mined is much lower than that required for therapeutic effects in the CSF [8] and thus its clinical significance in modifying CNS functions remains to be demonstrated.

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Comparative inhibitory effects of mefloquine and primaquine on hepatic drug-metabolizing enzymes

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Mefloquine (MQ) and primaquine (PQ) are quinoline derivatives used mainly as antimalarial drugs. Several studies have shown that PQ is a potent inhibitor of many enzymes *in vitro* including those dependent on the hepatic cytochrome P-450 [1–5]. In an *in vivo* study in man, PQ has been reported to be a potent inhibitor of antipyrine metabolism [6]. However, little is known about the effect of MQ on hepatic drug metabolism, except in a recent study by Riviere and Back [7], who have demonstrated that both MQ and PQ could inhibit the metabolism of aminopyrine and ethinylestradiol *in vitro* as well as the metabolism of tolbutamide *in vivo*.

In the present communication, we have investigated and compared the inhibitory effects of these two antimalarial drugs on a wide range of hepatic drug-metabolizing enzymes *in vitro* and studied the nature of their inhibition on these enzymes. Their inhibitory effects on the *in vivo* metabolism of hexobarbital and zoxazolamine were also tested.

Methods

Male Wistar rats (150–200 g), obtained from the National Laboratory Animal Center of Mahidol University, were housed under standardized conditions of light (6:00 a.m. to 6:00 p.m.) and temperature (25–28°). All animals were untreated and allowed at least 5 days to become acclimated to the housing conditions prior to use in experiments. They were given free access to food (standard rat chow; Purina Laboratory Chow, Zuellig Pte. Ltd, Singapore) and tap water *ad lib.* until 14–16 hr before killing, during which they were allowed access to water only. Animals were killed by heart puncture under light ether anesthesia between 7.00 and 9.00 a.m. Livers were quickly removed and washed with ice-cold buffered KCl (1.15% KCl in 0.1 M Na^+/K^+ phosphate buffer, pH 7.4) to remove the blood. The liver was blotted dry with filter paper and weighed. All subsequent procedures were performed below 4°. After weighing, the liver was teased with scissors and then homogenized with 2–7 volumes of buffered KCl in a Potter–Elvehjem homogenizer using a Teflon pestle. The homogenate was centrifuged at 9000 *g* in a refrigerated centrifuge for 15 min using Beckman Centrifuge Model J-21B with JA-21 rotor. After centrifugation, the supernatant was carefully aspirated off with Pasteur pipette. Three milliliters of this post-mitochondrial fraction were further centrifuged at 105,000 *g* for 60 min in a Beckman Model L5-65 refrigerated ultracentrifuge, whereas the remaining portion (about 9.0 ml) was used as the enzyme source in the *in vitro* hepatic drug-metabolizing enzyme assays. After ultracentrifugation the soluble supernatant fraction was carefully removed with a Pasteur pipette and the microsomal pellet was rinsed and resuspended in 3.0 ml ice-cold 0.1 M Na^+/K^+ phosphate buffer, pH 7.4. This microsomal suspension was used for the determination of microsomal protein content.

The activities of aminopyrine *N*-demethylase, aniline hydroxylase, hexobarbital oxidase, and *p*-nitroanisole *O*-demethylase were measured in the presence of various cofactors according to the methods described in detail in previous publications [8, 9]. *p*-Chloro-*N*-methylaniline *N*-

demethylase was determined by the method of Kupfer and Bruggeman [10]. For all enzyme assays, conditions were established to ensure linearity of product formation with respect to protein concentrations and incubation time. Microsomal protein concentrations were determined by the method of Lowry *et al.* [11].

