kg in two dogs.
○ *misonidazole (i.v. route);* ● *misonidazole (oral route);* △ *Ro 05-9963 (i.v. misonidazole);* ▲ *Ro 05-9963 (oral misonidazole).*

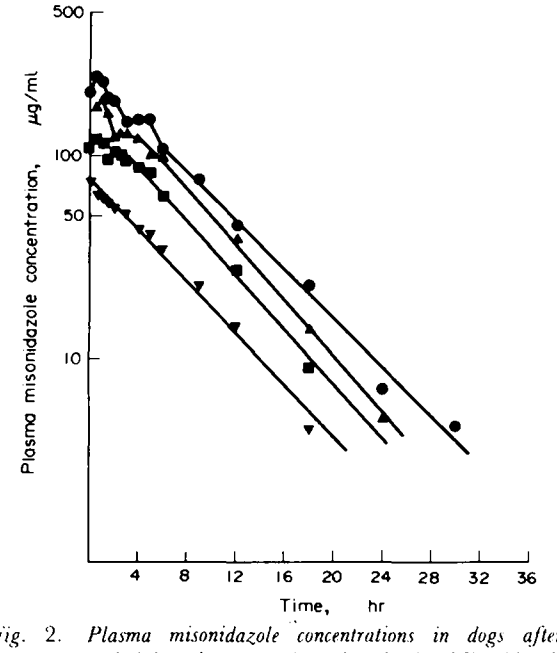

Fig. 2. Plasma misonidazole concentrations in dogs after intravenous administration at various dose levels. Misonidazole concentration is plotted on a logarithmic scale and time on a linear scale. ● 200 mg/kg; ▲ 150 mg/kg; ■ 100 mg/kg; ▼ 50 mg/kg.

In the case of intravenous administration there may be an initial distribution phase which may last between 1.5 and 3 hr (Fig. 2). However, this was not always apparent, particularly at the lower doses (50 and 100 mg/kg) despite the fact that several plasma samples were taken at the earlier time points (Fig. 2). The distribution phase, if present, is followed by an elimination phase which can be described by a single exponential function indicating apparent first order kinetics. When the drug was given orally there was an initial absorption phase, the duration of which varied considerably from 1 to 6 hr (e.g., Fig. 1).

We have determined a number of pharmacokinetic parameters for intravenous and oral misonidazole at the different dose levels and these are summarised in Table 1. Misonidazole peak time is expressed as the median of the four estimates for each dose level. The median value was used in order to avoid undue weighting caused by extreme values. Other pharmacokinetic parameters are expressed as the mean ± one standard error for the four estimates at each dose level. It should be noted, however, that some trends were seen over the four doses in the same dog and these are discussed in the appropriate section. Standard errors given in Table 1 may therefore include a component of variation due to such trends as well as normal experimental variation.

Peak misonidazole concentrations

Figure 3 illustrates the linear relationship between the peak plasma misonidazole concentrations and the administered dose. The correlation was good for both the oral route ($r=0.97$, $P<0.05$) and the intravenous route ($r=0.999$, $P<0.001$). However, the peak concentrations after oral dosage were generally lower than those for intravenous dosage.

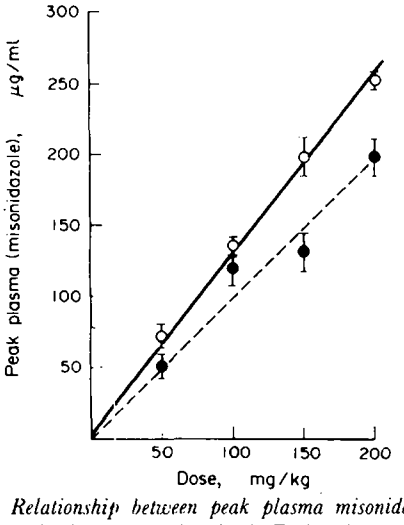

Fig. 3. Relationship between peak plasma misonidazole concentration and misonidazole dose level. Each point represents the mean and standard error for the four consecutive estimations. The correlation coefficient was calculated from the four points of each plot.

Oral, $r=0.97$; $P<0.05$.
i.v., $r=0.999$; $P<0.001$.
● *oral*; ○ *i.v.* ±*S.E.M.*

The coefficients of variation for peak misonidazole concentrations showed no tendency to vary with the dose. However, the coefficients of variation of the oral data were generally larger than those for the intravenous data.

