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DISPOSITION OF THE ANTIMALARIAL, MEFLOQUINE, IN THE ISOLATED PERFUSED RAT LIVER

MICHAEL D. COLEMAN,* LAWRENCE FLECKENSTEIN, LISA A. SHIPLEY and MELVIN H. HEIFFER

Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC 20307-5100, U.S.A.

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Abstract—The disposition of mefloquine has been investigated in the isolated perfused rat liver (IPRL) preparation after the administration of [^{14}C]mefloquine HCl (3.8 mg, 4 μCi, quinoline ring labeled). Mefloquine underwent avid hepatic uptake within 10 min of dosing. Also at this point, hepatic oxygen consumption was reduced markedly in four of the six IPRL preparations, but was restored completely by approximately 30 min post-dose. The drug concentration profile underwent a biexponential decline over the 4-hr study period, with a terminal $T_{1/2}$ of 1.0 ± 0.3 hr. The area under the perfusate plasma concentration/time curve ($AUC_{0-\infty}$) was 4.0 ± 1.8 $\mu g \cdot hr \cdot ml^{-1}$. Mefloquine was a high clearance compound (956.0 ± 390 ml/hr) with a large apparent volume of distribution (1416 ± 819 ml) in the IPRL. Biliary excretion accounted for 7.5 ± 6.5% of the dose. Mefloquine was quantitated by HPLC analysis as approximately half (3.3 ± 1.8%) of biliary label, the remainder consisting of highly polar metabolites of mefloquine. By 4 hr, a total of 64.8 ± 4.4% of the [^{14}C] dose was recovered from the livers. Subsequent HPLC analysis revealed this to be mostly unchanged mefloquine. Subcellular fractionation of the homogenized livers revealed that 50.6 ± 6.8% of the dose of mefloquine was located in the 10,000 *g* pellet. In summary, mefloquine was cleared rapidly from the IPRL and underwent avid hepatic uptake into the lipid-rich fractions of rat liver.

Mefloquine (Fig. 1) is a relatively new antimalarial compound that is highly effective against chloroquine-resistant *Plasmodium falciparum* malaria. The pharmacokinetics of mefloquine have been studied in dogs [1], rats [2] and humans [1, 3–6]. Mefloquine exhibits a large volume of distribution, is concentrated in soft tissues, is metabolized slowly, and undergoes considerable biliary excretion *in vivo* [1–3]. Mefloquine also inhibits hepatic metabolism of aminopyrine *in vitro* in rat microsomes [7].

Therefore, to investigate more fully the hepatic handling of mefloquine, we have chosen the isolated perfused rat liver (IPRL) preparation. This experimental model excludes the influence of other organs and routes of elimination present in the intact animal and has been of value in studies of the disposition of a number of compounds, e.g. cimetidine, propranolol, suramin and the antimalarial primaquine [8–11].

MATERIALS AND METHODS

Reagents. Mefloquine hydrochloride (WR 142,490) and WR 184,806, the internal standard for HPLC analysis, were both synthesized on contracts to the United States Army Medical Research and Development Command (USAMRDC) by Ash Stevens, Inc., Detroit, MI, and Starks Associates, Inc., Buffalo, NY, respectively. [^{14}C]Mefloquine hydrochloride (sp. act. 10 mCi/mmol) labeled at position 4 of the quinoline ring was obtained under contract to USAMRDC from the Chemistry and Life Sciences Division, Research Triangle Institute, Research Triangle Park, NC. Radiochemical purity was determined by HPLC and TLC to be 99%. NCS tissue solubilizer and hydrogen peroxide were supplied by Fisher Scientific (Fairlawn, NJ). Emulsifying liquid scintillant "Aquasol" was obtained from NEN Research Products, Boston, MA. All other reagents were of HPLC or analytical grade.

Animals. Male Sprague–Dawley rats (200–250 g, Walter Reed Army Institute of Research Breeding Colony) were housed in well ventilated cages and kept at a room temperature of approximately 24°. They were allowed to feed *ad lib.* on pelleted food

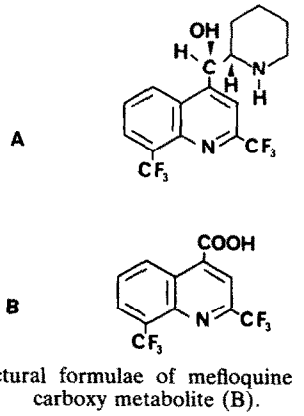

Fig. 1. Structural formulae of mefloquine (A) and its carboxy metabolite (B).