To study the inhibition of each of these five hepatic drug-metabolizing enzymes by primaquine or mefloquine, 1.0 ml of the postmitochondrial fraction and a complete supporting system were preincubated with the inhibitor, in a series of concentrations ranging from 10^{-3} to 10^{-6} M for 10 min before adding the substrate to start the reaction. Control incubation mixture containing equivalent components except for the omission of the inhibitor was also run simultaneously. In kinetic experiments, two fixed concentrations of each inhibitor were used (0.05 and 0.10 mM for mefloquine or 0.01 and 0.05 mM for primaquine). The reaction was started by addition of varying concentrations of the substrate (e.g., 0.25, 0.5, 1.0 and 2.0 mM for aminopyrine or 0.1, 0.2, 0.4, 0.8 and 1.6 mM for aniline). A Lineweaver–Burk plot for each enzyme was constructed [12]. To obtain the slope and intercept inhibition constants (K_{is} and K_{ii}) secondary plots of Lineweaver–Burk slopes and intercepts were plotted as a function of inhibitor concentrations, respectively. These inhibitor constants were then defined by the point of intersection of the line with the X-axis [13]. In this report, however, only one K_i was presented, as in Table 1, and it was the K_{is} since both approaches, using either intercept or slope in the secondary plot, gave similar value for the K_i.

Primaquine diphosphate was purchased from Sigma Chemical Co. (St Louis, MO). Mefloquine HCl was kindly given as a gift by Dr K. Webster, AFRIMS, Bangkok and also by Dr R. Reber-Liske, F. Hoffmann La Roche & Co., Basel, Switzerland. Other chemicals were obtained commercially.

Both hexobarbital sleeping time and zoxazolamine paralysis time were determined from the animals pretreated for 40 min with mefloquine HCl (50 mg/kg, p.o.) or primaquine diphosphate (50 mg/kg, i.p.) before the administration of hexobarbital sodium (120 mg/kg, i.p.) and zoxazolamine (60 mg/kg, i.p.), respectively.

For statistical analysis, the Student's unpaired t-test was used to determine the significance between the treatment and the control groups, with $P < 0.05$ [14]. Regression analysis by the method of least squares was used in drawing a line to obtain enzyme kinetic parameters (apparent K_m, V_{max}, K_{is} and K_{ii}).

Results and discussion

In this study, five model hepatic drug-metabolizing enzymes were selected to test their response to the inhibitory effects of both MQ and PQ. Although PQ is well known to be capable of inhibiting a wide range of enzymes [1–7], its inhibitory effect on hexobarbital oxidase, *p*-chloro-*N*-methylaniline *N*-demethylase and *p*-nitroanisole *O*-demethylase has not been reported. MQ has only recently been found to inhibit the hepatic metabolism of aminopyrine, ethinylestradiol and tolbutamide [7]. As shown in Table 1, PQ produced a concentration-dependent

Table 1. Dose-dependent inhibition of mefloquine and primaquine on hepatic drug-metabolizing enzymes

	Mefloquine (M)			Primaquine (M)		
	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
AND	54 ± 1	13 ± 1	4 ± 2	54 ± 2	29 ± 3	13 ± 3
HO	—	—	—	71 ± 4	45 ± 3	34 ± 6
AH	23 ± 2	5 ± 3	4 ± 3	72 ± 1	46 ± 3	28 ± 5
pCAD	8 ± 1	1 ± 3	—	10 ± 2	1 ± 3	—
pNAD	35 ± 1	12 ± 3	2 ± 1	63 ± 4	32 ± 5	14 ± 6

Values are expressed as mean per cent inhibition in the presence of each inhibitor at three different concentrations ± SE of four separate determinations, each from pooled livers of four rats. AND, aminopyrine *N*-demethylase; pCAD, *p*-chloro-*N*-methylaniline *N*-demethylase; HO, hexobarbital oxidase; pNAD, *p*-nitroanisole *O*-demethylase; AH, aniline hydroxylase.

Table 2. Summary of inhibition constants and type of inhibition of antimalarials on hepatic drug-metabolizing enzymes

Enzyme	K_i (mM)	
	MQ	PQ
Aminopyrine *N*-demethylase	0.054 ± 0.003 (NC)	0.027 ± 0.003 (C)
Hexobarbital oxidase	—	0.044 ± 0.006 (NC)
Aniline hydroxylase	—	0.033 ± 0.005 (NC)
p-Chloro-*N*-methylaniline-*N*-demethylase	—	0.347 ± 0.024 (C)
p-Nitroanisole *O*-demethylase	0.317 ± 0.170 (NC)	0.033 ± 0.005 (NC)

Each value represents mean ± SE of three separate determinations. The type of inhibition by each drug is indicated in parenthesis: C, competitive inhibition and NC, noncompetitive inhibition.

