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# The Effect of Fever on Quinine and Quinidine Disposition in the Rat

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**Abstract**—The pharmacokinetics of quinine and its diastereoisomer quinidine has been investigated in normal and febrile rats. Endotoxin-induced fever in rats resulted in an increased quinine clearance (CL) ($4{\cdot}49 \pm 1{\cdot}45$ vs $1{\cdot}38 \pm 0{\cdot}65$ L $h^{-1}$ $kg^{-1}$, $P < 0{\cdot}001$) and volume of distribution ($V_d$) ($42{\cdot}6 \pm 8{\cdot}8$ vs $28{\cdot}9 \pm 10{\cdot}3$ L $kg^{-1}$, $P < 0{\cdot}05$) with a concomitant shortening of the elimination half-life ($t_{\frac{1}{2}}$) ($7{\cdot}1 \pm 2{\cdot}5$ vs $15{\cdot}9 \pm 5{\cdot}9$ h, $P < 0{\cdot}01$). With quinidine, however, fever resulted in an increased CL ($3{\cdot}95 \pm 1{\cdot}05$ vs $1{\cdot}89 \pm 0{\cdot}60$ L $h^{-1}$ $kg^{-1}$, $P < 0{\cdot}002$) with no change in $V_d$ and a significant decrease in $t_{\frac{1}{2}}$ ($5{\cdot}1 \pm 0{\cdot}7$ vs $10{\cdot}1 \pm 2{\cdot}8$ h, $P < 0{\cdot}001$). In both studies there was no significant difference in hepatic microsomal protein or cytochrome P450 content. Neither drug accumulated in the liver but low concentrations of quinidine were present in the heart 24 h after administration. In-vitro studies suggest that temperature does not alter the binding of either drug. These data suggest that fever enhances the clearance of quinine and quinidine. These findings may offer some additional explanation of the lack of serious quinine and quinidine toxicity during the treatment of malaria infection, even after large dosages of the drug administered during the initial period of treatment when fever is most intense.

Studies of the influence of fever on the pharmacokinetics of antimalarial drugs have been limited (Mackoviak 1989). Trenholme et al (1976) studied the disposition of quinine during fever and suggested hepatic metabolic dysfunction was the basis for the enhanced incidence of quinine toxicity in febrile patients. Osifo & DiStefano (1978) reported increased toxicity of chloroquine in pyrogen-treated rats. However, the mechanism of chloroquine toxicity during fever is unclear since its metabolism in rats is not altered by bacterial pyrogen (Osifo 1979). Studies of the influence of fever on the pharmacokinetics of various drugs have also suggested that a complex relationship exists between fever and drug metabolism and distribution (Song et al 1972; Pennington et al 1975; Elin et al 1975; Ladefoged 1977; Abdullah & Baggot 1984). Although not conclusive, these reports suggest that fever can influence drug disposition, and this is of special importance with antimalarials since these agents are administered primarily to patients during the initial period of the illness when fever and other manifestations of the disease are most intense. The present study aims to investigate what effect *Escherichia coli* endotoxin-induced fever would have on the pharmacokinetic parameters of quinine and quinidine in rats. This bacterial pyrogen has been used successfully to induce fever in the rat (Gorodischer et al 1976; Osifo 1979).

## Materials and Methods

*Study design*

Quinine dihydrochloride, quinidine hydrochloride monohydrate and *Escherichia coli* lipopolysaccharide were obtained from Sigma Chem., Poole, UK. *E. coli* lipopolysaccharide was dissolved in sterile 0·9% NaCl (saline) and administered intraperitoneally (2 mg $mL^{-1}$ $kg^{-1}$) to a group of male Wistar rats (200–250 g, n = 6). Quinine (or quinidine) (50 mg $kg^{-1}$) was administered orally and serial blood samples taken from the tail veins, beginning 1 h after exposure to pyrogen and plasma removed following centrifugation for drug quantification by HPLC. Rats were conscious throughout the study except during blood sampling when they were anaesthetized lightly with diethyl ether (BDH, Poole, UK). Rectal temperature was measured throughout the study with a rectal temperature probe (Cormack Thermometer, Rustington, UK) inserted at least 4 cm into the rectum. The experiments lasted for 24 h after the drug challenge. Control animals were injected with sterile saline (1 mL $kg^{-1}$) and matched for age, weight and sex. At the end of the experiment, rats were anaesthetized with diethyl ether and the liver and heart excised and homogenized with an Ultra-Turrax (Janke and Kynkel, IKA Laboratories, Germany). The suspensions were centrifuged at 3000 rev $min^{-1}$ for 10 min and 3 mL of the top layer suspension from each organ was used for assay of quinine (or quinidine) by HPLC. Plasma and tissue homogenate blanks were obtained from control rats and carried through each assay protocol. Hepatic microsomal protein (Lowry et al 1951) and cytochrome P450 content (Omuro & Sato 1964) were also determined in controls and pyrogen-treated rats.