* Author to whom all correspondence should be addressed.

(Ziegler Brothers, Gardner, PA) 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).

Isolated perfused rat livers (IPRL). Rats were anesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and their livers were isolated using standard techniques and then perfused in a constant flow (15 ml/min) recirculating system at 37° as previously described [8]. The perfusate (100 ml) comprised 10% washed human red cells, 1% (w/v) bovine serum albumin (Sigma Chemicals Co., St. Louis, MO) and 0.1% glucose in a standard electrolyte solution [8]. The principal indices of liver viability were steady oxygen consumption (1.5 to 2 μmol/g liver/min), sustained bile flow (0.2 to 0.6 ml/hr), constant perfusion pressure (6 to 8 cm H_2O), reproducible liver function tests (i.e. determination of perfusate sodium, potassium, total protein, alanine aminotransferase and gamma-glutamyl transferase concentrations), and normal visual appearance.

Protocol. The disposition of mefloquine in the IPRL was studied for 4 hr following a 3.8 mg bolus dose of mefloquine HCl containing a trace dose (4 μCi) of ^{14}C-labelled drug (N = 6). Mefloquine was added as a solution in 35 μl of dimethyl sulfoxide directly into the perfusate reservoir, thereby simulating systematic dosage. Samples (1 ml) were removed from the perfusate reservoir pre-dose and at 10, 20 and 30 min, and 1, 1.5, 2, 2.5, 3, 3.5 and 4 hr post-dosage. An equal volume of fresh perfusate was added to the reservoir to replace that removed by sampling. After centrifugation (1100 *g*, 3 min), the perfusate plasma was removed and stored at −20° until assayed for mefloquine and radioactivity. Bile was collected at half-hour intervals into preweighed vials, and the bile volume was determined by weight (assuming 1 ml bile = 1 g) before freezing at −20°. Sodium taurocholate (30 μmol/hr, Sigma) was infused into the perfusate reservoir to maintain bile flow. Perfusate gases (CO_2 and O_2) were measured, and liver function tests were performed before and after each experiment to assess liver viability. The subcellular localization of mefloquine and [^{14}C] radioactivity was determined for each liver at the end of each experiment at 4 hr post-dose as described below.

Preparation of liver tissue fractions. At the conclusion of each experiment, the livers were flushed with 0.9% 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. The 25% (w/v) homogenate was centrifuged at 10,000 *g* for 25 min at 4°. The resulting supernatant fraction was decanted without disturbing the pellet and centrifuged at 105,000 *g* for 60 min at 4°. The 105,000 *g* and 10,000 *g* pellets were then resuspended in three times their weight of phosphate buffer. Prior to HPLC analysis of mefloquine in the various liver fractions, separate mefloquine standard curves were prepared in drug-free whole liver homogenate, 10,000 *g* pellet (resuspended), 10,000 *g* supernatant, 105,000 *g* pellet (resuspended) and 105,000 *g* supernatant.

Analytical procedures. Perfusate gases were measured using a Corning 168 Blood Gas Analyzer. Mefloquine levels were determined by HPLC employing u.v. detection (Waters model 440) at 214 nm and a C_{18} (μBondapak, Waters Associates, Milford, MA) stainless steel column [12]. The mobile phase consisted of methanol/water (70/30, v/v), contained octane sulfonic acid (0.005 M) as an ion pair reagent, and flowed at 1 ml/min. The lower limit of sensitivity for this method was 10 ng/ml. Prior to HPLC analysis of whole perfusate, to indirectly measure drug and red cell concentrations, standard curves were prepared in drug-free whole perfusate. [^{14}C]Radioactivity was measured using a Packard Tri-Carb 4034 liquid scintillation spectrometer. Duplicate samples of perfusate plasma (10 μl) and bile (5 μl) were assayed directly for [^{14}C] after the addition of scintillant fluid (20 ml). Samples of whole liver homogenate and the various liver tissue fractions were solubilized and decolorized as previously described [13] before assay for [^{14}C] levels.

