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The disposition of pyrimethamine in the rat isolated perfused liver: the effect of suramin

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The pharmacokinetics of pyrimethamine (0·5 mg) were determined in the rat isolated perfused liver in the presence of suramin (17·75 mg). The clearance, half life, area under the curve, and volume of distribution of pyrimethamine were unaffected by the concurrent administration of suramin, as was the hepatic sub-cellular disposition of the drug. This report is of relevance to the future concurrent administration of suramin and pyrimethamine in West Africa.

The recent spread of the resistance of *Plasmodium falciparum* malaria to 4-aminoquinolines in East Africa (Doberstyn 1984) points to the eventual loss of effectiveness of these drugs in other African areas, and necessitates the use of alternatives such as pyrimethamine/sulphonamide combinations. In the event of chloroquine resistance occurring in West Africa, pyrimethamine is likely to be concurrently administered with the macrofilaricidal drug, suramin, which is already in use for the treatment of onchocerciasis. We have investigated the effect of suramin, a potent inhibitor of many enzyme systems (Hawking 1978), on the disposition of pyrimethamine in the rat isolated, perfused liver preparation (RIPL), a useful experimental model for the study of the hepatic component of possible drug interactions, free from the influence of other organs.

Materials and methods

Rat isolated perfused livers. Male rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) and their livers isolated using standard techniques and then perfused in a constant flow (15 ml min^{-1}) recirculating system at 37 °C as previously described (Mihaly et al 1982). The principal indices of liver viability were steady oxygen consumption (1·5–2·0 μmol (g liver)$^{-1}$ min^{-1}), sustained bile flow (0·2–0·6 ml h^{-1}), constant perfusion pressure (60–80 mm H_2O), reproducible liver function tests (i.e. determination of perfusate sodium, potassium, total protein, alanine amino transferase and γ-glutamyl transferase concentration), and normal visual appearance.

Methods. Two groups of five RIPL preparations were studied. To the first group of preparations pyrimethamine (0·5 mg) was added to the reservoir as a solution in dimethyl sulphoxide (DMSO, 10 μl), thereby simulating systemic dosage. To the second RIPL group, pyrimethamine (0·5 mg, DMSO, 10 μl) was co-administered with suramin (17·75 mg dissolved in water, 100 μl). Samples were removed from the perfusate reservoir pre-dose, and at 0·5, 1, 1·5, 2, 2·5, 3, 3·5, 4, 4·5 and 5 h. After centrifugation (1100*g*, 2 min) the perfusate plasma was removed and stored at −20 °C before assay for pyrimethamine by HPLC (Coleman et al 1984). An equal volume of fresh perfusate was added to the reservoir to replace that removed by sampling. Bile was collected at hourly intervals into pre-weighed vials and the bile volume determined by weight. To prepare a representative sample of total bile production of each RIPL preparation, 40% of each hourly bile sample was removed and combined. Pyrimethamine concentrations were determined by direct injection of bile samples (20 μl) onto the HPLC. At the conclusion of each experiment, the livers were flushed with 0·9% saline, weighed, and then homogenized in three times the liver weight of ice-cold 0·067 M phosphate buffer (pH 7·5 containing 1·5% KCl) using a Teflon-in-glass homogenizer. The 25% homogenate was centrifuged at 10 000*g* for 20 min at 4 °C. The resulting supernatant was decanted without disturbing the pellet and centrifuged at 105 000*g* for 60 min at 4 °C. The 105 000*g* and 10 000*g* pellets were then resuspended in three times their weight of phosphate buffer. Before pyrimethamine HPLC analysis of the various liver fractions, separate pyrimethamine standard curves were derived from drug-free whole liver homogenate, 10 000*g* pellet (resuspended), 10 000*g* supernatant, 105 000*g* pellet (resuspended) and 105 000*g* supernatant. Perfusate oxygen and carbon dioxide content were measured and liver function tests performed before and after each experiment to ascertain liver viability. The pharmacokinetic parameters for pyrimethamine [peak plasma concentrations (Cpk), terminal phase rate constant and half life ($t_{\frac{1}{2}}$), area under the curve from time zero to infinity (AUC), clearance (Cl) and volume of distribution (Vd)] were calculated as previously described (Coleman et al 1985). Statistical comparison between the two treatment groups was made by use of Student's *t*-test (unpaired). Statistical significance was accepted when $P \leq 0.05$. Tabulated data are presented as mean ± s.d.