The time of peak plasma concentration is dependant upon the route of drug administration. Rapid peaks are achieved when the drug is given intravenously with median peak time values ranging from 0.29 to 0.75 hr (Table 1A). Peak plasma concentrations occur later after oral administration and median values range between 1.5 and 3 hr (Table 1A). For both routes of administration there is a suggestion that peak times may occur later at higher doses. There was also a suggestion of a trend towards an increase in peak misonidazole concentrations with consecutive intravenous doses ($0.05<P<0.1$).

The longest peak time (6 hr) was recorded for the dog receiving 100 mg/kg orally and on this occasion the dog was fed inadvertantly before dosage.

Area under the curve

The total area under the curve (AUC) of the plasma misonidazole concentration against time plot was calculated using Simpson's Rule [3]. AUC values were similar for the oral and intravenous routes (Table 1A). The percentage systemic oral bioavailability of the drug was calculated from the ratio of the AUC for oral administration to that for intravenous administration at the same dose level [4]. Table 2 shows the percentage oral bioavailability values for the four dose levels and the overall mean value of 92±5%(S.E.). This indicates that there is good oral absorption of misonidazole with no significant pre-absorption metabolism by the gut flora or extensive first-pass metabolism by the liver and intestinal mucosa.

The dose dependence of AUC is demonstrated in Fig. 4. A close linear correlation was observed between dose and AUV for both oral administration ($r=0.97$, $P<0.05$) and the intravenous route. ($r=0.999$; $P<0.001$).

The data also indicated that there may be a trend towards increasing AUC values with consecutive doses, particularly for the oral

Table 1. *Pharmacokinetic parameters*

		Table 1A: Misonidazole							Table 1B: Ro 05-9963		
Dose (mg/kg)	Route	Peak conc (μg/ml)	Median peak time (hr)	AUC (μg/ml.hr)	$t_{\frac{1}{2}}$ (hr)	*Ke* (hr^{-1})	*Vd* (l/kg)	*Pcl* l/kg/hr	Peak conc (μg/ml)	Median peak time (hr)	*AUC* (μg/ml.hr)
50	i.v.	73.2 ±8.7	0.29	546 ±39	5.13 ±0.36	0.14 ±0.02	0.65 ±0.06	0.1 ±0.005	6.9 ±0.3	3	107 ±8
50	oral	52.3 ±6.6	1.5	496 ±40	4.95 ±0.36	0.14 ±0.013	0.68 ±0.06	0.1 ±0.005	6.7 ±0.6	3.5	112 ±4
100	i.v.	133.8 ±4.1	0.5	1066 ±96	3.96 ±0.31	0.18 ±0.005	0.5 ±0.07	0.1 ±0.005	11.2 ±0.3	6	172 ±9
100	oral	118.4 ±9.4	2.5	969 ±61	3.77 ±0.16	0.17 ±0.02	0.4 ±0.04	0.1 ±0.005	10.9 ±0.7	5.5	172 ±9
150	i.v.	196.8 ±11.8	0.58	1583 ±103	4.92 ±0.26	0.14 ±0.01	0.66 ±0.07	0.1 ±0.005	8.9 ±0.2	4	148 ±16
150	oral	131.5 ±12.4	3	1269 ±158	4.42 ±0.15	0.16 ±0.005	0.65 ±0.08	0.13 ±0.02	9.3 ±0.1	5.5	142 ±14
200	i.v.	252.4 ±5.5	0.75	2099 ±175	5.09 ±0.25	0.14 ±0.005	0.66 ±0.05	0.1 ±0.01	9.9 ±0.9	5	169 ±12
200	oral	198.9 ±12.5	3	2214 ±268	5.38 ±0.53	0.13 +0.01	0.57 ±0.07	0.09 ±0.01	10.6 ±0.5	5	187 ±14

Table 2. Oral bioavailability for various dose levels of misonidazole

Dose mg/kg	Percentage bioavailability
50	89 ±4
100	91 ±3
150	82 ±12
200	105 ±3
Mean	92 ±5

Values are means of 4 determinations ± S.E.
Oral bioavailability = 100 × Oral AUC/Intravenous AUC.