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*Drug analysis*

The concentration of quinine and quinidine in samples of plasma, heart or liver homogenates was estimated by a selective and sensitive HPLC procedure adapted from Mihaly et al (1987a) and reported previously (Mansor et al 1990).

*Pharmacokinetic analysis*

The elimination rate constant ($\beta$) of quinine and quinidine was obtained by least square regression analysis of the plasma concentration vs time curve, and elimination half-life ($t_{\frac{1}{2}}$) was calculated from the ratio $0{\cdot}693/\beta$. Other pharmaco-

kinetic parameters were calculated using standard model-independent formulae (Rowland & Tozer 1989). Statistical comparisons were made using unpaired Student's $t$-test, accepting $P<0.05$ as significant. Comparisons of multiple means were made using a one-way analysis of variance. Data in the text and tables are presented as mean ± s.d. and graphically as mean ± s.e.m.

*Equilibrium dialysis*

A sample (0·9 mL) of quinine (or quinidine) (1 and 5 μg $mL^{-1}$) in rat plasma was dialysed against the same volume of 0·05 M phosphate buffer, pH 7·4 (Bowmer & Lindup 1979) in a Dianorm apparatus (Diachema AG, Zurich, Switzerland). The apparatus consists of two teflon half-cells (1 mL capacity each), separated by a semipermeable membrane. One compartment was loaded with the drug-protein solution whilst the other contained only phosphate buffer. Incubations were carried out in a water bath at 19, 30, 37 and 44°C and rotated at 8 rev $min^{-1}$ for 4 h. Equilibrium was reached after 2·15 h and the unbound fraction of the drug was found to be constant up to 6 h. The dialysis membranes (Visking tubing, mol. wt cut-off 10000 daltons) were obtained from Medicell International, London, UK. At the end of the dialysis, the buffer compartment was examined for protein contamination using Albustix (Ames Division, Miles Laboratories, Slough, UK; sensitivity 0·05 g $L^{-1}$). The plasma and buffer pH were checked in all samples and were found to be within ±0·1 pH units of the predialysis value. Quinine (or quinidine) was measured in the plasma and buffer sides by an HPLC procedure previously described (Mansor et al 1990). The binding of quinine, or quinidine, to rat plasma was calculated as the ratio of the bound drug to the total drug concentration.

## Results

*Preliminary investigations*

Preliminary studies involved monitoring the temperature response to various doses of the *E. coli* pyrogen. One hour after administration, bacterial pyrogen (2 mg $mL^{-1}$ $kg^{-1}$; i.p.) resulted in a typical rise in the rectal temperature from a basal level of 37·50 ± 0·30 to 38·80 ± 0·25°C. Febrile response did not occur in control rats injected with sterile saline (1 mL $kg^{-1}$; i.p.). This dose amounts to two times that which produced a febrile response in the studies of Osifo (1979).

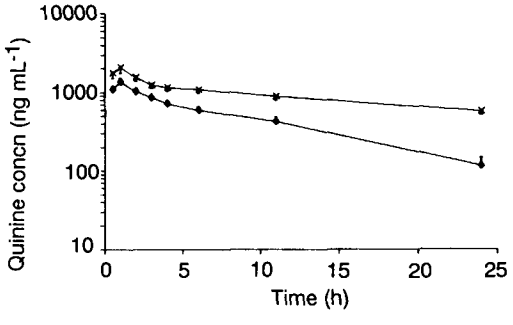


FIG. 1. Mean concentrations of quinine after an oral dose (50 mg $kg^{-1}$) in normal (×) and febrile (◆) rats. Values are mean (s.e.m.) of 6 animals.

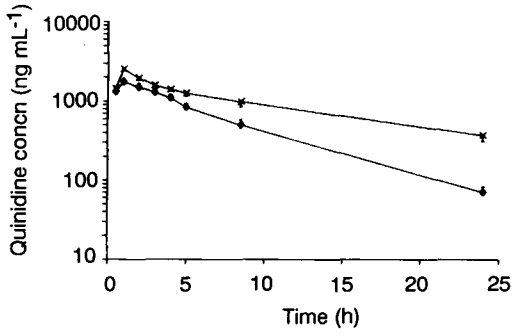


FIG. 2. Mean concentrations of quinidine after an oral dose (50 mg $kg^{-1}$) in normal (×) and febrile (◆) rats. Values are mean (s.e.m.) of 6 animals.

