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PHARMACOKINETICS AND DISPOSITION

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Paracetamol disposition in Thai patients during and after treatment of falciparum malaria

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Abstract Investigations in animals have suggested that conjugation of paracetamol may be reduced in malaria. We have measured plasma concentrations and the urinary excretion of paracetamol and its phase II metabolites in eight Thai patients during uncomplicated falciparum malaria and in convalescence, following a 1000 mg single oral dose.

The apparent oral clearance (Malaria, 3.6; Convalescence, 3.9; $ml \cdot min^{-1} \cdot kg^{-1}$), the elimination half-life (Malaria, 3.8; Convalescence, 3.7 h) and apparent volume of distribution (Malaria, 1.2; Convalescence, 1.2; $l \cdot kg^{-1}$) of paracetamol were similar during malaria and convalescence. In addition, the urinary excretion of paracetamol and its major phase II metabolites and their formation clearances from paracetamol were not significantly different between the two study phases.

These data show that clinical malaria infection has no effect on the conjugation of paracetamol in man.

Key words Paracetamol, Malaria; pharmacokinetics, phase II conjugation, glucuronidation, sulphation

Hepatic phase II conjugation reactions are known to be reduced by experimental malaria infections in animals and perfused organ systems [1, 2, 3, 4]. The aim of this investigation was to study the influence of clinical malaria on phase II conjugation reactions in man using paracetamol as a probe. Previous work [5, 6] has shown that malaria infection can impair paracetamol glucuronidation in rat liver microsomes in vitro and rat in vivo. Paracetamol is selected because it is extensively metabolized by the liver. An oral dose of 1000 mg is excreted mostly as glucuronide (55%) and sulphate conjugates (30%) with only 2 to 5% excreted unchanged in the urine [7]. Moreover, it is an analgesic commonly given to malaria patients for the relief of joint pain, headache and fever and is a model drug for studying the effects of diseases on glucuronidation and sulphation in humans. The effects of renal [8], thyroid [9] and liver diseases [10, 11] on paracetamol conjugation reactions have been described.

While there are previous reports [12, 13, 14, 15, 16] on the disposition of antimalarial agents (which undergo phase I metabolism) in patients with malaria, studies on phase II conjugation reactions in malaria patients have not been conducted. The present work compares the disposition of paracetamol and its metabolites in patients during uncomplicated malaria and in convalescence.

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Materials and methods

Subjects

Eight male Thai patients with uncomplicated clinical falciparum malaria aged between 19 and 41 years, weighing 44–80 kg and with median (range) parasite counts of 16560 (3180–84280)/μl were recruited to the investigation which was carried out at the Hospital for Tropical Diseases, Bangkok, Thailand. Each gave informed consent and the study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Prior to drug administration, each subject had a physical examination, evaluation of vital signs, a 12-lead electrocardiogram and routine blood examinations (haematology, clinical chemistry) and urinalysis.

Treatment

The patients were randomised to receive two oral regimens; (a) Artemether (Artenam®, Profarma n.v., Belgium; 50 mg/tablet) at an initial dose of 300 mg, followed by 100 mg daily for 4 consecu-