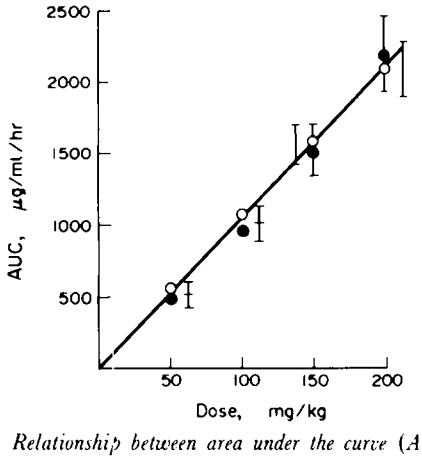

Fig. 4. Relationship between area under the curve (AUC) for plasma misonidazole and misonidazole dose level. Each point represents the mean and standard error for the four consecutive estimations. The correlation coefficient was calculated from the four points of each plot
Oral, $r=0.97$; $P<0.05$.
i.v., $r=0.99$; $P<0.001$.
● *oral*; ○ *i.v.*; ± *S.E.M.*

route of administration ($P<0.01$ for oral route: $0.05<P<0.1$ for i.v. route).

Elimination rate constant and half-life

The rate constant for the elimination phase (*Ke*) is given by the slope of the elimination phase of the log plasma concentration against time plot (see Fig. 2). The line of best fit was obtained by the method of least squares linear regression analysis. The half-life for the elimination phase ($t\frac{1}{2}$) was calculated from the equation $t\frac{1}{2}=(\ln 2)/Ke$.

It may be seen (Table 1A) that the values for *Ke* and $t\frac{1}{2}$ are similar for the oral and intravenous routes. Overall mean values for *Ke* were $0.151\pm0.028\,hr^{-1}$ (S.E.) and $0.148\pm0.026\,hr^{-1}$ (S.E.) for oral and intravenous routes respectively. Mean values for $t\frac{1}{2}$ were 4.6 ± 0.86 hr (S.E.) for the oral route and 4.7 ± 0.72 hr (S.E.) for the intravenous route.

It was found that *Ke* and $t\frac{1}{2}$ were independent of dose level for both routes of administration and there was no evidence of a trend with consecutive intravenous doses. There was, however, some suggestion of a decrease in the elimination constant and an increase in the half-life for consecutive oral doses ($0.01<P<0.02$).

Plasma clearance

The plasma clearance value (*Pcl*) is sometimes preferred to the half-life as an indication of the rate of elimination of a drug from the whole body. It was derived using the formula $Pcl=D/AUC_{0-\infty}$. Values showed little variation between 0.09 and 0.13 l/kg/hr with an overall mean value of $0.11\,l/kg/hr\pm0.01$. Values for the oral and intravenous routes were found to be similar and were independent of dose.

Altered dose levels

In a further study the two dogs given misonidazole at 50 mg/kg were used to ascertain the effects of altered dose levels upon the pharmacokinetics in the same dog. A dose level of 200 mg/kg was used and the drug administered for a further 2 weeks.

For both orally and intravenously dosed dogs the values of the misonidazole elimination rate constant, half-life, volume of distribution and plasma clearance were similar to those obtained at the 50 mg/kg dose level in the same dogs and presented in Table 1A. For example, the half-life for oral misonidazole was 4.95 ± 0.36 hr (S.E.) at 50 mg/kg and 5.79 ± 1.44 hr (S.E.) at 200 mg/kg. The corresponding values for the intravenous route were 5.13 ± 0.36 hr (S.E.) for the lower dose and 6.3 ± 0.42 hr (S.E.) for the higher dose level.

As would be expected, the values of the misonidazole peak concentration and AUC were different from those seen at the lower dose, but were similar to those obtained in the dogs receiving the oral and intravenous 200 mg/kg doses. The kinetic parameters for the *O*-demethylated metabolite were also similar to those obtained in the other dogs receiving 200 mg/kg (see next section).

O-Demethylated metabolite Ro 05-9963

The *O*-demethylated metabolite of misonid-

azole, Ro 05-9963, was detected in the plasma of all dogs administered misonidazole, both orally and intravenously (see Fig. 1). Plasma metabolite concentration and AUC values were considerably lower than the corresponding values for misonidazole. Peak metabolite concentrations occurred later than the misonidazole peaks having median time ranges of 3.5–6 hr and 4–6 hr for the intravenous and oral routes respectively (see Table 1B). Values for the peak metabolite concentration time of peak concentration and AUC were similar for oral and intravenous routes at all dose levels. Figure 5 illustrates the relationship between peak metabolite concentration and misonidazole dose level. It can be seen that the peak for the 50 mg/kg dose is lower than those at the higher doses. It was also found that the peak occurred earlier and was associated with a reduced AUC at this dose level (Table 1B).

