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DECREASED HEPATIC ELIMINATION OF PYRIMETHAMINE DURING MALARIA INFECTION

STUDIES IN THE ISOLATED PERFUSED RAT LIVER

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Abstract—The elimination of the antimalarial drug pyrimethamine was studied in isolated liver preparations from young rats (80–100 g) infected with merozoites of *Plasmodium berghei* two weeks earlier. Perfusate half-life of pyrimethamine was increased in livers from M.I. rats ($t_{\frac{1}{2}}\beta$ control group = 56 ± 11 min vs M.I. group = 101 ± 12, $P < 0.01$), reflecting a decrease in hepatic clearance (3.6 ± 1.1 ml/min vs 1.9 ± 0.5 ml/min, $P < 0.01$). There was no significant difference in volume of distribution between livers from M.I. and control groups. Intrahepatic concentration of unchanged drug at 3 hr was 4–5-fold greater in livers from infected rats (control group = 4725 ± 2287 ng/ml vs M.I. group =22,324 ± 6824 ng/ml), while liver:perfusate concentration ratios were not significantly different (control group = 30.8 ± 24.1 vs. M.I. group = 35.6 ± 20.3). We conclude that the hepatic elimination of pyrimethamine is substantially impaired in the malaria-infected rat.

Patients with malaria become acutely symptomatic during the erythrocytic phase of the disease, and it is then that drug treatment is usually instituted. During this phase the liver shows biochemical and pathological evidence of damage [1, 2], and the possibility of impaired drug metabolism arises [3]. We have recently shown that isolated liver preparations from rats infected with *P. berghei* show up to 50% reduction in primaquine clearance, depending on the extent of red cell parasitaemia that has been achieved in the intact animal [4]. The present study examines the impact of the erythrocytic stage of *P. berghei* infection on the elimination and hepatic distribution of the widely used anti-malarial pyrimethamine in the isolated perfused rat liver.

MATERIALS AND METHODS

Experimental preparation

Isolated perfused rat livers (IPRLs) were prepared from six healthy rats, 5–7 weeks of age and weighing 80–100 g (control group) and from six rats (age and weight matched) that had two weeks previously been infected with the rodent malaria parasite *P. berghei* (M.I. group), using procedures previously described [4]. Perfusate (100 ml) comprised 10% v/v washed human red cells, 1% w/v bovine serum albumin (Commonwealth Serum Laboratories, Melbourne) and 0.1% glucose in Krebbs–Henseleit buffer and was pumped at constant flow (10 ml/min) in a recycling system at 37°. The mean level of parasitaemia produced in the M.I. rats (19.5 ± 13.4%) ranged from 3 to 35% of erythrocytes infected. As previously reported, the infection led to pigmentation and enlargement of the liver (M.I. group = 5.8 ± 1.1 g vs control = 4.8 ± 0.4 g) and spleen (M.I. group = 3.1 ± 0.6 g vs control = 0.6 ± 0.1 g) in all animals. Indices of viability of IPRLs from control and M.I. rats were not significantly different as assessed by oxygen consumption (control group = 2.0 ± 0.7 μmol O_2/g liver/min vs M.I. group = 1.5 ± 0.9 μmole O_2/g liver/min), by initial bile flow (0.47 ± 0.12 ml/hr vs 0.40 ± 0.09 ml/hr) or by initial perfusion back pressure (8.4 ± 2.2 cmH_2O vs 8.8 ± 2.5 cm H_2O).

Experimental design

1. *Elimination of pyrimethamine from perfusate.* Pyrimethamine was injected into the perfusate reservoir (simulating bolus systemic dosage) of both control and M.I. liver preparations, as a solution in dimethylnitrosamine (20 mg/ml; 25 μl = 0.5 mg). Perfusate was sampled predose and again at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min for measurement of pyrimethamine concentration. An equal volume of drug-free perfusate was added to the reservoir to replace that volume of perfusate removed by sampling. Bile was collected hourly over 3 hr.

2. *Hepatic uptake of pyrimethamine.* At the conclusion of the 3 hr perfusion, the hepatic content of unmetabolised pyrimethamine was examined in 5 of the IPRL preparations studied above (M.I. = 3, control = 2) and a further 7 comparable IPRLs preparations (M.I. = 3, control = 4) treated in the same way as for perfusate elimination studies. At 3 hr after flushing with ice-cold buffer (5 ml), the liver was

immediately immersed in ice-cold solution of 250 mM sucrose. All subsequent steps were carried out at 4°. Liver (5 g) was minced and then homogenised in 15 ml of buffer with four strokes of a Potter Elvejheim homogeniser at 1000 rpm. An aliquot of the homogenate was frozen and later subjected to HPLC analysis of tissue pyrimethamine levels.