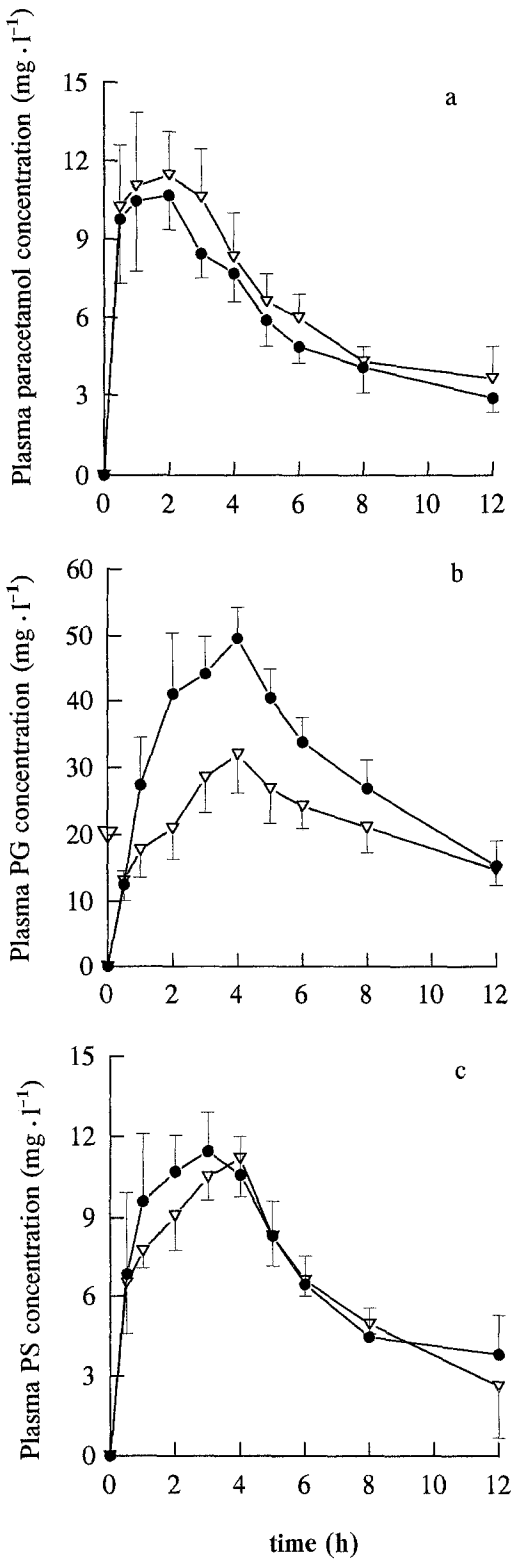

Fig. 1a–c Plasma concentration vs time profiles for paracetamol, paracetamol glucuronide (PG) and paracetamol sulphate (PS) following a single oral dose of paracetamol (1000 mg) in Thai patients during malaria (▽) and in convalescence (●). Values are mean with SEM ($n = 8$)

tive days or (b) Artemether 300 mg, followed by mefloquine (Lariam®, Hoffman La-Roche, 250 mg per tablet) given in two divided doses of 750 and 500 mg at 24 and 30 h after artemether. Three patients received diphenhydramine and 1 patient penicillin V as essential supportive therapy. Blood parasitaemia was assessed six-hourly post-treatment until it fell below the level of microscopic detection in a thick smear, then twice daily until day-28 (Artemether alone) or day-42 (Artemether/Mefloquine). Complete blood count, urinalysis and blood biochemistry tests were performed on admission, then weekly until day-28 or day-42.

Study design

Paracetamol (Government Pharmaceutical Organization of Thailand, 1000 mg; 2 × 500 mg per tablet) was given simultaneously with the first dose of artemether in any regimen. The tablets were given under supervision with a glass of water. Blood samples (1 ml) were collected via an indwelling cannula inserted into a forearm vein prior to and at 0.5, 1, 2, 3, 4, 5, 6, 8 and 12 h post dose and immediately centrifuged (600 × g; 15 min) to obtain plasma. Urine was collected predose and in intervals of 0–6, 6–12, 12–18 and 18–24 h. All plasma and urine samples were stored at –80 °C until assayed. The protocol was repeated during convalescence when the patients were aparasitaemic (day 28 or day 42). Each patient therefore served as his own control.

Analytical method

Paracetamol and 3-acetamidophenol (internal standard) were obtained from Sigma Chemical Co., Poole, Dorset, UK. Paracetamol glucuronide and paracetamol sulphate were generously donated by Sterling Winthrop, Alnwick, UK. Paracetamol cysteine and paracetamol mercapturate were gifts from Dr M. S. Lennard, University of Sheffield, UK. Ethyl acetate and methanol (HPLC grade) were obtained from BDH (Poole., UK). All other chemicals were of analytical reagent grade.

Concentrations of paracetamol, paracetamol glucuronide and paracetamol sulphate in plasma and concentrations of paracetamol, paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine and paracetamol mercapturate in urine were measured by reversed-phase HPLC [17, 18]. The interassay coefficients of variation for paracetamol ranged from 5–10 % at plasma and urine concentrations of 0.5–20 mg · l^{-1}The corresponding values for the individual metabolites in urine were as follows: paracetamol glucuronide 3.5 % at 10 mg · l^{-1}; paracetamol sulphate 4.5 % at 10 mg · l^{-1}; paracetamol cysteine and mercapturate 5–10 % at 10–80 mg · l^{-1}.

