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The Disposition of Quinine in the Rat Isolated Perfused Liver: Effect of Dose Size

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Abstract—We have investigated the pharmacokinetics of both free and total quinine in the rat isolated perfused liver at three doses, 6·25, 12·5 and 25 mg. The plasma concentrations of free and total quinine decayed biexponentially over 4 h. However, on increasing dose, the terminal half-life of free and total quinine showed marked increases ranging from 12·4 ± 3·7 min at 6·25 mg to 176·0 ± 153 min at 25 mg (total quinine). Quinine clearance was reduced approximately by half as the dose was doubled. At 10 min post dosage, quinine extraction at the 6·25 mg dose (56 ± 16·3%) was more than twice that of the highest dose (25 mg, 25·0 ± 6·5%). Free quinine at the 6·25 mg dose was cleared at ≈ 100% of perfusate flow, whereas at 25 mg, clearance was less than one fifth of that value. Unchanged quinine elimination in bile was low, with less than 1% of the parent drug being detected at the 12·5 and 25 mg doses. Relatively little parent drug was recovered from the liver at 4 h. At the 25 mg dose, ≤ 6% was recovered as parent drug. HPLC analysis revealed some polar metabolites of quinine in the bile and in the liver homogenates. Dose dependent kinetics of quinine were demonstrated in this study, as hepatic extraction of quinine decreased with increasing dose and input concentration.

Quinine is an effective treatment for chloroquine-resistant falciparum malaria (WHO 1984) and for severe cerebral malaria (Franke et al 1987). Recently, to overcome drug resistance, it has been necessary to increase quinine plasma concentrations. However, the drug's half-life in man has been reported to increase markedly with dose (Berlin et al 1975). In addition, the pharmacokinetics of the diastereoisomer, quinidine, also used as an antimalarial, have been shown to be dose-dependent in the rat isolated perfused liver (Yu et al 1982). In man, non-linear quinine pharmacokinetics may cause difficulty in the accurate prediction of plasma drug concentrations, possibly leading to toxicity. As the major site of elimination of quinine is the liver (Webster 1985), we wished to investigate the pharmacokinetics of quinine in the perfused liver preparation across a dose range.

Materials and Methods

Chemicals

Quinine sulphate (Standard Reference Material 936) was obtained from the National Bureau of Standards, Washington D.C., USA. Of many sources of quinine tested, this was the only material free from contamination with dihydroquinine. The internal standard quinidine (anhydrous), was obtained from the Aldrich Chemical Company, Milwaukee, WI, USA. Sodium dihydrogen phosphate, sodium perchlorate and phosphoric acid (85%) were supplied by Fisher Scientific, Fair Lawn, NJ, USA. All other solvents were of HPLC grade and were purchased from American Burdick and Jackson, Muskegon, MI, USA.

Animals

Male Sprague-Dawley rats (200–250 g, Harlan Sprague Dawley Inc., Indianapolis, IN, USA) were housed in well ventilated cages and kept at a room temperature of approximately 24°C. They were allowed free access to pelleted food (Ziegler Brothers, Gardner, PA, USA) and tap water. Animals were cared for in accordance with the principles of The Guide for the Care and Use of Laboratory Animals (Department of Health, Education and Welfare, No. NIH 85–23).

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Isolated perfused livers (IPL)

Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) and their livers were isolated using standard techniques and then perfused in a constant flow (15 mL min^{-1}) recirculating system (Mihaly et al 1982); a pilot study revealed extensive drug binding (≥ 50%) to the plastic tubing of the IPL apparatus, and this was replaced with glass tubing. Before each experiment, with the exception of the silastic membrane oxygenator, the entire system was siliconized (AquaSil, Pierce Chemical Company, Rockford, IL, USA). The oxygenator was rinsed with a concentrated (1 mg mL^{-1}) quinine solution, then flushed with distilled water (60 mL). Analysis of pre-dose samples revealed no detectable levels of quinine in the IPL system, and total drug binding to the glass and plastic of the system was reduced to less than 7%. The perfusate (100 mL) comprised 10% washed human erythrocytes, 1% (w/v) bovine serum albumin (Sigma Chemicals Co, St. Louis, MO, USA) and 0·1% glucose in a standard electrolyte solution (Krone et al 1974). The principal indices of liver viability were steady oxygen consumption (1·5 to 2 μmol O_2(g liver)$^{-1}$ min^{-1}), sustained bile flow (0·2–0·6 mL h^{-1}), constant perfusion pressure (6–8 cm H_2O), reproducible liver function tests (i.e. determination of perfusate sodium, potassium, total protein, alanine aminotransferase, and γ-glutamyl transferase concentrations), and normal visual appearance.

