Biochemical Pharmacology, Vol. 36, No. 2, pp. 225–228, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

THE EFFECT OF MALARIA INFECTION ON PRIMAQUINE ELIMINATION IN THE ISOLATED PERFUSED RAT LIVER

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(*Received* 4 *June* 1986; *accepted* 1 *August* 1986)

Abstract—Most antimalarial drugs are eliminated by hepatic metabolism. However, the influence of malaria infection on the hepatic elimination of these drugs has not been examined. In the present study the elimination of the antimalarial primaquine has been examined in isolated perfused rat livers (IPRL) of malaria-infected Sprague–Dawley rats (90–110 g) (MI group; N = 6) and age- and weight-matched healthy rats (control group; N = 7). IPRL preparations for the MI group were established 12–15 days after rats were infected with merozoites of *Plasmodium berghei* (150×10^6 parasites/ml; 0.2 ml i.p.). At the time of study there was marked variation in the degree of parasitaemia achieved in the rats used in the MI group, from 2 to 27% of erythrocytes being infected. Livers were isolated using standard techniques and perfused at 10 ml/min in a 100 ml recycling system for 4 hr. In the control group, the perfusate disappearance of primaquine was biphasic with a mean $t_{\frac{1}{2}}\beta$ of 0.77 ± 0.10 hr. This was prolonged in the MI group (mean $t_{\frac{1}{2}}\beta = 1.06 \pm 0.09$ hr; $P < 0.05$). There was no significant difference in the volumes of distribution of primaquine between the MI group (mean = 320 ± 73 ml) and the control group (mean = 284 ± 79 ml). Although there was a trend to lowered primaquine clearance in the MI group (mean 217 ± 26 ml/hr), it was not significantly different from that seen in the control group (mean = 277 ± 42 ml/hr; $0.10 < P > 0.05$). However, there was an inverse linear correlation between primaquine clearance and the percentage parasitaemia ($r = 0.722$, $P < 0.05$). These results suggest that the extent to which primaquine elimination had been compromised was related to the severity of malaria infection, and that in severe infections reduced efficiency of elimination raises the possibility of drug toxicity.

Drug treatment remains the most important approach to combatting malaria [1–3]. Little, however, is known about the pharmacokinetics of commonly used antimalarials in malaria-infected patients [4]. It would be expected that the disposition of antimalarial drugs might change during malaria infection, since there is clinical and pathological evidence of liver damage associated with both the exo-erythrocytic (tissue) and erythrocytic (blood) stage of the parasites life cycle in the human host [5, 6]. Furthermore, since many antimalarials are extensively eliminated by hepatic metabolism, and most are low-clearance drugs, their elimination would be sensitive to any decrease in drug metabolising enzyme function occasioned by liver damage [7]. In one recent *in vitro* study in mice, the metabolism of benzo[a]pyrene and ethylmorphine in liver homogenates was significantly impaired during the erythrocytic stage of infection (i.e. using merozoites) of the rodent parasite *P. berghei* [8].

To study this question in more detail we have developed an isolated perfused rat liver (IPRL) preparation using livers from rats infected with merozoites of *P. berghei*. In the present experiments we have examined the hepatic disposition of the low-clearance antimalarial drug, primaquine [9, 10] in IPRLs derived from healthy (i.e. control) and from malaria-infected rats.

METHODS

Experimental preparations. Isolated perfused rat livers were prepared by standard techniques [11] as previously described [12]. Essentially this involved cannulation of the bile duct with polypropylene tubing (o.d. = 0.61 mm, i.d. = 0.28 mm) and clearing of connective tissue surrounding the liver, before cannulation of the portal vein (Argyle Medicut, 14G; Sherwood Indust.) which was then flushed with heparinised saline (50 U/ml). The inferior vena cava was tied off above the renal vein and cava cannulated with polypropylene tubing (o.d. = 1.90 mm, i.d. = 1.40 mm) beyond the hepatic vein. The liver was then removed and placed on a glass platform inside a humidified, thermostatically (37°) maintained environment. Perfusate comprising washed human red cells (10% v/v), bovine serum albumin (1% w/v) and glucose (0.1% w/v) in Krebs–Henseleit buffer was pumped through the liver at a constant flow rate of 10 ml/min.

Perfused livers were obtained from seven healthy Sprague–Dawley rats, 5–7 weeks of age and weighing 90–110 g, comprising the control group, and six age- and weight-matched rats with parasitaemia, produced by *P. berghei*, constituting the malaria infected (MI) group.