Pharmacokinetic analysis

Peak plasma concentration (C_{max}) and time to C_{max} (t_{max}) for paracetamol were noted directly. The area under the plasma concentration vs time curve (AUC) of paracetamol, paracetamol glucuronide and paracetamol sulphate was calculated by linear trapezoidal summation with extrapolation to infinity. Total clearance (CL/f) of paracetamol was calculated as the ratio of the dose and AUC. The elimination half-life ($t_{1/2}$) was calculated from the slope of the terminal phase of the log drug concentration vs time curve and the apparent volume of distribution (V_z/f) from ln 2 · CL/$t_{1/2}$.

The formation clearances of paracetamol metabolites were calculated from the product of CL and the fractional recovery of each metabolite in the urine. The Wilcoxon signed-rank test for paired samples was used for statistical analysis taking $P < 0.05$ as significant.

Table 1 Pharmacokinetic parameters describing the disposition and urinary excretion of paracetamol and formation clearances of paracetamol metabolites during malaria and convalescence in 8 Thai patients following an oral dose of paracetamol (1000 mg)

	Malaria	Convalescence	Median difference (95 % C.I.)
Pharmacokinetic parameter			
C_{max} (mg · l^{-1})	15.2 (3.8)	14.5 (5.8)	0.73 (–3.99 to 5.20)
t_{max} (h)	1.0 (0.5 – 3.5)	1.0 (0.5 – 3.5)	0.50 (–1.50 to 2.00)
$AUC_{(0-\infty)}$ (mg · l^{-1} · h)	95.6 (39.9)	88.1 (36.9)	7.53 (–10.80 to 27.50)
$t_{1/2}$(h)	3.8 (1.2)	3.7 (1.1)	0.04 (–0.68 to 0.93)
V_z/f (l · kg^{-1})	1.2 (0.2)	1.2 (0.7)	–0.13 (–0.39 to 0.59)
CL/f (ml · min^{-1} · kg^{-1})	3.6 (1.7)	3.9 (1.3)	–0.38 (–1.04 to 0.36)
Excretion of metabolites (% of dose)			
Glucuronide	36.4 (12.4)	43.0 (9.8)	–5.70 (–20.05 to 8.75)
Sulphate	23.3 (9.7)	24.2 (9.3)	–1.90 (–7.50 to–7.40)
Cysteine	7.9 (5.5)	5.7 (2.4)	3.58 (–2.80 to 11.50)
Mercapturate	3.5 (1.1)	1.2 (0.5)	1.25* (0 to 2.55)
Glutathione	10.7 (7.0)	6.7 (2.5)	1.40 (–2.50 to 9.10)
Unchanged	4.2 (1.6)	5.3 (3.6)	0.30 (–3.35 to 2.35)
Formation clearance of metabolites (ml · min^{-1} · kg^{-1})			
Glucuronide	1.3 (0.6)	1.6 (0.4)	–0.37 (–0.91 to 0.36)
Sulphate	0.7 (0.2)	0.9 (0.3)	–0.18 (–0.30 to 0.06)
Cysteine	0.2 (0.2)	0.2 (0.1)	0.02 (–0.13 to 0.30)
Mercapturate	0.2 (0.1)	0.1 (0.1)	0.02 (–0.02 to 0.14)
Glutathione	0.5 (0.5)	0.3 (0.1)	0.20 (0 to 0.56)

Results are given as means (SD) except for t_{max} values which are expressed as medians and ranges
Significantly different from control values: $^*P < 0.05$ (Wilcoxon's signed-rank test for paired samples

Results

There were no biochemical abnormalities on admission and all patients made a full recovery as assessed by complete clearance of fever and parasitaemia. Plasma concentrations of paracetamol, paracetamol glucuronide and paracetamol sulphate in patients during malaria and in convalescence are shown in Fig.1 (a,b and c). The $AUC_{(0-\infty)}$ of paracetamol (Malaria, 95.6 (39.9); Convalescence, 88.1 (36.9); mg · l^{-1} · h), paracetamol glucuronide (Malaria, 419 (101.1); Convalescence, 470 (182) mg · l^{-1} · h) and paracetamol sulphate (Malaria, 111 (43.4); Convalescence, 84.5 (38.0); mg · l^{-1} · h) were not significantly different between the two phases. Pharmacokinetic parameters for paracetamol in malaria and convalescence are shown in Table 1. There were no statistically significant differences in the values of any of these parameters. However there was a significant reduction (Convalescence, 361 (109); Malaria 245 (87.1) in AUC_{0-12h} for paracetamol glucuronide (Median difference –109; 95 % C.I. –180 to –41; $P = 0.016$)

The urinary recovery of paracetamol and its metabolites in malaria and convalescence is shown in Table 1. Approximately 72 % of the dose was recovered in urine in 24 h in each phase of the study. Urinary excretion of paracetamol metabolites, with the exception of paracetamol mercapturate, was not significantly different in convalescence when compared with values obtained during infection. The urine flow rate of the patients during malaria was not significantly different from that in convalescence (Malaria, 1.6 (0.7); Convalescence, 1.8 (0.5) ml · min^{-1}).