Analysis of bile. Mefloquine was determined in each half-hour bile sample by direct injection of 5 μl of bile onto the HPLC column. In addition, a representative aliquot (40%) of the total bile production of each half-hour sample was removed and combined. A further aliquot (500 μl) was removed from the pooled bile and was subjected to enzymatic hydrolysis (pH 5.0, 37°, 16 hr) with β-glucuronidase preparation from bovine liver (Sigma) (1 unit/μl of bile) and aryl sulfatase preparation obtained from *Helix pomatia* (Sigma: 1 unit/μl bile). Control incubations contained 0.1 M sodium acetate buffer (pH 5.0). Prior to hydrolysis of the pooled bile samples, mefloquine was found to be stable to hydrolysis by this enzyme preparation. A 5-μl aliquot from each pooled bile sample was injected directly onto the HPLC. Effluent fractions corresponding to the void volume (0 to 4.5 min), void volume to mefloquine (4.5 to 6.50 min) and mefloquine (6.5 to 8 min) were collected and assayed for [^{14}C] after the addition of liquid scintillant.

Pharmacokinetic calculations and statistical analysis. The area under the perfusate concentration/time curve (AUC) for mefloquine and [^{14}C] from time = 0 to time = 4 hr (AUC_{0-4hr}) was calculated by the trapezoidal rule [14]. The AUC from 4 hr to infinity was calculated from the ratio C_4/β where C_4 was the perfusate concentration at time 4 hr. The area under the curve from zero to infinity ($AUC_{0-\infty}$) for mefloquine was obtained from the sum of the two areas. The terminal phase elimination rate constant (β) was determined by least squares regression analysis of the post-distributive perfusate mefloquine concentration–time data and the terminal phase half-life ($T_{1/2}$) from the ratio $0.693/\beta$. Mefloquine clearance (Cl) from perfusate plasma was calculated from the equation

$$Cl = \frac{\text{Dose}}{AUC_{0-\infty}}$$

and the apparent volume of distribution (V_d) for mefloquine from

$$V_d = \frac{\text{Dose}}{AUC\,\beta_{0-\infty}}$$

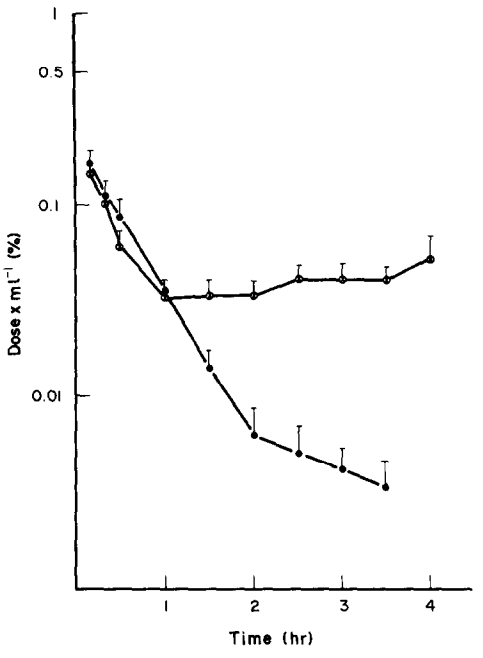

Fig. 2. Perfusate concentrations (% dose/ml ± SEM) of mefloquine (●—●) and [^{14}C] (⊗—⊗) after systemic administration of [^{14}C]mefloquine HCl (3.8 mg, 4 μCi) to the IPRL.

Biliary clearance was calculated by dividing the amount of mefloquine eliminated in bile over each 30-min collection interval by the perfusate plasma concentration of mefloquine at the mid-point of that interval. To determine drug accumulation in red cells (10% of perfusate volume), whole perfusate (W) to perfusate plasma (P) concentration ratios (W/P) were determined from the quotient of whole perfusate and perfusate plasma drug levels. Statistical comparisons between two groups were made by the use of Student's *t*-test. Data were tabulated as mean ± SD and presented graphically as mean ± SEM. Statistical significance was accepted at the $P < 0.05$ level.