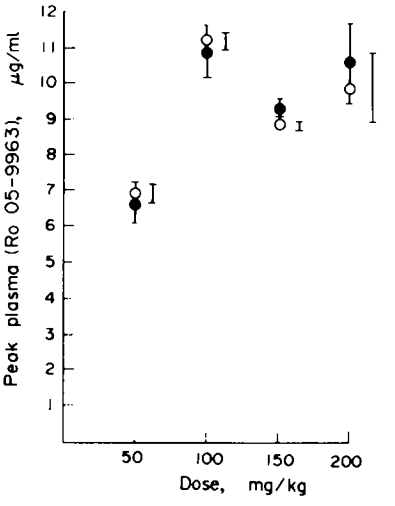

Fig. 5. Relationship between peak plasma Ro 05-9963 concentration and misonidazole dose level.
● *oral;* ○ *i.v.;* ± *S.E.M.*

Urinary excretion

The data for the urinary excretion for the 50 and 200 mg/kg doses are summarised in Table 3. Misonidazole and Ro 05-9963 were detected in the free and glucuronide conjugated forms. In one dog, however, no Ro 05-9963 glucuronide was detected. Urinary excretion was monitored for 72 hr and during that period 15–20% of the original dose of misonidazole was excreted. Of this, the majority (93–100%) was excreted during the first 48 hr. The amount excreted as unmetabolised misonidazole was about 5–7%; this figure was independent of dose and route of administration.

Toxicity

Retching and vomiting were occasionally seen 1–2 hr after the administration of misonidazole at all dose levels, but only in those dogs receiving the drug intravenously. No neurological symptoms were noted in any of the dogs during the study and in the subsequent 4 weeks.

Haematological parameters, blood urea and glucose, and serum alanine transaminase levels remained within normal limits for all dogs, with the exception of the dog given 200 mg/kg misonidazole orally, in which the serum alanine transaminase levels were raised following the third and fourth dose but subsequently returned to normal.

DISCUSSION

We have used HPLC analysis to study the pharmacokinetics of misonidazole when administered both orally and intravenously to

Table 3. Urinary excretion of the free and o-glucuronide forms of misonidazole and Ro 05-9963

	Percentage of dose excreted in 72 hr				
	Misonidazole		Ro 05-9963		
	Free	Conjugated	Free	Conjugated	Total
50 mg/kg i.v.	5.1 ±2.5	0.6 ±0.3	13.8 ±3.6	0.8 ±1.2	20.0 ±4.6
50 mg/kg Oral	4.5 ±1.2	1.3 ±1	13.0 ±5.2	1.7 ±2.8	18.9 ±6.3
200 mg/kg i.v.	7.4 ±5.9	3.5 ±2.7	6.2 ±2.9	4.4 ±3.2	18.5 ±7.7
200 mg/kg Oral	6.2 ±1.28	0.6 ±0.75	8.3 ±1.66	0	15.1 ±2.65

Figures quoted as mean of 4 determinations ± S.E.

the dog at various doses. The effects of repeated dosage and some toxicological observations are also recorded.

Searches of the literature have failed to reveal previously published detailed pharmacokinetic data of misonidazole in the dog. We can, however, compare our results with the unpublished data of Flockhart [5] and those given in a preliminary report by Lu *et al.* [6].

After intravenous administration of misonidazole there may be an initial distribution phase, more marked at higher dose levels, followed by a terminal or β-phase. Similar disposition kinetics were observed by Lu *et al.* [6].

Following oral administration a fairly rapid absorption phase is seen with peak misonidazole concentrations occurring at 1.5–3 hr, this is similar to the range of values seen in man [7–10]. The absorption phase is followed by a terminal elimination phase similar to that for the oral route.

The peak misonidazole concentration was directly proportional to dose over the range 50–200 mg/kg; this relationship was also seen when the dose was expressed in terms of surface area (g/m^2). Whilst this was true for both the oral and intravenous routes of administration, peak levels were rather lower for the oral route. In man a similar relationship between peak plasma concentration and dose has been observed for oral doses up to 100 mg/kg; however, the correlation becomes less clear at doses approaching 200 mg/kg [11]. It is also interesting to note that the peak misonidazole levels in dog and man are quite similar for a particular dose given on weight basis [8–10].