Results and discussion

The calculated pharmacokinetic parameters of pyrimethamine are shown in Table 1. After administration of

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Table 1. The pharmacokinetic parameters of pyrimethamine (0·5 mg) before and after the concurrent administration of suramin (17·75 mg) to the rat isolated perfused liver.

	n	C_pK μg ml^{-1}	$t_{\frac{1}{2}}$ h	AUC μg h ml^{-1}	V_d ml	Cl ml h^{-1}
Pyrimethamine	5	1·5 ± 0·4	2·7 ± 1·1	5·3 ± 1·1	252·2 ± 66·2	98·4 ± 20·0
Pyrimethamine/suramin	5	1·3 ± 0·4	4·1 ± 0·9	6·8 ± 1·9	364·0 ± 110·0	77·6 ± 18·5
$P \leq 5$		NS	NS	NS	NS	NS

pyrimethamine alone to the RIPL, drug concentrations decayed monoexponentially, with a half-life of 2·7 ± 1·1 h; the AUC for pyrimethamine was 5·3 ± 1·1 μg h ml^{-1}. The systemic clearance of pyrimethamine (98·4 ± 20·0 ml h^{-1}) represented only 10·2% of liver perfusate flow, indicating pyrimethamine to be a low clearance drug in the RIPL. The apparent volume of distribution of pyrimethamine (252·2 ± 66·2 ml) significantly exceeded the circuit volume (100 ml plus liver volume) implying considerable hepatic drug uptake. This was reflected in the HPLC analysis of the whole liver and various fractions. Of the dose of pyrimethamine, 20·0 ± 12·9% was recovered as parent drug from the livers at 5 h. Analysis of the pooled bile aliquots revealed only low level elimination of unchanged pyrimethamine (2·5 ± 1·1%). These observations are in agreement with a previous report (Coleman et al 1985).

The coadministration of suramin with pyrimethamine to the RIPL caused an increase in both the half-life (2·7 ± 1·1 to 4·1 ± 0·9 h) and volume of distribution 252·2 ± 66·2 to 364·0 ± 110·0 ml). However, these differences did not attain significance and the values for the two treatment groups for area under the curve (5·3 ± 1·1 and 6·8 ± 1·9 μl h ml^{-1}) clearance (98·4 ± 20·0 and 77·6 ± 18·5 ml h^{-1}), and peak plasma concentration (1·5 ± 0·4 and 1·3 ± 0·4 μg ml^{-1}) showed no significant differences, hence suramin did not alter the perfusate plasma disposition of pyrimethamine. The biliary elimination of unchanged pyrimethamine (2·5 ± 1·1%) was also unaffected by the presence of suramin (2·4 ± 1·7%). There were no significant differences in either the percentage of pyrimethamine recovered from the whole livers (20·0 ± 12·9, 19·8 ± 4·0%) or the levels of drug measured in the various liver tissue fractions (Table 2). Hence suramin altered neither the hepatic disposition nor the sub-cellular localization of pyrimethamine.

Table 2. The percentage of unchanged pyrimethamine present in whole liver and fractions before and after the concurrent administration of suramin (17·75 mg) to the rat isolated perfused liver.

Tissue	Pyrimethamine alone	Pyrimethamine with suramin
Whole liver	20·0 ± 12·9	19·8 ± 4·0
10 000*g* supernatant	6·7 ± 2·4	7·8 ± 2·0
10 000*g* pellet	14·0 ± 11·1	12·5 ± 5·0
105 000*g* supernatant	3·4 ± 1·0	2·8 ± 0·8
105 000*g* pellet	2·9 ± 0·6	2·3 ± 1·1

In summary, the disposition of pyrimethamine in the RIPL was unaffected by the presence of suramin. It is hoped that this report will be of relevance to the future concurrent administration of these drugs in endemic areas of both malaria and onchocerciasis.

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