The febrile response was not consistent using the smaller dose of pyrogen as used by Osifo. After establishing this pyrexial response, quinine or quinidine, was administered to the rats 1 h after exposure to bacterial pyrogen.

*Disposition of quinine and quinidine*

The mean (s.e.m.) plasma concentration-time profiles for quinine and quinidine are shown in Figs 1 and 2. *E. coli* endotoxin-induced fever resulted in lower plasma concentrations of both drugs in febrile as compared with control rats. Table 1 lists the mean (s.d.) pharmacokinetic parameters for quinine and quinidine. In the case of quinine, fever results in a significant increase in clearance (CL) (226%) and volume of distribution ($V_d$) (47%) compared with controls. The combined effects of these changes resulted in a shortening of the elimination half-life ($t_{\frac{1}{2}}$) (55%) in pyrogen-treated rats. In contrast, with respect to quinidine, fever produced a significant increase in CL (108%) without any change in Vd. This resulted in a significant decrease in $t_{\frac{1}{2}}$ (50%) in febrile rats compared with controls.

*Liver and heart homogenates*

Quinine, after a dose of 50 mg $kg^{-1}$, was not detected in the heart and did not appreciably accumulate in the liver (fever: 489 ± 333 vs control: 919 ± 623 ng (g liver)$^{-1}$, $P>0.05$). With quinidine, however, a small quantity of drug was detected in the heart (fever: 189 ± 33 vs control: 198 ± 29 ng (g heart)$^{-1}$, $P>0.05$) but did not accumulate in the liver (fever: 318 ± 67 vs control: 409 ± 100 ng (g liver)$^{-1}$, $P>0.05$).

*Microsomal protein and cytochrome P450*

There was no significant difference ($P>0.05$) in hepatic microsomal protein (quinine (fever: 15·4 ± 3·8 vs control: 17·2 ± 2·0 mg (g liver)$^{-1}$); quinidine (fever: 16·7 ± 2·1 vs control: 17·1 ± 2·0 mg (g liver)$^{-1}$)) or cytochrome P450 (quinine (fever: 0·64 ± 0·09 vs control: 0·61 ± 0·08 nmol P450 (mg protein)$^{-1}$); quinidine (fever: 0·61 ± 0·09 vs control: 0·63 ± 0·61 ± 0·07 nmol P450 (mg protein)$^{-1}$)) in livers between groups.

*Effect of temperature on binding*

For quinine and quinidine, one-way analysis of variance suggests that temperature does not alter the plasma protein binding at the concentrations studied (1 and 5 μg $mL^{-1}$) (Table 2).

Table 1. Pharmacokinetic parameters of quinine and quinidine in normal and pyrogen-treated rats. Values are mean (±s.d.) from 6 rats.

| | Quinine | | Quinidine | |
|---|---|---|---|---|
| Parameters | Control | Fever | Control | Fever |
| Clearance (CL) ($L\ h^{-1}\ kg^{-1}$) | 1·38 ±0·65 | 4·49*** ±1·45 | 1·89 ±0·60 | 3·95† ±1·05 |
| Volume of distribution ($V_d$) ($L\ kg^{-1}$) | 28·9 ±10·3 | 42·6* ±8·8 | 25·8 ±2·5 | 28·8 ±6·8 |
| Half-life ($t_{\frac{1}{2}}$) (h) | 15·9 ±5·9 | 7·1** ±2·5 | 10·1 ±2·8 | 5·1†† ±0·7 |

* $P<0·05$, ** $P<0·01$, *** $P<0·001$, † $P<0·002$, †† $P<0·001$, significantly different compared with controls.