Drug analysis

Perfusate and liver tissue concentrations of pyrimethamine were estimated by a sensitive and selective high performance liquid chromotographic procedure as previously described [5].

Calculations and statistical assessments. Data in the text and table are presented as mean ± standard deviation and graphically as mean ± standard error of the mean. The pharmacokinetic parameters were calculated as follows [6]: half-life was calculated from the slope of the terminal elimination phase, clearance from the ratio of dose to area under the curve from the time of dosing to infinity and volume of distribution from the product of clearance and half life divided by ln 2 (i.e. 0.693). Statistical comparisons were made with the Wilcoxon's rank test, accepting $P < 0.05$ as significant.

RESULTS

Following pyrimethamine dosage to control perfused livers, drug levels fell biexponentially (Fig. 1). Initially the decline in concentrations was due substantially to hepatic tissue uptake of the drug [7]. Thereafter perfusate levels fell more gradually and monoexponentially, reflecting drug elimination. The table summarises mean pharmacokinetic parameters and shows a significantly lower clearance and longer half-life in M.I. perfused livers when compared with controls ($P < 0.01$). The values for volume of distribution did not differ between M.I. and control groups, but were seen to be 2–3-fold the value of the perfusate volume (100 ml), indicating drug uptake into liver tissue (Table 1).

Table 1. Pharmacokinetic parameters of pyrimethamine in perfused livers from control and M.I. groups of rats

Parameter	Control	M.I.
Clearance (ml/min)	3.6 ± 1.1	1.9 ± 0.5†
Half-life (min)	56 ± 11	101 ± 12†
Volume of distribution (ml)	284 ± 80	287 ± 78
Perfusate concentration at 3 hr (ng/ml)	298 ± 316	723 ± 250†
Liver concentration* at 3 hr (ng/ml liver)	4725 ± 2287	22324 ± 6824†

* Using specific gravity of liver of 1.0 g/ml.
†Denotes significant difference ($P < 0.05$).

The decreased clearance of pyrimethamine in liver preparations from malaria infected rats is reflected by the 2–3-fold higher pyrimethamine perfusate concentrations at 3 hr in the M.I. group compared to those seen in controls (Table 1). At 3 hr the hepatic concentration (using the specific gravity of liver tissue of 1.0 g/ml) of pyrimethamine was 4725 ± 2287 ng/ml and 22324 ± 6824 ng/ml in livers from control and

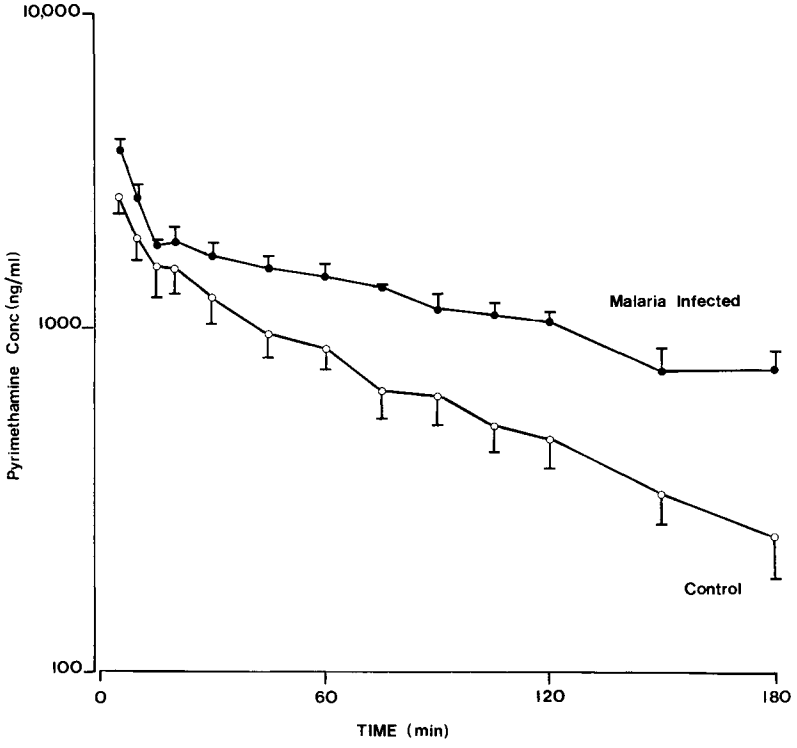

Fig. 1. Mean perfusate elimination profiles for pyrimethamine in control (○—○) and malaria infected (●—●) isolated perfused rat liver preparations. Pyrimethamine clearance in perfused livers from malaria infected animals was reduced by about 50% of the control value.

