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Pyrimethamine pharmacokinetics and its tissue localization in mice: effect of dose size

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The plasma pharmacokinetics and mass fate of [^{14}C]pyrimethamine were investigated in the mouse, following dosage with 12·5, 25, 50, and 75 mg kg^{-1} (i.p.). Peak plasma concentrations of pyrimethamine were reached between 1 and 2 h and then declined monoexponentially. The mean values for AUC 0 → 30 h increased linearly in relation to the administered dose of pyrimethamine ($r = 0{\cdot}979$, $P \leq 0{\cdot}001$). The mean values for intraperitoneal clearance and half-life were not significantly different between dose groups, indicating that the plasma pharmacokinetics of pyrimethamine were independent of dose. The percentage of the administered dose excreted in urine as pyrimethamine (1·3–3·5%) and ^{14}C-radioactivity (21·7–29·1%) did not change with increasing dose. In contrast, the cumulative percentage of the dose excreted as ^{14}C-radioactivity in faeces (16·7–22·8%) after the three highest doses 25, 50 and 75 mg kg^{-1} was significantly less than that seen with the lowest dose of 12·5 mg (50·3%). This suggests extensive biliary excretion of radioactivity, and that the capacity of this process may have been exceeded with the highest doses. Seven days after the administration of each of the three highest doses, a significantly greater percentage of [^{14}C]pyrimethamine was localized in the soft tissues; i.e. heart, lung and kidney (7·8–13·8%), gut (5·4–9·4%) and particularly the liver (25·0–27·9%) when compared with the lowest dose of the drug (1·2, 1·0, 0·3% respectively). Following each dose, between 85 and 97% of the administered radioactivity was accounted for. These studies indicate, that with higher doses of pyrimethamine, the parent drug and/or metabolites may accumulate in soft tissue, particularly the liver, but without appreciable effects on the plasma disposition and urinary excretion of the drug.

Pyrimethamine-sulphonamide combination therapy is a first choice in areas of chloroquine resistant *Plasmodium falciparum* malaria. The pharmacokinetics of pyrimethamine remain poorly documented, since suitably selective and sensitive analytical methods were, until recently, unavailable. We have recently reported a method (Coleman et al 1984) which satisfies these criteria, and in this paper describe a series of experiments designed to investigate the disposition of pyrimethamine in the mouse; a host for the experimental rodent malarial parasite *Plasmodium berghei*.

The objectives of these studies were firstly to investigate the disposition of pyrimethamine in the mouse, and secondly, to establish the effect of dose size on (a) the pharmacokinetics of the drug and (b) the mass fate and tissue localization of ^{14}C-radiolabelled pyrimethamine.

MATERIALS AND METHODS

Reagents

Pyrimethamine base, and pyrimethamine 3-*N*-oxide were supplied by Wellcome UK (Beckenham, Kent). Proguanil hydrochloride, the internal standard, was supplied by ICI Pharmaceuticals (Alderley Edge, Cheshire). [^{14}C]Pyrimethamine base (specific activity 54 mCi mmol^{-1}) labelled in position 2 of the pyrimidine ring, was obtained from the Radiochemical Centre, Amersham International, (Bucks. UK). NCS tissue solubliser and hydrogen peroxide (30% w/v) were supplied by BDH Chemicals Ltd. (Poole, Dorset, UK). Emulsifying liquid scintillant (ES 299) was obtained from the Packard Instrument Company Ltd, (Caversham, Reading, UK). All other reagents were of HPLC grade (Fisons, Loughborough, UK).

Determination of pyrimethamine

Pyrimethamine and pyrimethamine 3-*N*-oxide concentrations in plasma and urine were determined by a selective and sensitive micro-analytical HPLC method (Coleman et al 1984).

Analysis of radioactivity

The levels of ^{14}C-radioactivity in samples were determined, in duplicate, by liquid scintillation counting using an Intertechnique SL30 liquid scintillation spectrometer.

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Animal studies

Eight groups of male white TFW mice (mean wt 25 g) were provided with an Oxoid 41B diet and free access to drinking water. The plasma disposition of pyrimethamine was determined in four of these groups. Groups A–D ($n = 5$; in each group) were each dosed with pyrimethamine base (suspended in 5% w/v Tween 80) at 12·5, 25, 50 and 75 mg kg^{-1} i.p. respectively. Blood samples (40–80 μl) were removed from the tail vein at 1, 2, 4, 6, 8, 10, 12, 24, 26, and 30 h into heparinized microhaematocrit tubes (20 μl capacity, Hawksley, Lancing, UK) which were then sealed. Following centrifugation (1100*g*, 5 min) volumes of plasma (20–40 μl), accurately measured using a 100 μl capacity syringe (Hamilton Ltd, Reno, Nevada, USA), were placed in microcap tubes (1·5 ml, L.I.P. Equipment and Services, West Yorkshire, UK).