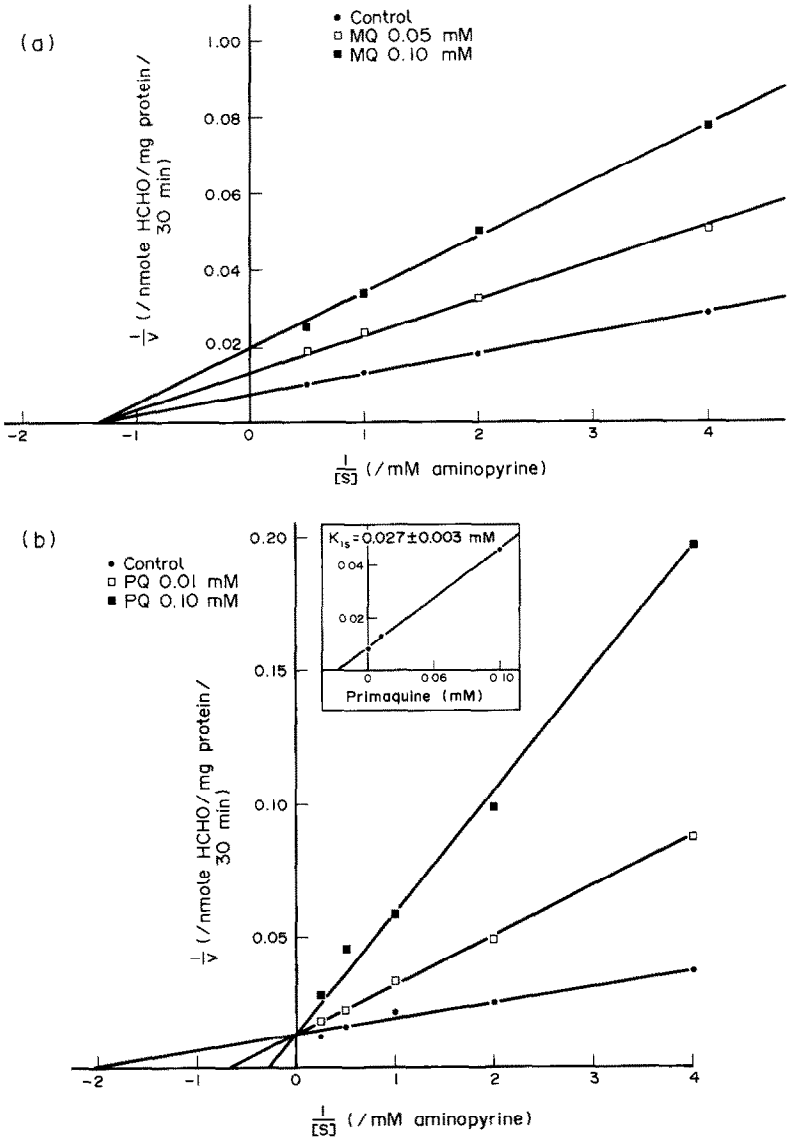

Fig. 1. Double reciprocal plot illustrating the effect of mefloquine (MQ) and primaquine (PQ) on aminopyrine *N*-demethylase from rat liver *in vitro*. (A) mefloquine; (B) primaquine. The enzyme activity was assayed both in the absence and in the presence of two different inhibitor concentrations. Each point is mean of three to four separate determinations, each from pooled livers of four rats. The inset shows a typical secondary plot of slope or intercept against different inhibitor concentrations from which the inhibition constants (K_{is} or K_{ii}) may be obtained.

inhibition on four out of the five hepatic drug-metabolizing enzymes examined; *p*-chloro-*N*-methylaniline *N*-demethylase was little affected by PQ. In contrast, MQ caused a concentration-dependent inhibition on aminopyrine *N*-demethylase and *p*-nitroanisole *O*-demethylase (Table 1); aniline hydroxylase was slightly inhibited by 10^{-4} M of MQ while hexobarbital oxidase and *p*-chloro-*N*-methylaniline *N*-demethylase were unaffected by MQ at any concentrations of the drug used.