We have established that the half-life of the plasma misonidazole terminal elimination phase is independent of dose over the range studied. In addition the plasma half-life is similar for oral and intravenous routes, the overall mean value being 4.7 hr (individual value range 3.2–6.9 hr). For doses up to 150 mg/kg Flockhart [5] observed a value of 6.79 ± 1.71 hr (S.E.). However, the polarographic assay used in that study measured total nitroimidazole concentration, including the metabolite Ro 05-9963 which we have shown to be present in significant levels in the plasma of dogs given 50–200 mg/kg misonidazole. The polarographic analysis, therefore, tends to overestimate the misonidazole half-life. Lu *et al.* [6] observed a terminal half-life of 5.5 hr after the intravenous administration of carbon-labelled misonidazole (100 mg/kg or $2 g/m^2$).

Half-life values for oral misonidazole in man, determined by HPLC analysis, vary from 4 to 18 hr with a mean of approximately 12 hr [9, 10]. There is some overlap, therefore, between the upper values for the dog and the lower values for man. Notably, the plasma half-life in the dog is similar to the half-life value of 4.5–5.5 hr determined in the baboon by a carbon-labelled study [11], but is considerably longer than the values recorded for the mouse and rat by various techniques [11, 12].

A further indication of the rate of drug elimination from the whole body is given by the plasma clearance value. Values for the plasma clearance were remarkably constant, being independent of dose and route of administration.

The apparent volume of distribution (*Vd*) gives an estimate of tissue penetration. We have shown that *Vd* is independent of dose and route of administration. The overall mean value was 0.6 l/kg. Significantly this is similar to the total body water volume (intracellular and extracellular fluid); this suggests that misonidazole is distributed uniformly in the body water with good tissue penetration.

The area under the curve (AUC) appears to be a particularly important pharmacokinetic parameter in relation to the neurotoxicity of the drug in man. Dische *et al.* [1] have suggested that the tissue exposure, and hence the probability of toxicity, can be estimated from the AUC. We have shown that the AUC in dogs is directly proportional to dose expressed both on a weight and a surface area basis for both oral and intravenous routes of administration.

Apart from the preliminary report by Lu *et al.* [6] no data have been published on the oral bioavailability of misonidazole in any species. In man only oral preparations have been used. We have shown the oral bioavailability is independent of dose, the mean value being $92 \pm 5\%$ (S.E.). Thus the bioavailability of misonidazole is essentially complete with no suggestion of extensive first-pass metabolism by the liver and intestinal mucosa. This also rules out any significant pre-absorption metabolism by the gut flora. Lu *et al.* [6] reported a much lower value of 25% for the oral bioavailability after a dose of $2 g/m^2$.

In the present study food was withheld from the dogs for at least 12 hr before dosage. The influence of the presence of gastric content was suggested by the data obtained on

one occasion when the dog was inadvertantly fed 1 hr before oral dosage. This resulted in a considerable delay in the misonidazole peak time (6 hr) associated with a lower AUC value than would be expected (oral bioavailability = 74%). Hence the time of the last feed may be an important factor in oral bioavailability studies with misonidazole and may account for variations between individual laboratories. This has obvious relevance to clinical studies in man in which oral route is used.

The pharmacokinetic data for the *O*-demethylated metabolite Ro 05-9963 showed that the peak plasma concentration and the time at which it was attained were independent of both the route of administration and of dose, for misonidazole doses of more than 50 mg/kg. This was also true for the metabolite AUC values. At dose levels of 100, 150 and 200 mg/kg misonidazole the plasma concentrations of Ro 05-9963 were maintained at a fairly constant level of approximately 10 μg/ml for several hours before declining slowly. However, at the 50 mg/kg dose level the plasma metabolite concentration level was rather lower. We concluded that this effect was not due to variation between dogs since it was seen for both the orally and intravenously dosed dogs at 50 mg/kg. Moreover, when these dogs subsequently received doses of 200 mg/kg higher metabolite concentrations were observed which were similar to those seen in the other dogs receiving doses greater than 50 mg/kg. The most likely explanation of these data is that at doses of greater than 50 mg/kg the enzyme systems catalysing the *O*-demethylation of misonidazole become saturated and the rates of Ro 05-9963 production and elimination remain roughly similar; this would result in a steady state concentration of the metabolite. An alternative possibility is that the plasma clearance of the metabolite increases proportionally with increased metabolite production. As a result of either of these effects Ro 05-9963, itself an active radiosensitiser, represents a higher proportion of the total nitroimidazole present in the plasma at 100 mg/kg dose level than at 200 mg/kg. For all dose levels the relative proportion of Ro 05-9963 was greater at the later time points.