At 30 h, the mice were killed, exsanguinated and the blood from the animals placed in heparinized microcap tubes. Plasma and red cells were separated by centrifugation (1100*g*, 5 min), and a 200 μl aliquot assayed for both pyrimethamine and pyrimethamine 3-*N*-oxide.

The urinary and faecal excretion and mass fate of [^{14}C]pyrimethamine were determined in the remaining four groups, E–H ($n = 6$ in each case) which were dosed with pyrimethamine base (suspended in 5% w/v Tween 80) at 12·5, 25, 50, 75 mg kg^{-1} i.p. respectively. These doses each contained a tracer dose (0·5 μCi, 2·2 μg) of [^{14}C]pyrimethamine base. The mice were then housed in individual metabolism cages, and urine and faeces collected serially for 7 days. Urine volumes were recorded and aliquots were assayed for pyrimethamine and pyrimethamine 3-*N*-oxide concentrations by HPLC (Coleman et al 1984). On completion of the study, the mice were removed from the cages and killed immediately. The heart, lung, kidneys, gut and liver were removed from each animal and homogenized using an Ultra Turrax blender, and the residual carcasses were cut into small pieces and homogenized for approximately 7 min in a Waring blender.

Sample preparation for determination of ^{14}C

Samples of urine (20–200 μl) and cage washings (500 μl) were directly assayed for ^{14}C after addition of scintillant fluid (5 ml). Aliquots of the homogenates (200 μl) of liver, gut, the combined heart, lung and kidney, faeces and remaining whole carcass were incubated overnight at room temperature with NCS tissue solubilizer (200 μl). Discolouration was achieved by a further 2 h incubation of the digest with hydrogen peroxide (200 μl) before the addition of emulsifying liquid scintillant (5 ml). Corresponding blank and spiked samples (see below) were processed in identical fashion.

To estimate background radioactivity, an untreated mouse was processed as described above, and the values obtained after counting these samples ($n = 5$ for each tissue) were subtracted from the experimental results. These control values were found to be less than 2% of the observed radioactivity in any of the experimental tissue homogenates. In order to determine recovery of ^{14}C from the various tissue homogenates, another untreated mouse was processed as described. Each of the tissues were spiked with 0·1 μCi (0·44 μg) of pyrimethamine immediately before the samples were homogenized and prepared for liquid scintillation counting.

Pharmacokinetic calculations and statistical analysis

Peak plasma concentrations (C_{pk}) and the times at which they were reached (T_{pk}) were obtained graphically. The elimination rate constants were determined by least squares regression analysis of the log plasma pyrimethamine concentration-time decay curves. The area under the plasma concentration-time curve from $t = 0$ to $t = 30$ h (AUC $0 \rightarrow 30$ h) was determined by the trapezoidal rule (Gibaldi & Perrier 1982). In the present study, the systemic availability from the i.p. dosage site and hence systemic clearance, of unchanged pyrimethamine, could not be determined. Consequently, the clearance of drug after i.p. dosage (Cl i.p.) was used as a measure of elimination efficiency for the different dose levels, and was calculated from the ratio of the dose and AUC $0 \rightarrow 30$ h (Rowland & Tozer 1980). The data were tabulated as mean ± s.d. and evaluated statistically using a one-way analysis of variance, accepting $P \leq 0{\cdot}05$ as significant.

RESULTS

Plasma disposition of unlabelled pyrimethamine

The mean log pyrimethamine plasma concentration-time curves for the four dosage groups are shown in Fig. 1 and the resultant pharmacokinetic parameters listed in Table 1. After i.p. administration, maximum measured plasma concentrations of pyrimethamine were reached within 1–2 h, after which they declined monoexponentially in all groups. At the two highest doses (50 and 75 mg kg^{-1}) considerably greater inter-animal variation in drug levels was encountered compared to that seen with the 12·5 and 25 mg kg^{-1} doses. This was reflected in the larger variance of the parameters for maximum

