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EFFECT OF MEFLOQUINE ON HEPATIC DRUG METABOLISM IN THE RAT: COMPARATIVE STUDY WITH PRIMAQUINE

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Abstract—The effect of the new antimalarial drug mefloquine (MQ) on hepatic drug metabolism in the rat has been studied *in vitro* and *in vivo* using three different substrates, aminopyrine, ethinyloestradiol and tolbutamide. Comparative studies have been performed with primaquine (PQ). *In vitro*, both MQ and PQ inhibited aminopyrine N-demethylase activity and the concentration required to produce 50% inhibition was 0.2 mM for MQ and approximately 0.1 mM for PQ. Lineweaver–Burk plots indicated inhibition by both antimalarials to be non-competitive. Both MQ and PQ produced comparable inhibition of ethinyloestradiol metabolism *in vitro* with the percentage recovery of the major metabolite, 2-hydroxyethinyloestradiol being reduced from 49.3 ± 10.8 to 5.1 ± 3.1 (0.5 mM MQ) and 1.5 ± 0.4% (0.5 mM PQ, mean ± S.D.). Following acute administration of MQ and PQ to rats (25 mg kg^{-1}) recovery of hydroxytolbutamide the major metabolite of tolbutamide, was reduced. In the period 0–8 hr, MQ caused a reduction in recovery from 54.4 ± 3.1 to 9.3 ± 3.4% and PQ from the control level to 32.2 ± 14.1%. There is therefore clear evidence that MQ inhibits hepatic microsomal enzymes both *in vitro* and *in vivo*. The more pronounced effect of MQ *in vivo*, in comparison with PQ, is probably a reflection of differences in the kinetics of the two antimalarials. The range of substrates studied indicate a non-selective and widespread inhibitory effect of these drugs on oxidative enzymes

Mefloquine (MQ) [D,L-erythro-α-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol] is a new antimalarial drug used for treatment and prevention of chloroquine-resistant strains of falciparum malaria. Chloroquine resistance in south-east Asia, South America, and also Africa and India is now very widespread and is posing serious problems in these areas. Evidence is also emerging that there is an increasing occurrence of parasites resistant to other antimalarial drug combinations. Thus there is an immediate need for alternative antimalarial drugs which can be used against chloroquine-resistant and multi-resistant strains.

MQ was developed by the United States Army and is structurally related to quinine. It is thought to act on malaria parasites in a similar way to quinine but does not intercalate with DNA as do quinine and other antimalarial drugs [1]. MQ is thus an effective blood schizonticidal compound, and is used not only for malaria chemotherapy, but also as a suppressive prophylactic [2].

It is important to establish if antimalarial drugs interact with other drugs which may be used concurrently. In a previous study the effect of chloroquine (CQ) and primaquine (PQ) on rat liver microsomal drug metabolism was investigated *in vitro* and *in vivo* [3]. The main finding was that PQ and to a much lesser estent CQ inhibited hepatic microsomal drug metabolism *in vitro* and *in vivo*. A further study in man showed that PQ but not CQ, inhibited antipyrine metabolism [4].

MQ is at present in an advanced state of development [5], and studies have shown that not only is it effective against CQ-resistant strains of the parasite, but it is also very effective in a single dose [6, 7]. Thus MQ is potentially very useful as a new antimalarial drug, and it is therefore important to ascertain whether it has the same inhibitory effects on microsomal drug metabolism as PQ. We have investigated the effects of MQ on drug metabolism in the rat *in vitro* and *in vivo*.

MATERIALS AND METHODS

Chemicals. Drugs and chemicals used were: Primaquine diphosphate and chlorpropamide Sigma Chemical Co. London, U.K.), [6,7-^{3}H]17 α-Ethinyloestradiol (New England Nuclear Corp., F.R.G.), tolbutamide and hydroxytolbutamide (Hoechst), aminopyrine, semicarbazide hydrochloride, and NADPH (Sigma). Ethinyloestradiol (EE_2) was a gift from Schering A.G. (Berlin, F.R.G.). Mefloquine hydrochloride was a gift from Hoffmann–LaRoche (Basel, Switzerland). All other reagents were obtained from B.D.H. (Poole, U.K.)

Animals. Adult male rats of the Wistar strain, weighing 200–350 g were housed in well ventilated cages and kept at a temperature of approximately 24°. They were allowed to feed ad lib. on pelleted food (Oxoid Breeding Diet, Oxoid Ltd., London, U.K.), and tap water.

Preparation of rat liver microsomes. Rats were killed by cervical dislocation, the livers rapidly removed and weighed, and placed in ice-cold 1.15%

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(w/v) KCl. The livers were roughly chopped and homogenised in ice-cold 1.15% KCl, using a glass homogeniser tube with a motor driven Teflon pestle. The 25% homogenate was centrifuged at 13,000 g for 20 min at 4° in an MSE Prepspin 65 ultracentrifuge. The resulting supernatant was carefully decanted without disturbing the pellet and centrifuged at 105,000 *g* for 60 min at 4°. The microsomal pellet was then resuspended in 7 ml of 1.15% KCl and centrifuged at 105,000 *g* for 60 min at 4°. The washed microsomes were resuspended in 0.2 ml M/15 phosphate buffer, pH 7.4. Microsomal protein content was determined by the method of Lowry *et al.* [8].