RESULTS

Perfusate disposition of mefloquine and [^{14}C]. The mean log-mefloquine perfusate concentration/time curve is shown on Fig. 2. Within 10 min of drug administration, drug and [^{14}C] were rapidly removed from the perfusate. Also at this point in four of the six IPRL preparations studied, oxygen consumption declined steeply from the mean pre-dose value of 1.8 ± 0.3 μmol $O_2 \cdot (g\ liver)^{-1} \cdot min^{-1}$ to almost zero at 20 min post-dose. In addition, bile production was slowed briefly. However, within 30 min post-dose, mean measured oxygen consumption was restored to pre-dose levels [1.73 ± 0.3 μmol $O_2 \cdot (g\ liver)^{-1} \cdot min^{-1}$]. At 4 hr the mean measured oxygen consumption [1.69 ± 0.19 μmol $O_2 \cdot (g\ liver)^{-1} \cdot min^{-1}$] did not differ significantly from the 30-min value (1.73 ± 0.3) nor the pre-dose value (1.8 ± 0.3). Mefloquine concentrations decayed biexponentially with a terminal elimination half-life of 1.0 ± 0.3 hr; $AUC_{0-\infty}$ was calculated to be 4.0 ± 1.8 $\mu g \cdot hr \cdot ml^{-1}$. The clearance of mefloquine (956 ± 393 ml/hr) represented approximately 106% of liver perfusate flow, indicating mefloquine to be a very high clearance compound in this experimental model. The apparent volume of distribution (1416 ± 819 ml) greatly exceeded the actual volume of the circuit (100 ml perfusate plus liver volume), implying avid hepatic uptake of mefloquine. A summary of the mass balance data is shown in Table 1. During the course of the study, only a small proportion of the administered [^{14}C] was eliminated in the bile (7.5 ± 6.5%). At the conclusion of each experiment the majority of the [^{14}C] and unchanged mefloquine were located in the liver (Table 2). From Table 1 and Fig. 1 it is apparent that only a minimal proportion of the [^{14}C] located in the perfusate at the conclusion of the study could be accounted for as parent drug. These findings indicate the presence of considerable quantities of unidentified metabolites of mefloquine in the perfusate plasma. A subsequent binding determination revealed that 18% of the mefloquine dose that was unaccounted for at the conclusion of the study had become bound to the plastic tubing of the IPRL circuit over 4 hr. The whole perfusate to perfusate plasma concentration ratios (W/P) were greater than unity for mefloquine and [^{14}C] at 1 and 2.5 hr, but close to unity at 4 hr (mefloquine, t = 1 hr, W/P = 2.1 ± 0.7; t = 2.5 hr, W/P = 3.00 ± 1.0; t = 4 hr, W/P = 1.0 ± 1.0. [^{14}C]radioactivity, t = 1 hr, W/P = 1.1 ± 0.33; t = 2.5 hr, W/P = 2.0 ± 0.9; t = 4 hr, W/P = 1.1 ± 0.7). As the hematocrit of the perfusate was only 10%, this indicates considerable accumulation of parent drug and metabolites in red cells during the initial phase of the study.

Table 1. Mass balance: Percentage mefloquine and [^{14}C] at 4 hr post-administration of [^{14}C]mefloquine (3.8 mg, 4 μCi) to the IPRL

	Mefloquine (HPLC identified) (%)	Total [^{14}C] radioactivity (%)
Liver	60.9 ± 17.3	64.8 ± 4.4
Bile	3.3 ± 1.8*	7.5 ± 6.5
	2.8 ± 1.6†	
Perfusate plasma	0.75 ± 0.6	7.6 ± 4.0
Total recovery	62.4 ± 16.5	80.0 ± 5.7

Values are means ± SEM; N = 6.
* Represents free compound.
† Represents free plus deconjugated compound.

Table 2. Percentage of [^{14}C] and mefloquine present in whole liver and fractions at 4-hr post-administration of [^{14}C]mefloquine (3.8 mg, 4 μCi) to the IPRL

	Recovery (%)	
Tissue	[^{14}C]	Mefloquine
Whole liver	64.8 ± 4.4	60.9 ± 17.3
10,000 *g* Pellet	56.0 ± 4.8	50.0 ± 6.8
10,000 *g* Supernatant	5.0 ± 3.1	7.0 ± 3.6
105,000 *g* Supernatant	3.7 ± 2.0	4.7 ± 4.2
105,000 *g* Pellet	1.6 ± 0.7	3.2 ± 1.4

Values are means ± SEM; N = 6.

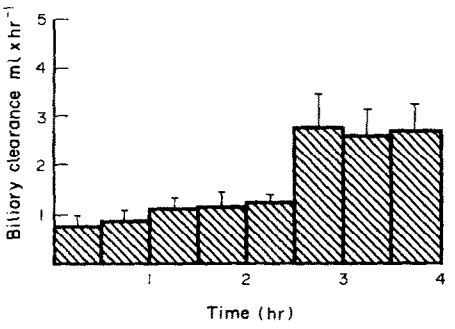

Fig. 3. Biliary clearance (ml/hr^{-1} ± SEM) of mefloquine plotted against time (hr) after the administration of [^{14}C]mefloquine HCl (3.8 mg, 4 μCi) to the IPRL.