When the nature of inhibition caused by these two drugs on the two most sensitive enzymes was investigated, it was found that MQ inhibited aminopyrine *N*-demethylase in a noncompetitive manner, whereas PQ produced a competitive inhibition on this enzyme (Fig. 1). Both MQ and PQ were found to inhibit *p*-nitroanisole *O*-demethylase noncompetitively (Fig. 2). The inhibition constants (K_i), as determined from the secondary plot of slope versus inhibitor concentrations, of these drugs on the five enzymes examined are compared in Table 2.

In concordance with the *in vitro* results, MQ had no effect on either hexobarbital sleeping time or zoxazolamine paralysis time (Table 3). This finding, however, is different from that of Riviere and Back [7], who found a more pronounced inhibitory effect of MQ than PQ on tolbutamide metabolism *in vivo*.

A possible explanation for this discrepancy is that the

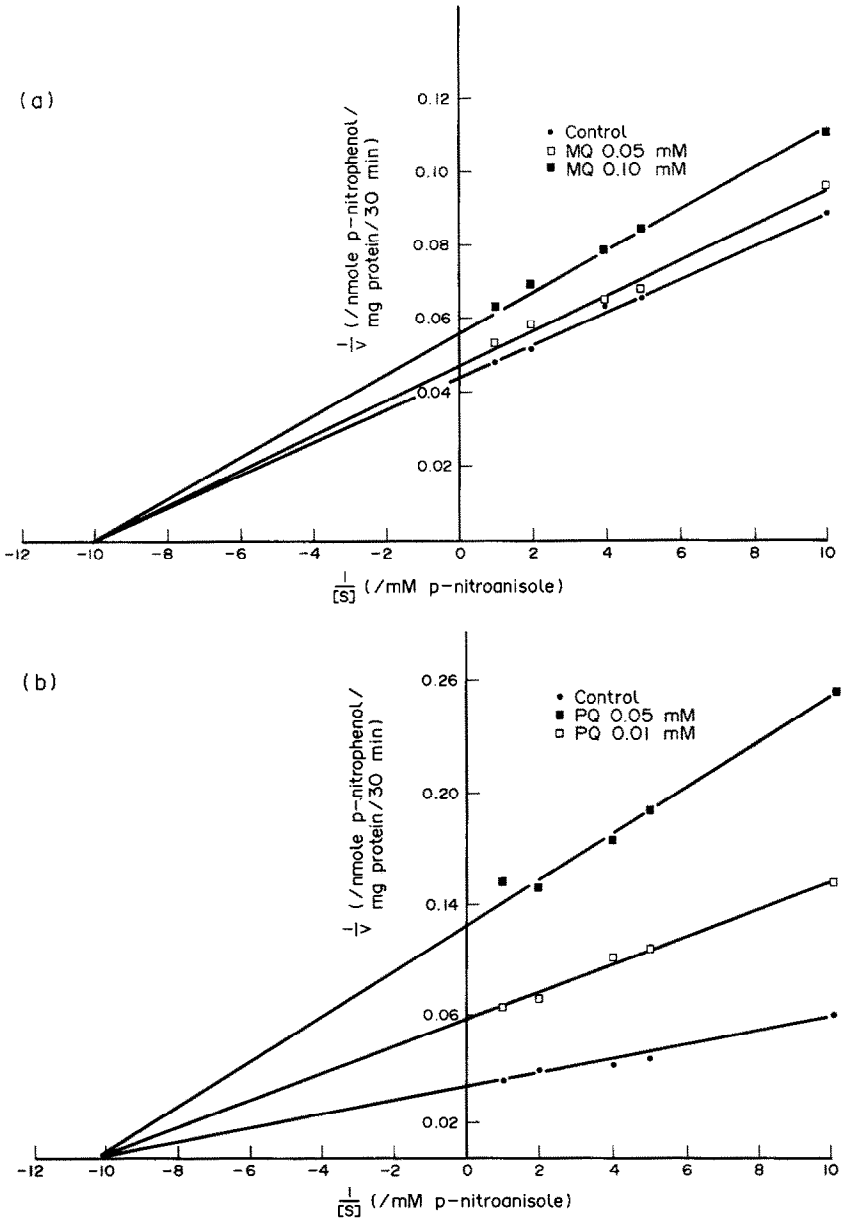

Fig. 2. Double reciprocal plot illustrating the effect of mefloquine (MQ) and primaquine (PQ) on *p*-nitroanisole *O*-demethylase from rat liver *in vitro*. (A) mefloquine; (B) primaquine. The enzyme activity was assayed both in the absence and in the presence of two different inhibitor concentrations. Each point is mean of three to four separate determinations, each from pooled livers of four rats.

cytochrome P-450 isozymes responsible for the hydroxylation of tolbutamide interact more favorably with MQ than PQ. It is also equally possible that MQ is more concentrated in the vicinity of the hydroxylating enzyme for tolbutamide metabolism. Elimination of MQ is slow and there is evidence of significant enterohepatic circulation of this drug [15].

The difference in the ability of these two antimalarial drugs on hepatic drug metabolism may be explained by stereochemistry. Both MQ and PQ contain a quinoline nucleus, which imparts some affinity for the P-450 active center as a consequence of its lipophilic factor but which itself exerts little inhibitory effect. As pointed out by Murray [16], the nature of quinoline substitutes appears to be the primary determinant of the observed potency. Thus, the different inhibitory effects of MQ and PQ can be explained mechanistically in terms of stereochemistry [6, 16]. The asymmetric center of the side chain of primaquine is removed from the point of interaction of the drug with high-spin P-450, whereas steric hindrance precludes an effective interaction between the pyridine nitrogen atom in mefloquine and high-spin ferric P-450.