M.I. groups, respectively. Hence, the decreased clearance of pyrimethamine from the perfusate of M.I. liver preparations leads to a higher proportion of the administered dose of unmetabolised pyrimethamine persisting in liver tissue of these preparations. Thus, the amount of unmetabolised pyrimethamine in the perfusate at 3 hr (expressed as percentage of administered dose) in control and M.I. preparations was $6.0 \pm 6.3\%$ and $14.5 \pm 5.0\%$, respectively. Similarly, the amount in control and M.I. liver tissue was $3.9 \pm 1.2\%$ and $20.2 \pm 4.5\%$, respectively. The extensive hepatic localisation of pyrimethamine is further illustrated by the large concentration gradient at 3 hr between liver tissue and perfusate plasma (μg/ml liver : μg/ml perfusate), seen in both control (30.8 ± 24.1) and M.I. livers (35.6 ± 20.3).

DISCUSSION

During the erythrocytic stage of malaria infection, the rapid multiplication of merozoites within red blood cells causes severe haemolysis. Liver damage follows from the phagocytosis of cellular and parasite debris, which has been released into the systemic circulation [3]. The accumulation of "malaria pigment" (i.e. breakdown products of haemoglobin haem [8, 9]) and the hyperplasia of Kupffer cells leads to discolouration and enlargement of livers. It has been established that malaria infection may result in striking pathological changes in the liver [1]. However, the suggested sequelae between malaria infection and impaired hepatic drug metabolism is not well defined and the influence of malaria on the extent of hepatic drug localisation into liver tissue has not been examined.

In the present study the hepatic clearance of pyrimethamine was decreased by nearly 50% in perfused livers derived from infected animals. This impairment of elimination efficiency was translated into a twofold increase in half-life, as no change in overall drug distribution was evident. These results are consistent with our earlier findings in this experimental model [4]. In those studies the clearance of primaquine was seen to decrease progressively as the severity of infection increased. Thus drug elimination was compromised by up to 50% in those perfused liver preparations that had been established from rats with the most extreme blood cell malaria infections. In the present study, unlike our study with primaquine, there was no clear relationship between pyrimethamine clearance and the extent of parasitaemia.

Previous *in vitro* studies in liver microsomes from erythrocyte stage, malaria infected mice showed similar impairment of drug metabolism [3]. There is also clinical evidence that in patients with cerebral malaria, the systemic clearance of unbound quinine is only one-third that seen in convalescent patients [10, 11], presumably due to a reduced hepatic elimination of the drug during the parasitic infection. These experimental and clinical studies indicate that reduced efficiency of drug elimination, particularly by the liver, may follow the erythrocytic stage of malaria infection. It is not possible, however, to predict the likely effect of malaria infection on the elimination of other antimalarial drugs of diverse metabolic fate. This issue with other antimalarial drugs could be readily examined using this perfused liver experimental model.

Pyrimethamine undergoes extensive hepatic uptake as indicated by the large value for volume of distribution and by the substantial gradient from liver tissue to perfusate in both M.I. and control group (Table 1). Due to the slower elimination of pyrimethamine in the M.I. group, the concentration of pyrimethamine in liver tissue at 3 hr, was substantially greater in infected livers than control preparations. Consequently, at the conclusion of the experiment the liver content of unmetabolised pyrimethamine accounted for one-fifth of the dose in M.I. preparations and only one-twentieth of the administered dose in control livers. These results imply the likelihood of increased pyrimethamine accumulation in liver tissues, particularly upon chronic administration, after malaria infection. At present there is no information on the extent of accumulation expected with other drugs used during malaria infection.

The present study has confirmed, for pyrimethamine, the impairment of drug metabolism, and the potential for drug accumulation in liver tissue, following the erythrocytic stage of malaria infection. These results suggest that the possibility of drug toxicity may need to be considered in designing dosage regimen in patients with severe infections.

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