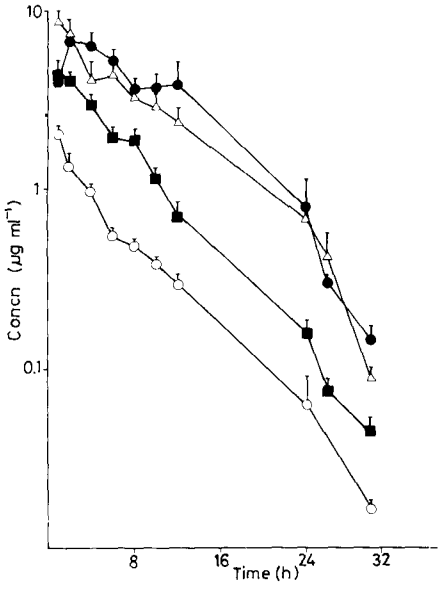

FIG. 1. Semi logarithmic plots of plasma concentration (mean ± s.e.m.) of pyrimethamine against time after the following doses: (mg kg^{-1}) 12·5 (○—○) 25 (■—■) 50 (△—△) 75 (●—●) (n = 5 for each dose).

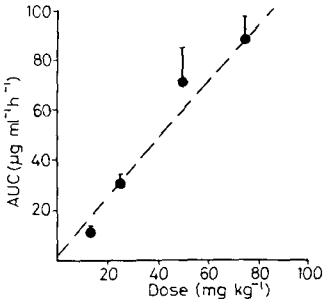

FIG. 2. Mean AUC 0 → 30 h (±s.e.m.) plotted against increasing dose of pyrimethamine (n = 5 for each dose). Broken line represents regression analysis (r = 0·979; $P \leq 0{\cdot}001$).

plasma concentrations (C_{pk}) area under the curve (AUC 0 → 30 h), and intraperitoneal clearance (Cl i.p.). Nonetheless, there was a dose dependent increase in mean AUC 0 → 30 with increasing doses of pyrimethamine (Fig. 2) and neither Cl i.p. nor elimination half life ($t_{\frac{1}{2}}$) differed between dosage groups.

Excretion and mass fate of [^{14}C]pyrimethamine

The cumulative excretion of both pyrimethamine in urine and ^{14}C-radioactivity in urine and faeces over 168 h is shown in Fig. 3. In the first 72 h approximately 90% of the drug and ^{14}C-radioactivity, that was ultimately excreted in urine and faeces, had been recovered. Over the ensuing 96 h only trivial, but persistent, levels of drug and radioactivity were excreted in the urine and faeces.

There were no appreciable differences in the cumulative urinary excretion of either pyrimethamine or ^{14}C-radioactivity between dosage groups. In contrast, the cumulative faecal excretion of ^{14}C-radioactivity after the lowest dose of pyrimethamine (12·5 mg kg^{-1}) was more than twice that obtained for the three highest doses (Fig. 3, Table 2).

Although pyrimethamine 3-*N*-oxide was detected in some samples of mouse plasma and urine, it rarely exceeded either the minimum detectable concentration (600 ng ml^{-1}) in plasma or 0·5% of the dose excreted in urine.

At the completion of the serial collection of urine and faeces after 168 h, the residual levels of ^{14}C-radioactivity were determined in mouse liver, gut, combined heart, lung and kidney, and in the remaining carcass as well as washings from the metabolism cages (Table 2). This allowed the mass balance of administered radioactivity to be determined. At the three highest doses of pyrimethamine (25, 50 and 75 mg kg^{-1}) a significantly greater percentage of the administered ^{14}C-radioactivity was localized in the gut, lung, kidney and heart, and particularly the liver when compared to the lowest pyrimethamine dose (12·5 mg kg^{-1}). However, there was little residual ^{14}C-radioactivity in the remaining carcasses, and in the cage washings, at all

Table 1. Pharmacokinetic parameters of pyrimethamine over the dose range 12·5–75 mg kg^{-1} listing mean values (± s.d.; n = 5 in each group), for time to peak concentration (T_{pk}), peak concentration (Cpk), elimination half-life ($t_{\frac{1}{2}}$), area under the curve from time = 0 to time = 30 h, (AUC 0 → 30 h) and intraperitoneal clearance (Cl i.p.)