Discussion

Clinical malaria is known to influence the pharmacokinetics of antimalarials, many of which undergo phase I metabolism before excretion. For example, plasma concentrations of quinine are increased during malaria, suggesting impaired hepatic metabolism, occuring indirectly as a result of reduced hepatic blood flow [12]. White et al. [13] also found decreased quinine clearance in malaria-infected patients but attributed it to a decreased V due to increased plasma protein binding of quinine to α_1-acid glycoprotein. The pharmacokinetics of mefloquine are altered in malaria. V and CL are both decreased whereas $t_{1/2}$ is reduced [19]. This was ascribed to an impairment in the enterohepatic circulation of mefloquine in malaria-infected patients resulting in a reduced elimination half-life.

There are no previous reports of the effect of clinical malaria on Phase II drug metabolism. Studies to date have examined the effect of experimental malaria (*Plasmodium berghei*) infection on Phase II conjugation reactions in animal models. Most of these [1, 2, 3, 4, 5, 6] have shown a consistent decrease in glucuronidation in malaria with sulphation being largely unaltered.

In the present study, the pharmacokinetic parameters (C_{max}, t_{max}, V_z/f and CL/f) of paracetamol for the patients during convalescence are similar to those reported previously in healthy Thai subjects [20]. However, the mean urinary excretion of paracetamol glucuronide and paracetamol sulphate in this study are closer to values in healthy Caucasians [18]. The urinary excretion of paracetamol cysteine and paracetamol mercapturate combined is approximately the amount normally recovered in Caucasians [18, 21].

Malaria had no effect on the pharmacokinetics of paracetamol. Since urine flow and excretion of parace-

tamol glucuronide were not influenced by malaria, the formation clearance of paracetamol glucuronide was not significantly different between the two study phases. This is in contrast to the results obtained in rat in vivo [6] where experimentally-induced malaria significantly decreased the glucuronidation of paracetamol. The disparity could be due to differences in the severity of infection or in the availability of uridine diphosphoglucuronic acid (UDPGA), a critical co-factor in hepatic glucuronidation. UDPGA (formed from UDP glucose) could conceivably be reduced in malaria where the ability to synthesize glycogen from exogenous glucose is decreased [22] and depression of glucose in malaria patients may be less marked than in malaria-infected animals. In Thailand, although hypoglycaemia is a frequent complication of falciparum malaria in adults, it occurs primarily in patients who are pregnant or severely ill [23]. Alternatively, the rapid clearance of peripheral parasitaemia and reduction in fever produced by artemether [24] may bring the severity of infection below that required to influence glucuronidation.

The increase in AUC_{0-12h} for plasma paracetamol glucuronide in convalescence suggests some improvement in the initial rate of excretion of this metabolite in urine but overall, recovery was unaltered. Since the urinary excretion of paracetamol sulphate in malaria was not significantly different from that in convalescence, the formation clearance of paracetamol sulphate was unchanged. Sulphation has consistently been shown to be unaffected by malaria [2, 3] due presumably to the cytosolic location of phenol sulfotransferases (PSTs). Malaria affects the smooth endoplasmic reticulum and mitochondria but not other organelles [25].

Although the urinary excretion of paracetamol mercapturate was significantly different in patients between the two study phases, the quantity of this metabolite excreted as a percentage of the dose administered is too small to suggest this is of any clinical significance.

In conclusion, malaria has no significant effect on the conjugation of paracetamol in man. Lack of an effect on Phase II conjugation despite significant alterations in Phase I reactions has been observed in other disease states. Influenza vaccine and natural viral influenza infections have been known to impair the Phase I oxidation processes of some drugs [26, 27]. However, the administration of influenza vaccine to healthy volunteers had no effect on the elimination of lorazepam and paracetamol, both cleared by phase II conjugation [28]. Most importantly, the results of this investigation confirm findings that suggest alterations in drug handling in malaria are more often due to reductions in hepatic blood flow [12], gastrointestinal absorption [29] or perturbations in plasma protein binding [13] than to the changes in drug metabolism [30] which might be predicted from investigations in animals.

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