Table 3. Effect of mefloquine and primaquine on the metabolism of hexobarbital and zoxazolamine

Drug	Hexobarbital sleeping time (min)	Zoxazolamine paralysis time (min)
Control	33.4 ± 2.8 (9)	117.7 ± 9.2 (16)
Mefloquine HCl	36.6 ± 3.2 (8)	116.0 ± 9.3 (8)
Primaquine $diPO_4$	70.4 ± 4.5 (7)*	245.1 ± 9.7* (8)

The animals were pretreated for 40 min with either mefloquine HCl (50 mg/kg, p.o.) or primaquine $diPO_4$ (50 mg/kg, i.p.). Hexobarbital (120 mg/kg, i.p.) or zoxazolamine (60 mg/kg, i.p.) was then given. Control animals received an equal volume of normal saline. The result is expressed as mean ± SE from the number of rats indicated in parenthesis.

* $P < 0.05$ (from Control).

In conclusion, the present data suggest that the two quinoline derivatives (MQ and PQ) have different specificities in their inhibitory action on hepatic drug-metabolizing enzymes and that PQ is a more universal inhibitor of these enzymes than MQ.

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Characterization of [^{3}H]Ro 16-6491 binding to digitonin solubilized monoamine oxidase-B and purification of the enzyme from human platelets by affinity chromatography

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It is now generally accepted that the two forms of the FAD-containing enzyme monoamine oxidase (MAO*; EC 1.4.3.4.), namely MAO-A and MAO-B, are two structurally different proteins coded by distinct genes. A decisive breakthrough in clarifying the structure of MAO-A and MAO-B has come from molecular biology studies. Bach *et al.* [1] in fact have obtained two distinct cDNA encoding human liver MAO-A and MAO-B, showing that the two isoenzymes differ in primary structure, with a relatively high degree of homology (about 70%). Most of the evidence available suggests that the two isoenzymes have native molecular masses of about 120 kDa, being composed by two very similar or identical subunits. In contrast to previous findings [2, 3], recent studies indicate that each of these two subunits have covalently linked FAD as coenzyme [4].

MAO-A and MAO-B are both located in the outer mitochondrial membrane. Detergents are required for their

* Abbreviations used: MAO, monoamine oxidase; PEA, phenylethylamine.