Treatment Group	Dose mg kg^{-1}	T_{pk} h	C_{pk} μg ml^{-1}	$t_{\frac{1}{2}}$ h	AUC 0 → 30 h μg h ml^{-1}	Cl i.p. ml min^{-1}
A	12·5	≤ 1	2·0 ± 0·7	5·6 ± 1·3	11·6 ± 1·4	0·45 ± 0·12
B	25·0	≤ 1	4·6 ± 1·3	4·6 ± 0·9	31·4 ± 5·4	0·34 ± 0·10
C	50·0	≤ 1	8·9 ± 4·5	5·3 ± 0·8	71·8 ± 30·1	0·38 ± 0·25
D	75·0	≤ 2	7·0 ± 4·3	5·4 ± 1·1	81·6 ± 37·5	0·44 ± 0·16

Table 2. Excretion in urine and faeces and tissue localization at 168 h, after ^{14}C pyrimethamine administration to mice. (n = 6 in each group)

Treatment Group	Dose mg kg^{-1}	% Recovered as Unchanged Pyri-methamine	% ^{14}C labelled pyrimethamine recovered							% Total Recovered
			Urine	Faeces	Liver	Gut	† H/L/K	Carcass	Cage Wash	
E	12·5	3·3 ± 3·5	29·1 ± 19·4	50·3 ± 13·3*	0·3 ± 0·25*	1·0 ± 0·5*	1·2 ± 2·2*	2·5 ± 1·4	0·8 ± 0·6	85·6 ± 26·2
F	25	2·8 ± 2·1	25·4 ± 9·9	22·8 ± 1·3	25·1 ± 12·8	6·1 ± 2·8	7·8 ± 2·2	0·7 ± 0·3	0·3 ± 0·1	88·2 ± 18·9
G	50	1·3 ± 1·2	21·7 ± 19·5	22·9 ± 5·9	27·9 ± 12·9	9·4 ± 4·0	13·8 ± 13·0	1·6 ± 0·8	0·2 ± 0·1	97·7 ± 22·1
H	75	3·2 ± 1·9	28·8 ± 12·8	16·7 ± 4·2	27·4 ± 20·2	5·4 ± 3·8	7·8 ± 5·9	4·7 ± 4·2	1·2 ± 0·8	91·4 ± 32·9

* denotes significant difference ($P \leq 0{\cdot}05$) from other dosage groups.
† H/L/K denotes combined heart/lung/kidney homogenate.

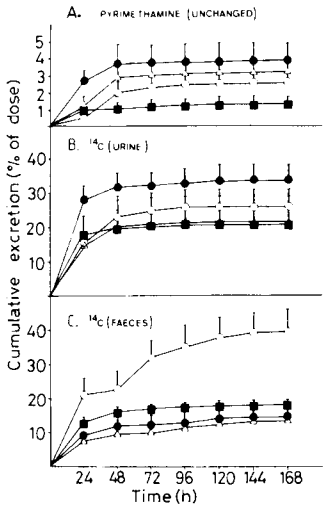

FIG. 3. Cumulative excretion profiles of ^{14}C and pyrimethamine plotted over 168 h: (A) unchanged pyrimethamine in urine (B) ^{14}C in urine (C) ^{14}C in faeces (mg kg^{-1}) 12·5 (○—○) 25 (■—■) 50 (△—△) 75 (●—●) (n = 6 for each dose).

dose levels. Therefore, as there was an appreciably greater faecal excretion of radioactivity at 12·5 mg kg^{-1}, the total recovery of administered radioactivity was comparable for all dose levels and virtually complete (≥85% for all dose levels; Table 2).

DISCUSSION

Pyrimethamine has been widely employed in malaria prophylaxis and is used in prophylaxis therapy when combined with a sulphonamide or sulphone, especially in areas of chloroquine resistance. However it is also used less frequently to treat toxoplasmosis and coccidiosis and more recently meningeal leukaemia (Lock 1981; Trier et al 1974; Geils et al 1971). In spite of its widespread use, there is little information on the pharmacokinetics and mass fate of this drug, or on the influence of dose size on these parameters.

After intra-peritoneal dosage to mice, pyrimethamine appeared rapidly to enter the systemic circulation (Fig. 1). Maximum plasma concentrations were achieved within 1–2 h and plasma levels subsequently declined monoexponentially, with an elimination half-life of 4·6–5·6 h for each of the four doses (Fig. 1, Table 1). These values are in broad agreement with results from an earlier study in mice which reported a half-life of 6·5 hours after a 10 mg kg^{-1} dose of pyrimethamine (Cavallito et al 1978).