The ANKA strain of *P. berghei* used in these

experiments was originally obtained from the Liverpool School of Tropical Medicine, and after one passage in mice, the strain was preserved at −170°, at the Army Malaria Research Unit, Ingleburn, Australia. Blood was collected from mice used for routine passage of the strain and diluted with sodium citrate solution (3.8%) as anticoagulant, to a concentration of 150×10^6 parasitised erythrocytes per ml [13]. Of this suspension, 0.2 ml was injected i.p. into 4-week-old rats which, after 15–20 days, led to a variable degree of parasitaemia with between 2 and 27% of the rats' erythrocytes infected. Although *P. berghei* tends to infect immature red cells, in the present study the observed parasitaemia was >20% in three of the six preparations. This indicates that mature red cells were also infected. The infection led to pigmentation and enlargement of the liver and spleen in all animals. The increased liver size (liver weight of control group = 5.6 ± 1.6 g vs MI group = 9.6 ± 1.6 g) was accompanied by a marked enlargement of Kupfer cells in which there were large amounts of malaria pigment. There were no abnormalities noted in hepatocytes upon light microscopic examination. Viability of IPRLs derived from control livers was not significantly different from that in the MI group as assessed by oxygen consumption at the end of perfusion (i.e. 4 hr) (Control group = 2.1 ± 0.2 μmol O_2/g liver/min vs MI group = 1.7 ± 0.4 μmol O_2/g liver/min), by initial bile flow (0.42 ± 0.22 ml/hr vs 0.42 ± 0.16 ml/hr) or by initial perfusion back pressure (5.0 ± 0.5 cm H_2O vs 4.8 ± 0.6 cm H_2O).

Experimental design. Primaquine diphosphate was administered into the perfusate reservoir (simulating bolus systemic dosage) of both control and MI liver preparations, as an aqueous solution (1 mg/ml, 1 ml; equivalent to 0.57 mg of primaquine base). Perfusate was sampled (1.5 ml) pre-dose and again at 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min for measurement of primaquine concentrations. An equal volume of blank perfusate was added to the reservoir to replace that removed by sampling. Bile was collected hourly over 4 hr.

Drug analysis. Perfusate concentrations of primaquine were estimated by sensitive and selective HPLC procedures as previously described [14].

Calculations and statistical assessments. Data in the text are presented as mean ± standard deviation, and in figures as mean ± standard error of the mean. The pharmacokinetic parameters, half life, clearance and volume of distribution were calculated by standard model independent pharmacokinetic formulae [7] as described in detail previously [10]. Statistical comparisons were made with the paired Student's *t*-test accepting $P < 0.05$ as significant.

RESULTS

Following primaquine dosage to control perfused livers, drug levels fell swiftly (Fig. 1) due primarily to extensive hepatic tissue uptake of the drug [10]. Thereafter drug levels showed a more gradual monoexponential decline, reflecting drug elimination. The terminal elimination half-life in controls of 0.77 ± 0.26 hr, was significantly prolonged in the MI group to 1.06 ± 0.22 hr ($P < 0.05$). Although there was a trend to a significantly reduced clearance in the MI group (217 ± 63 ml/hr) compared to that in

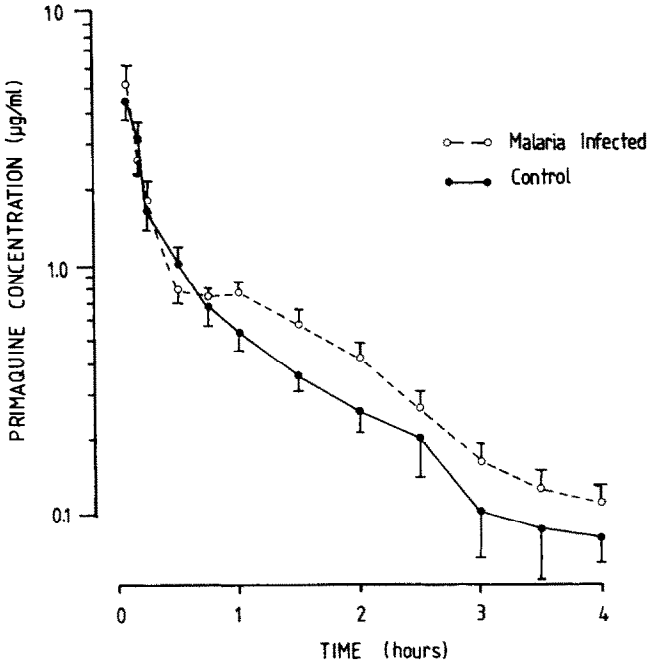

Fig. 1. Mean perfusate concentrations of primaquine plotted against time for malaria infected and healthy (control) isolated perfused liver preparations. Primaquine elimination was impaired by malaria infection.