Similar levels of Ro 05-9963 have been observed in human plasma following oral misonidazole administration [9].

After 72 hr. 15–20% of the total misonidazole dose was excreted in the urine as the free and glucuronide conjugated forms of misonidazole and Ro 05-9963. The majority of this was excreted in the first 48 hr. In a human volunteer 48% of a dose of 30 mg/kg misonidazole was recovered from the urine in similar proportions to those in our study and a further 29% of the carbon-labelled material was recovered from the urine in an unidentified form [11]. However, in human patients the total recovery was much lower [13]. The urinary free and conjugated forms of misonidazole and Ro 05-9963 accounted for 45% of the dose of 100 mg/kg given intraperitoneally in mice and 34% of an oral dose of 200 mg/kg in a baboon [11].

Ings *et al.* [14] showed that following dosage with metronidazole one of the major urinary metabolites in dogs was the carboxylic acid derivative. Assandri *et al.* [15] also recovered the carboxylic acid and hydroxyl derivatives of 5-isopropyl-1 methyl-2 nitro-1 H-imidazole from the urine of dogs. Thus it is possible that some of the misonidazole unaccounted for in this study may be excreted as a carboxylic acid derivative. In addition, some may be excreted as the amine produced by nitroreduction and detected as a minor metabolite in human urine [11].

The dog is reported to be particularly susceptible to the neurotoxic effects of the nitroimidazole series [16]. However, using the present treatment regimes we have not encountered any neurological symptoms other than the emetic effect seen after intravenous dosage. Nevertheless, we have recorded symptoms of ataxia and nystagmus in a "giant" dog following three weekly intravenous doses of misonidazole at 150 mg/kg (6 g/m^2). It should be noted, therefore, that we have included only healthy male dogs of medium weight range with little breed difference in this study. Variations in the toxic side-effects and indeed in the pharmacokinetics of misonidazole may be encountered as a result of differences in such factors as sex, breed, weight and general condition.

In view of the detailed pharmacokinetic findings presented here we believe that the dog may be a useful species for the study of misonidazole as a radiosensitising agent. Several aspects of the pharmacokinetics are similar to those in man and the reproducibility of the kinetic data, both within and between individual dogs, was very encouraging. However, the main advantage over the rodent species commonly used for experimental studies is the longer plasma half-life in the

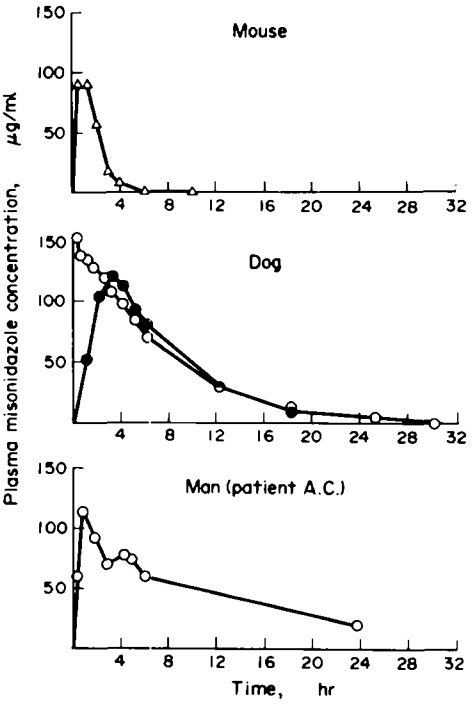

Fig. 6. Comparison of plasma misonidazole concentrations in mouse (100 mg/kg *i.p.*), *dog* (100 mg/kg *oral and i.v.*) *and men* (60 mg/kg *orally*).

dog which is closer to the values observed in man. The differences in the plasma clearance of misonidazole in the dog, mouse and man are well illustrated in Fig. 6. The relatively short half-life of misonidazole in mice is thought to be the reason for the rather poor penetration into mouse tumours by the drug [1]. The longer plasma half-life in the dog should allow better tumour penetration and we are currently investigating this possibility.

Acknowledgements—We would like to thank Mrs. Jane Donaldson for her technical assistance and Miss C. M. Wright for typing.

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