In the present study the individual values obtained for both AUC 0 → 30 h and Cl i.p. after 50 and 75 mg kg^{-1} showed a greater variance than those observed following administration of 12·5 and 25 mg kg^{-1} doses. This may reflect differences in the absorption or efficiency of elimination of the drug at the two higher doses. Nonetheless the values for AUC 0 → 30 h rose proportionally with increasing dose (Fig. 2; r = 0·979; $P \leq 0{\cdot}001$). Hence the values calculated for Cl i.p. exhibited no significant change between dose groups (Table 1). This finding, together with the similarities in mean values for half-lives between groups, implies that the kinetics of pyrimethamine elimination from plasma are first order, even at doses approaching the LD50 for this drug in mice (80 mg kg^{-1}; Rees et al 1976).

The cumulative excretion of both pyrimethamine and ^{14}C-radioactivity over 7 days and the tissue localization of the residual radioactivity was determined in parallel groups of mice, each receiving a [^{14}C]pyrimethamine tracer dose (i.p.) in an equivalent dosage schedule (i.e. 12·5, 25, 50, 75 mg kg^{-1} pyrimethamine). It can be seen from the cumulative excretion profiles (Fig. 3) that in excess of 90% of the radioactivity ultimately excreted, was recovered within the first 72 h. There were no differences in the urinary excretion of pyrimethamine or ^{14}C-radioactivity between groups of mice. Although 20–30% of the dose appeared as ^{14}C-radioactivity in urine, only one tenth of this amount was present as the parent drug. Trace levels only of the 3-*N*-oxide

metabolite of pyrimethamine were detected in these urine samples, suggesting that this is a trivial route of metabolism for pyrimethamine in the mouse. This is in agreement with a previous report in rats, where only 1·4% of the dose (1 mg kg^{-1}) was recovered as unconjugated 3-*N*-oxide (Hubbell et al 1978). The remaining radioactivity in the urine samples of our study would be in the form of one or more pyrimethamine metabolites other than the 3-*N*-oxide. As the major metabolite in rat urine has been identified as the hydroxylated derivative of the 3-*N*-oxide (Hubbell et al 1978), it is probable that this compound is also present in considerable levels in mouse urine. After the lowest dose of pyrimethamine (12·5 mg kg^{-1}), 50·3% of the administered radioactivity was recovered in the faeces, implying considerable biliary excretion of the drug and/or its metabolites (Table 2). Significantly less radioactivity (22·8, 23·4 and 16·7% of the dose) appeared in the faeces after 25, 50 and 75 mg kg^{-1} respectively, suggesting that the capacity of the biliary excretion process had been exceeded at the higher doses. This was reflected in the substantially elevated residual levels of radioactivity in the livers of the three higher dose groups (Table 2). Furthermore, considerably greater levels of radioactivity were located in the gut, heart, lung and kidneys of mice receiving 25 mg kg^{-1} or more of pyrimethamine. As the plasma concentrations of pyrimethamine declined monoexponentially (Fig. 1) and the cumulative urinary excretion of unchanged drug was virtually complete (Fig. 3), the residual radioactivity located in the soft organs (after doses of 25 mg kg^{-1} or above), is unlikely to represent the parent drug, but rather metabolites of pyrimethamine. Only small amounts of radioactivity were found in the animal carcasses and cage washings for all treatment groups, and consequently between 86 and 98% of the administered radioactivity could be accounted for (Table 2).

Studies with radiolabelled pyrimethamine (Hubbell et al 1978) indicate that this drug and/or its metabolites undergo considerable biliary excretion. Our findings, using ^{14}C labelled pyrimethamine support these observations, and suggest that at chronic or higher doses, this route of elimination may be capacity limited. This may lead to accumulation of pyrimethamine and/or its metabolites in body tissues, particularly the liver (Table 2). It is interesting to note that pyrimethamine exerts a considerable primary exoerythrocytic schizontocidal effect within the liver during antimalarial therapy (Bruce-Chwatt 1981).

Enterohepatic recirculation has been demonstrated in various species for pyrimethamine (Cavallito et al 1978) and also for the structurally related compounds trimethoprim and minoxidil (Hubbell et al 1978; Thomas et al 1975). This recycling process may contribute to the long acting therapeutic effect of this drug, and to the continued low level of urinary excretion of unchanged pyrimethamine on day 7 of the present study.

We have investigated the plasma pharmacokinetics of pyrimethamine in the mouse and found them to be independent of dose. However, our studies with ^{14}C labelled pyrimethamine suggest that, with higher doses and during chronic therapy, the drug and/or its metabolites may accumulate in various body tissues particularly the liver.

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