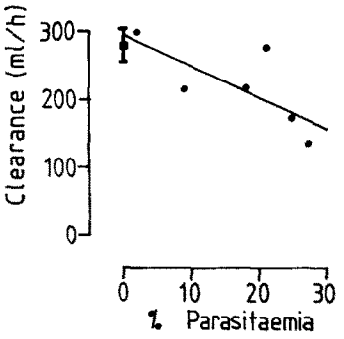

Fig. 2. Plot of primaquine clearance, derived from malaria infected isolated perfused rat livers (●), against percent parasitaemia ($r = 0.722$; $P < 0.05$). The mean (±SEM) clearance in control preparations (i.e. zero parasitaemia) is also shown (■). Results suggest that the extent to which primaquine clearance is impaired, is related to the severity of malaria infection.

control preparation (277 ± 112 ml/hr), this did not reach significance ($0.10 < P < 0.05$). However, when individual clearance values in the MI group were related to the severity of malaria infection (% parasitaemia) a significantly negative correlation was observed (Fig. 2). No significant difference was noted in the volume of distribution (control = 284 ± 79 ml; MI = 320 ± 73 ml).

DISCUSSION

Pathological changes occur in the liver in both the exoerythrocytic and erythrocytic stages of infection of the mammalian host. The malaria parasite first invades the liver (exoerythrocytic phase) as the sporozoite, where it develops into many thousands of merozoites which ultimately cause rupture of infected hepatocytes. Relatively few liver cells are destroyed in this process, and in one *in vitro* study examining drug metabolism in hepatic microsomal preparations from mice infected with *P. berghei*, no disturbance of drug metabolism was demonstrated during the exoerythrocytic phase [8]. It none the less remains uncertain whether this phase of infection may lead to impairment of drug metabolism in the intact animal or whole organ preparation.

During the erythrocytic phase of the infection, the rapid multiplication of merozoites within red blood cells causes severe haemolysis. The liver becomes involved in the phagocytosis of cellular and parasite debris, particularly by Kupffer cells [8]. It becomes discoloured and enlarged in association with Kupffer cell hyperplasia and the accumulation of 'malaria pigment'. This material has been identified by some investigators as haematin and/or haemozoin—breakdown products of haemoglobin haem [15, 16]. The potential significance of these substances has been indicated by a recent study where intravenous injection of haem to rhesus monkeys was shown to cause reduced liver content of cytochrome P-450 enzymes, by direct or indirect means and diminished *in vitro* metabolism of several test substances [17]. It would seem, therefore, that although the striking light microscopic changes of the erythrocytic phase of malaria infection are seen in Kupffer cells, there is an important inhibitory effect of circulating haem or its breakdown products on drug metabolising enzymes in liver cells.

A recent *in vitro* study demonstrated that the metabolism of the test substrates ethylmorphine and benz[a]pyrene was impaired in microsomal preparations derived from mice during the erythrocytic phase of infection [8]. In a separate study conducted in humans, elevated plasma levels of quinine during the erythrocytic phase of infection were presumed to be due to impaired hepatic elimination of the drug—though alterations in drug distribution due to changes in plasma protein binding of quinine, or the effects of pyrexia, may also have been in part responsible [18].

In the present study we have established a whole organ system to examine the effect of merozoite infection (i.e. erythrocytic phase) on hepatic drug elimination, and have used it to study the hepatic elimination of primaquine. Primaquine is subjected to extensive hepatic drug elimination by the hepatic cytochrome P-450 mixed function oxidase system [10]. Since its hepatic clearance in the IPRL is low, the efficiency of elimination will be sensitive to changes in the activity of the drug metabolizing enzymes.

The significantly prolonged half-life and trend towards a reduced clearance found in MI rat livers suggests that the hepatic disposition of primaquine has been impaired by merozoite infection (Fig. 1). This conclusion is supported when the graded effect of the severity of the parasitaemia is taken into account. The parasitaemia produced in the group of rats we studied was variable (2–27% of erythrocytes infected), and Fig. 2 indicates that the more extensive the parasitaemia, the more profound the reduction in primaquine elimination; the most severe parasitaemia leading to a reduction in primaquine clearance of >50%.

Since it is usual for drug therapy to be instituted in malaria infected humans during the acute exacerbation of the erythrocytic phase of infection, this study has potentially important implications for the drug treatment of malaria. The toxicological implications of compromised drug metabolism may need to be considered in designing appropriate dosage regimen in patients treated during the erythrocytic phase of malaria infection.

Acknowledgements—We wish to thank the Army Malaria Research Unit, Ingleburn, N.S.W., 2174 Australia, and in particular Sergeant Keith Newman for providing malaria-infected rats. We are grateful to Ms Jane Bell for expert typing of this manuscript.

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