Protocol

The disposition of quinine in the IPL was studied in three

dose groups (n = 5 per group) over 4 h. Groups I, II and III received a bolus dose of quinine sulphate at 6·25, 12·5 and 25 mg, respectively. Each dose was added as a solution in 0·9% NaCl (saline) (80 μL) directly into the reservoir, thereby simulating systemic dosage. Samples (0·5 mL) were removed from the perfusate reservoir pre-dose and at 1, 3, 5, 10, 15, 20, 25 and 30 min, and at 1, 1·5, 2 and 3 h post dosage. In addition, samples (0·5 mL) were also removed from the portal inflow and the inferior vena cava outflow simultaneously to derive an estimate of hepatic extraction. An equal volume of fresh perfusate was added to the reservoir to replace that removed by sampling. Sodium taurocholate (Sigma) was infused at 30 μmol h^{-1} into the perfusate reservoir to maintain bile flow. Perfusate gases (CO_2 and O_2) were measured and liver function tests performed before and after each experiment to assess liver viability. At the conclusion of each experiment, the livers were flushed with saline, blotted dry, weighed and then homogenized in ice cold 0·007 M phosphate buffer (pH 7·5 containing 1·5% KCl) using a Teflon-in-glass homogenizer. Homogenates were stored at −20°C until assay by HPLC.

Analysis of total quinine in perfusate and liver homogenates

Samples of perfusate plasma and liver homogenates were analysed for total quinine concentrations according to Patel (1982) with some modification. The term 'total quinine' denotes all drug in perfusate, including that bound to albumin and free 'unbound' drug. Samples of perfusate (100 μL) or liver homogenate (80 μL) were placed in microcap tubes. Following the addition of internal standard (5–15 μL) and methanol (500 μL), the samples were centrifuged at 10 000 *g* for 10 min. The decanted supernatant was filtered using Nalgene nylon (45 μm) syringe filters (Daigger Scientific, Alexandria, VA, USA) before injection onto the HPLC system. Assay sensitivity was 0·05 μg mL^{-1} for quinine. Recovery was 95%. Co-efficients of variation were 2·2% at 25 μg mL^{-1} and 5·6% at 12·5 μg mL^{-1} (n = 6). Day to day coefficients of variation were 6% at 10 μg mL^{-1}.

Analysis of total quinine in bile

Samples of bile were analysed for total quinine using a solid phase extraction described as follows. 50 μL samples were spiked with internal standard (2–5 μL). Following the addition of 400 μL of 37% HCl, the samples were heated at 65°C in a water bath for 10 min. After the samples were cooled to room temperature, borate buffer (0·2 M) was added (pH 9·2, 1·3 mL). Purification of samples was achieved using Bond Elut 3 cc solid phase diol columns (Analytichem International, Harbor City CA, USA). The columns were first conditioned with 2 mL H_2O followed by 2 mL methanol and 2 mL ammonia water (pH 9). 500 μL of each sample was added to a conditioned diol column and aspirated at 15 mm Hg using an Analytichem 'Vac Elut' SPS-24. The columns were washed with 1 mL (0·6 M) phosphate buffer (pH 6·3), and eluted with 4 × 500 μL of methanol. The samples were evaporated under nitrogen, reconstituted with 0·5 mL of mobile phase and filtered through a 45 μm nylon syringe filter. Assay sensitivity was 0·08 μg mL^{-1} for total quinine in bile. Recovery was 90%: coefficients of variation were (within day) 5·0% at 10 μg mL^{-1} and 8·7% at 10 μg mL^{-1} for day to day variation.