Analysis of biliary metabolites. Overall, 7.5 ± 6.5% of the dose of [^{14}C] was eliminated in bile over 4 hr. However, only 3.3 ± 1.8% of the [^{14}C] was determined to be mefloquine. The remaining radioactivity was distributed approximately equally between the void volume (1.2 ± 0.6%) and the fraction which eluted between the void volume and mefloquine (1.5 ± 1.3%). Flushing the HPLC system with methanol at the end of each sample run accounted for a negligible quantity of [^{14}C] (0.001%).

The disposition of [^{14}C] was unaffected by subjecting the bile to deconjugating enzymes, indicating that the excretion of mefloquine as glucuronide and sulfate conjugates was not an important route of elimination. The analysis of biliary clearance (Fig. 3) revealed steady elimination of intact mefloquine over the first 2 hr of the study followed by a marked increase in drug excretion at 2.5 hr. This elevated level of clearance was maintained until the end of the study.

Liver fractionation studies. HPLC analysis of whole liver homogenates revealed that the [^{14}C] found in the livers consisted almost exclusively of parent drug (Table 1). The vast majority of the [^{14}C] and mefloquine were located in the 10,000 *g* pellet (Table 2). There was no significant difference in the distribution of mefloquine and [^{14}C] between either the 105,000 *g* pellet or supernatant.

DISCUSSION

In a recent study in the intact rat, co-administration of mefloquine with pyrimethamine was associated with a significant change in the tissue distribution of pyrimethamine specifically in the liver and lung [15]. It has also been reported that mefloquine is taken up extensively into soft tissues in rodents, including the liver [16]. Hence, we wished to investigate in detail the pharmacokinetics, metabolism, biliary excretion and hepatic subcellular localization of mefloquine in the IPRL preparation.

Within 10 min of drug administration, less than 20% of the [^{14}C] and mefloquine remained in the perfusate (Fig. 2), indicating extremely rapid clearance of drug. However, a considerable variability was encountered in the clearances of the individual IPRL preparations. Previous studies with this compound, both in rodents [2] and humans [3, 4], have also exhibited marked data variation. This might be due, in part, to the long half-life and extensive soft tissue uptake and redistribution of the drug.

In the present study, in some of the IPRL preparations, clearance actually exceeded perfusate flow. In addition to avid hepatic drug uptake, this may be accounted for by rapid drug accumulation by perfusate erythrocytes. This is supported by the whole perfusate to perfusate plasma concentration ratios of mefloquine. In addition, a previous study has indicated mefloquine red cell levels to be 5- to 6-fold those of plasma [16].

In humans, mefloquine is highly plasma protein bound (>98%) predominantly to albumin (>95%) [2]. However, V_d is high in both animals and humans; tissue to plasma concentration ratios have been determined to be as high as 40:1 [2]. These findings, together with the avid hepatic and red cell uptake reported in the present study, may be accounted for by the known high affinity of mefloquine for membrane phospholipids [17].

Mefloquine is known to be metabolized slowly *in vivo* [3]; hence, it might be expected that the high levels of [^{14}C] located in the liver at 4 hr would be parent drug; indeed this proved to be the case. Furthermore, sub-cellular fractionation of the homogenized livers at 4 hr revealed the vast majority of the mefloquine to be located in the 10,000 *g* pellet. This is the most lipid-rich fraction in homogenized liver consisting of cell nuclei, mitochondria and cell wall debris [18], which is consistent with the known high lipid solubility of mefloquine (octanol/water partition coefficient, 250:1).

Analysis of the collected bile samples revealed that over half the eliminated [^{14}C] consisted of polar metabolized derivatives of mefloquine. These metabolites could not be accounted for as glucoronides or sulfates; hence, they may have been carboxylated or aryl hydroxylated derivatives of mefloquine as described in a previous report [19], which describes the major metabolite as a carboxylated derivative (Fig. 1). It may be that the small quantity of unidentified perfusate metabolites of mefloquine were also of a polar nature.

To our knowledge, the brief but reversible reduction in oxygen consumption observed in many of the liver preparations in this study has not been reported previously. As metabolism of mefloquine is slow and the [^{14}C] present in the liver was entirely accounted for as mefloquine, it is plausible that this phenomenon is mediated by high concentrations of the parent drug.

In conclusion, our studies indicate that mefloquine was cleared very rapidly from perfusate plasma and underwent avid uptake into red cells and the lipid-rich fractions of rat liver. Metabolism of the drug, however, was sparing, the majority of the polar drug derivatives being excreted into bile.

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