Table 2. The effect of temperature on the binding of quinine and quinidine to rat plasma in-vitro. Each point is the mean (±s.d.) of four measurements.

| | 19°C | 30°C | 37°C | 44°C |
|---|---|---|---|---|
| Quinine ($\mu g\ mL^{-1}$) | | | | |
| 1 | 21·0±2·2 | 21·3±2·5 | 21·3±2·8 | 21·8±1·7 |
| 5 | 21·8±2·8 | 21·3±2·7 | 21·5±3·1 | 21·5±2·4 |
| Quinidine ($\mu g\ mL^{-1}$) | | | | |
| 1 | 21·5±2·1 | 21·8±3·0 | 21·8±3·0 | 21·8±1·7 |
| 5 | 21·3±3·0 | 21·8±2·8 | 21·5±2·1 | 21·8±3·5 |

## Discussion

Previous investigations of drug disposition during pyrogen-induced fever in animals and man, suggested that this is a complex issue, which might be related to the apparently opposing influences of fever on hepatic metabolic function and hepatic blood flow (Song et al 1972; Elin et al 1975; Pennington et al 1975; Trenholme et al 1976; Abdullah & Baggot 1986; Ghezzi et al 1986; Prince et al 1989). In the present study, fever alters the disposition of quinine and quinidine in the rat model. In the case of quinidine, fever resulted in a significant increase in CL without any change in $V_d$ and these changes are reflected by a significant reduction in $t_{\frac{1}{2}}$ as compared with control. In the case of quinine, fever produced a significant increase in $V_d$ and CL with a concomitant decrease in $t_{\frac{1}{2}}$. The increase in CL is due presumably to increased hepatic drug metabolism via the cytochrome P450 hydroxylation pathway, the principal route of metabolism of both drugs (Brodie et al 1951). In view of the increase in hepatic blood flow as a result of an increase in cardiac output during fever (Bradley 1949), accelerated metabolism and elimination of drug might be expected to occur in febrile animals. Since these two cinchona alkaloids have an intermediate hepatic extraction ratio (Rowland & Tozer 1989), changes in hepatic blood flow might be expected to play an important role in determining their clearance in such disease states (Wilkinson & Shand 1975). Hepatic enzyme activities, as determined by measurement of microsomal protein and cytochrome P450, are not significantly different between each group of rats suggesting that the dosage of pyrogen used did not impair the function of the hepatic mixed-function system in any non-selective manner. Gorodischer et al (1976) reported, however, that a massive dose of pyrogen (four times greater than that used in the present study) produced pathological abnormalities of the liver in the endotoxin-treated animals as well as a reduction in microsomal cytochrome P450 and related drug metabolizing enzymes.

The increase in $V_d$ in the case of quinine is difficult to explain. There are pharmacokinetic differences between quinine and quinidine in the rat (Watari et al 1989), and in their binding properties to various components within the body (Kurz & Fichtl 1983; Mihaly et al 1987b; Watari et al 1989). These present studies demonstrate that neither drug accumulates in the highly perfused organs such as the heart and the liver and is in agreement with earlier works investigating their distribution pattern in various tissues in fowl and dog (Kelsey et al 1943; Hiatt & Quinn 1945). Quinine is, however, highly bound to muscle (Kurz & Fichtl 1983) and also has some affinity with the melanocytic system of cells in the skin, but not to the same extent as chloroquine and quinacrine (Lindquist & Ullberg 1972). Thus increase in cardiac output and the associated increase in perfusion of the musculoskeletal system may contribute to the increase in the $V_d$ of quinine. Earlier studies with drugs that bind mainly to albumin suggested an inverse relationship between temperature and protein binding (Ballard 1974). However, in our study, temperature did not alter the plasma protein binding of either drug in-vitro. Other investigators have observed an increase in $V_d$ of gentamicin (Pennington et al 1975; Halkin et al 1981) and theophylline (Prince et al 1989), suggesting that fever potentiates tissue penetration by these drugs since CL was changed only minimally. Since these drugs bind poorly to plasma protein (Vozeh et al 1988), a temperature-related reduction in binding (greater unbound drug) to plasma protein could not explain the potentiating effect of fever on the increased $V_d$ of both drugs. With respect to quinine, the differences in $V_d$ between the normal and the febrile animals might be related to either a higher tissue concentration or another and more extensive distribution to the tissues. These differences may also suggest that quinine concentrations may be higher in the tissues under febrile conditions. Finally our study in rats is not in agreement with that of Trenholme et al (1976) in man. This might be due to factors such as differences in the species studied and the type of agents used to induce fever. Examples involving the effect of such variables on the variation of response to fever and consequently effects on drug disposition have been

documented (Blaschke et al 1973; Elin et al 1975; Aarbakke et al 1978; Borsook et al 1978; Abdullah & Baggot 1984, 1986). In addition, the study of Trenholme et al (1976) had certain limitations such as using a relatively non-specific assay for quantification of quinine and only studying fever in two subjects.

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