Analysis of free perfusate quinine

Free quinine levels i.e. drug unbound to perfusate albumin, were measured by the method of Silamut et al (1985) with modification. Samples (100 μL) were ultracentrifuged using a Centrifree micropartition system (Amicon Corporation, Danvers MA, USA) for 20 min at 1500 *g*. The samples were filtered through a 45 μm nylon syringe filter and assayed. Quinine standard curves were prepared in either drug-free perfusate plasma, bile or liver homogenate before analysis of samples. Assay sensitivity for free quinine was 0·05 μg mL^{-1}. Recovery was 82% and coefficients of variation were 2·2% at 75 μg mL^{-1} and 3·4% at 40 μg mL^{-1} (n = 5). Extracts of quinine derived from all three extraction methods were analysed by HPLC using the following conditions. The mobile phase consisted of water–acetonitrile–sodium dihydrogen phosphate (1 M, monobasic)-sodium perchlorate (1 M): (83·5% : 12·5% : 2% : 1%). The pH was adjusted to 1·7 by the addition of phosphoric acid and flow was maintained at 1·5 mL min^{-1}. Chromatographic separation was achieved using a Beckman 5 μm octyl ultrasphere 4·6 mm × 15 cm column. Retention times for quinine and internal standard were 10·7 and 9·2 min, respectively. Detection of quinine and internal standard was with a McPherson FL-749 spectrofluorometer (excitation 351 nm, emission 440 nm) equipped with a xenon 150 W lamp. The assay was specific for quinine, separating dihydroquinine and the internal standard. The Waters 590 pump and Waters Intelligent Sample Processor (Model 712) were controlled by a Waters 840 Data System.

Pharmacokinetic calculations and statistical analysis

The perfusate concentration time data for quinine were computer-fitted to a two compartment model using PCNONLIN. Clearance was calculated using the formula:

$$CL = \frac{\text{Dose}}{\text{AUC}}$$

The volume of distribution of quinine at 4 h was calculated assuming a biexponential plasma decline using the formula:

$$V_{ss} = \frac{\text{Dose}}{\text{AUC}\beta}$$

Quinine hepatic extraction was calculated at 10, 30 and 60 min by subtracting the vena-caval (outflow) concentration from the portal (inflow) concentration and expressing this value as a percentage of the inflow concentration. Biliary clearance was calculated by dividing the amount of quinine eliminated in bile over each 30 min collection interval by their corresponding perfusate plasma concentrations at the mid point of the interval. Statistical comparisons between the three groups were made by the use of one way analysis of variance. Data are tabulated as mean ± s.d. and presented graphically as mean ± s.e.m. Statistical significance was accepted at the $P \leqslant 0{\cdot}05$ levels.

Results

Perfusate disposition of quinine

The mean total and mean free log-quinine perfusate concentration-time curves are shown in Figs 1, 2. The plasma concentrations of both free and total quinine in all three dose groups decayed biexponentially over 4 h. However, on

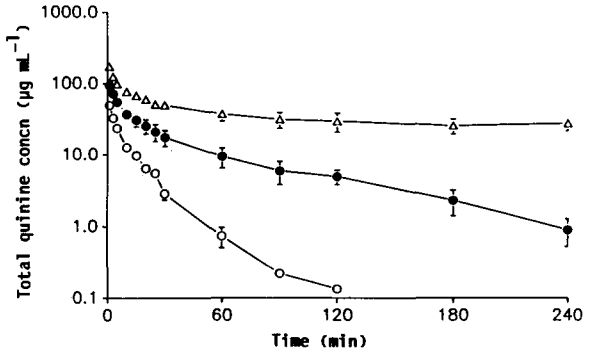

FIG. 1. Total quinine concentrations after the administration of 25 mg (△—△), 12·5 mg (●—●) and 6·25 mg (○—○) quinine sulphate to the rat isolated perfused liver (n = 5 per group, mean ± s.e.m.).

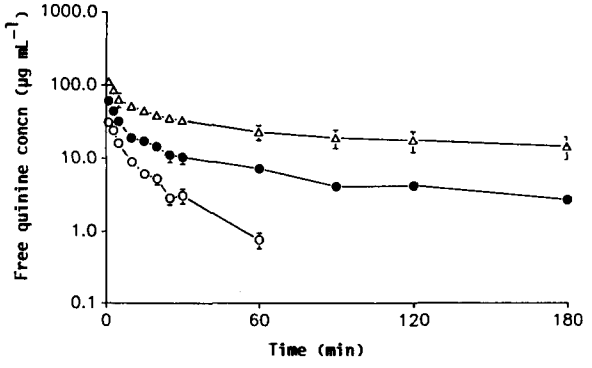

FIG. 2. Free quinine concentrations after the administration of 25 mg (△—△), 12·5 mg (●—●) and 6·25 mg (○—○) quinine sulphate to the rat isolated perfused liver (n = 5 per group, mean ± s.e.m.).

increasing dose, the half-life of both free and total quinine showed a marked increase (Tables 1, 2) ranging from 12·4 ± 3·7 min (free, 19·2 ± 4·4) at the 6·25 mg dose, to 176·0 ± 153 (free, 170 ± 123) min at the highest dose. In addition, both free and total quinine clearance was reduced approximately by half as the dose was doubled. At the lowest dose, total drug clearance was 87·4 ± 15·5% of perfusate flow, indicating quinine to be a high clearance drug in the IPL: in fact, free quinine clearance as a percentage of perfusate flow (107·5 ± 29·0%) indicated removal of free drug from perfusate to be complete at this dose. Uptake of quinine into red cells (10% of this perfusate) may well have accounted for a value in excess of 100%. At the 12·5 and 25 mg doses, total drug clearance as a percentage of perfusate flow fell to 40·4 ± 17·2% and 19·44 ± 17·7%, respectively. From Fig. 1 the low level of quinine elimination at the highest dose was underlined by the more than two fold increase in the $AUC_{0\text{-}4}$ (7981 ± 2088 μg min mL^{-1}) when calculated to infinity (AUC, 15624 ± 11459 μg min mL^{-1}). However, the initial volume of the central compartment (V_1) did not significantly change with dose with either free or total drug, and corresponded (for total drug) approximately to the volume of the circuit. The volumes of distribution calculated at 4 h (V_{ss}) greatly exceeded the circuit volume, indicating extensive hepatic drug uptake (Tables 1, 2).

The high clearance of total quinine at the lowest dose

Table 1. Pharmacokinetic estimates for total quinine derived after the administration of quinine to the rat isolated perfused liver (mean ± s.d., n = 5 per group).

	Dose		
	6·25 mg	12·5 mg	25 mg
AUC (μg h mL^{-1})	487·0 ± 89·0	2250·7 ± 757	15624 ± 11459
$t_{\frac{1}{2}}$ (min)	12·4 ± 3·7	40·2 ± 28	176·0 ± 153
V_1 (mL)	91·0 ± 24·0	104·0 ± 19·5	140·0 ± 48·0
V_{ss} (mL)	229·6 ± 50·5	307·1 ± 162·5	372·1 ± 122·0
CL (mL min^{-1})	13·1 ± 2·3	6·3 ± 3·1	2·9 ± 2·5
C_{max} (μg mL^{-1})	71·6 ± 20·7	121·3 ± 23·6	190·8 ± 47·2

Table 2. Pharmacokinetic estimates for free quinine derived after the administration of quinine to the rat isolated perfused liver (mean ± s.d., n = 5 per group).

	Dose		
	6·25 mg	12·5 mg	25 mg
AUC (μg h mL^{-1})	379·4 ± 132·0	1498 ± 493	9668 ± 7032
$t_{\frac{1}{2}}$ (min)	19·2 ± 4·4	55·6 ± 38·2	170·0 ± 123
V_1 (mL)	176·8 ± 39·0	170·8 ± 45·1	185·2 ± 29·2
V_{ss} (mL)	423·0 ± 95·2	535·0 ± 98·7	523 ± 153
CL (mL min^{-1})	16·3 ± 4·5	4·3 ± 4	4·7 ± 4·2
C_{max} (μg mL^{-1})	36·6 ± 8·6	78·0 ± 27·1	136·0 ± 20·5

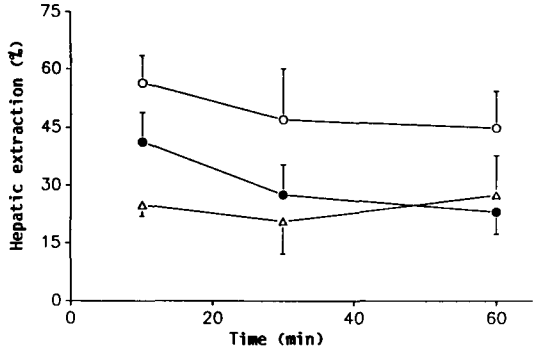

FIG. 3. % Hepatic extraction plotted against time after the administration of 25 mg (△—△) 12·5 mg (●—●) and 6·25 mg (○—○) quinine sulphate to the rat isolated perfused liver (n = 5 per group, mean ± s.e.m.).

(6·25 mg) was reflected in the initial extraction of the drug (Fig. 3). 10 min post dosage, quinine extraction at 6·25 mg (56·5 ± 16·3%) was more than twice that of the highest dose (25·0 ± 6·5%). However, at 30 and 60 min the trend of reduced drug extraction with increasing dose did not attain statistical significance.

Biliary elimination of quinine

The elimination of total quinine in bile was extremely low, with less than 1% of the parent drug being detected in bile at 12·5 and 25 mg doses (0·22 ± 0·09, 0·22 ± 0·07%). At the 6·25 mg dose, quinine levels (0·02 ± 0·009%) were significantly lower than the other two groups. The biliary clearance of quinine was significantly greater during the 0–30 min times at the 6·25 mg dose (0·104 ± 0·08 mL h^{-1}) compared with

$0{\cdot}33 \pm 0{\cdot}16$ mL h^{-1} and $0{\cdot}30 \pm 0{\cdot}14$ mL h^{-1} at the 12·5 and 25 mg doses, respectively. However, there were no significant differences between the groups during the remaining time points. Chromatograms of bile extracts revealed a number of unidentified derivatives of quinine.

Liver disposition of quinine

Relatively small quantities of quinine were recovered from the livers at 4 h. At the highest dose, $\leqslant 6\%$ of the dose was recovered from the livers at 4 h. There was no significant difference between the respective quantities of quinine measured in the liver homogenate of all three dose groups (6·25 mg, $5{\cdot}4 \pm 3{\cdot}8\%$: 12·5 mg, $2{\cdot}5 \pm 1{\cdot}9\%$: 25 mg, $5{\cdot}4 \pm 3{\cdot}8\%$). Again, HPLC analysis of the homogenates revealed some unknown polar metabolites of quinine.

Discussion

At the lowest dose, in the present study, the clearance of quinine was rapid, even at an initial perfusate concentration of $\geqslant 60$ μg mL^{-1} which is almost three times the concentration generally accepted to be approaching systemic toxicity (Franke et al 1987). The initial extraction of quinine ($\geqslant 55\%$) at 10 min was maintained at a high level ($\geqslant 45\%$) for 60 min post dose. As the drug was cleared so quickly by the IPL it is possible that at lower doses in this experimental model quinine would be even more rapidly eliminated. However, the clearance of free quinine at the 6·25 mg doses was $\approx 100\%$ of perfusate flow, suggesting that the hepatic elimination of quinine was probably close to linearity at this dose. At 12·5 mg kg^{-1}, perfusate clearance of total quinine was slowed markedly in comparison with the lowest dose. However, initial drug levels of over 120 μg mL^{-1} were reduced to below 10 μg mL^{-1} at 90 min post dose and clearance was maintained at $\approx 35\%$ of perfusate flow. At the highest dose, initial plasma values of ≈ 190 μg mL^{-1} were nearly ten fold higher than those thought to be approaching systemic toxicity in malarial patients (White et al 1982). Elimination was greatly reduced, with extraction measured at less than half that of the lowest dose. However, even at the highest dose, drug clearance was still considerable. The pharmacokinetics of the free quinine concentrations paralleled the progressive fall in quinine elimination with increasing dose. The volumes of distribution (V_{ss}) showed no significant changes across the dose range chosen, suggesting the dose dependent elimination of quinine was due to capacity limited hepatic metabolism.

The plasma disposition of quinine in man appears to vary widely according to disease state or dose, with reported half-lives ranging from 11·1 to 17·0 h (White et al 1982, 1983). Quinine is extensively biotransformed and undergoes a broadly similar metabolic fate in man, rabbit and rat: of the two major urinary metabolites in man, 2-hydroxyquinine and 2-quininone (Brodie et al 1951), the 2-hydroxy derivative was also excreted predominantly in the rabbit (Watabe & Kiyonaga 1972). Both derivatives were found in rat urine (Barrow et al 1980). In addition, the metabolism of the diastereoisomer, quinidine, in the rat has also been shown to be qualitatively similar to that in man (Rakhit & Mico 1985). The non-linearity of quinidine metabolism in the IPL (Yu et al 1982) and in rat liver microsomes (Rakhit & Mico 1985) correspond with the observation of dose-dependent pharmacokinetics in man (Bolme & Otto 1977; Guentert et al 1979). In the present study, quinine clearance in the IPL appeared to be linear some way above therapeutic concentrations in marked contrast to quinidine. Hence it is conceivable that the non-linearity of quinine disposition which has been reported to occur in man (Berlin et al 1975), may be due to processes other than capacity limited hepatic metabolism.

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