Microsomal incubation of aminopyrine with mefloquine. The N-demethylation of aminopyrine was carried out using the following reaction mixture: aminopyrine (2.5 mM), semicarbazide (9.37 mM), MQ (0.001–2.0 mM) or PQ (0.001–0.1 mM), microsomes (0.5 ml of 4 mg ml^{-1} suspension) and NADPH (0.6 mM). Incubation was at 37° for 10 min in an agitating water bath. Microsomal reactions were started by the addition of NADPH, and terminated by the addition of 15% zinc sulphate (2 ml). Formaldehyde production was measured with the Nash reagent [9] and absorbance determined at 415 nm. In another study the effect of two fixed concentrations of MQ (0.01 and 0.1 mM) on the kinetics of aminopyrine N-demethylation (0.25, 0.4, 0.75, 1.5 and 2.5 mM aminopyrine) was investigated.

Analysis of [^{3}H]EE_2 metabolites in microsomal incubations. The following reaction mixture was used: [^{3}H]-EE_2 (1 μCi), EE_2 (0.01 mM), ascorbic acid (1 mM), MQ (0.01, 0.1 and 0.5 mM) or PQ (0.5 mM) and NADPH (0.6 mM). Incubations were performed at 37° for 30 min in an agitating water bath. Microsomal reactions were stopped by extracting twice with ether (4 ml and 3 ml). Radiolabelled metabolites present in the ether extracts were analysed by h.p.l.c. substantially according to the method described by Maggs *et al.* [10]. A Spectra-Physics SP 8700 solvent delivery pump was used connected to an LKB 2112 Redirac fraction collector. A stainless-steel column was used packed with Partisil® 10/25 ODS-2 (25 cm × 0.46 cm i.d., Whatman Inc., Clifton, NJ). 10 μl samples were eluted at room temperature with a linear gradient of methanol in 0.5% (w/v) ammonium dihydrogen phosphate buffer, (pH 3.0), from 50 to 65% at 2%/min. This system is known to be suitable for separating oestrogens. The flow rate was 2 ml min^{-1}. The radioactive content of each sample was determined by liquid scintillation spectrometry using a Packard Tri-Carb 4640 liquid scintillation counter.

Urinary excretion of tolbutamide. Twelve rats were individually housed in metabolism cages. Four rats were controls and groups of four rats were given MQ or PQ (25 mg kg^{-1}) 30 min before tolbutamide. Tolbutamide (50 mg kg^{-1}) was injected i.p. and urine collected at 2, 4, 6, 8 and 24 hr. After measurement of the volume, aliquots were taken and stored deep frozen for analysis by h.p.l.c.

Assay of hydroxytolbutamide. Urinary hydroxytolbutamide concentrations were measured by h.p.l.c. substantially according to the method of Nation *et al.* [11]. A model 110A pump (Altex) linked to CE 2112 spectrophotometer (Cecil) monitoring at 230 nm was used. Separations were performed at room temperature on a Partisil® 10/25 ODS-2 (0.46 cm i.d. × 25 cm) protected by an in-line guard column (Whatman column survival kit) packed with Co: Pell ODS. The mobile phase used was methanol: 0.05% phosphoric acid in proportions 52:48 by volume. The flow rate was 1.5 ml min^{-1}.

Blank rat urine (50 μl) was spiked with known amounts of hydroxytolbutamide (5–120 μg). The internal standard (I.S.), chlorpropamide (20 μl of 6 mg ml^{-1} solution) was added followed by methanol (380 μl). The contents were vortexed for a few seconds and then centrifuged for 10 min at 2000 *g*; 20 μl of supernatant was injected on to the column. The ratio of the peak height of hydroxytolbutamide to I.S. was plotted against the concentration of the compound to provide a standard curve.

Samples of urine (50 μl) from rats injected with tolbutamide were pipetted into pyrex tubes (75 × 12 mm), followed by the addition of I.S. and methanol. Samples were then analysed as described above.

Statistical analysis. The Student's unpaired *t*-test was used to determine statistical significance between treatment groups and controls. Data are presented as mean ± S.D.

RESULTS

MQ and PQ both inhibited aminopyrine N-demethylase activity *in vitro* (Fig. 1). PQ was a more potent inhibitor at 0.001 and 0.01 mM with 28% and

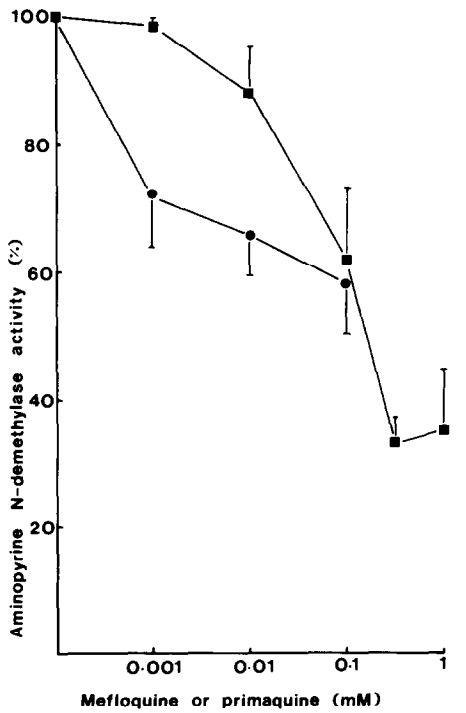

Fig. 1. Effect of mefloquine (■—■) and primaquine (●—●) on aminopyrine N-demethylase activity. The results are the mean ± S.D. of four experiments with four different rat liver microsomal preparations.

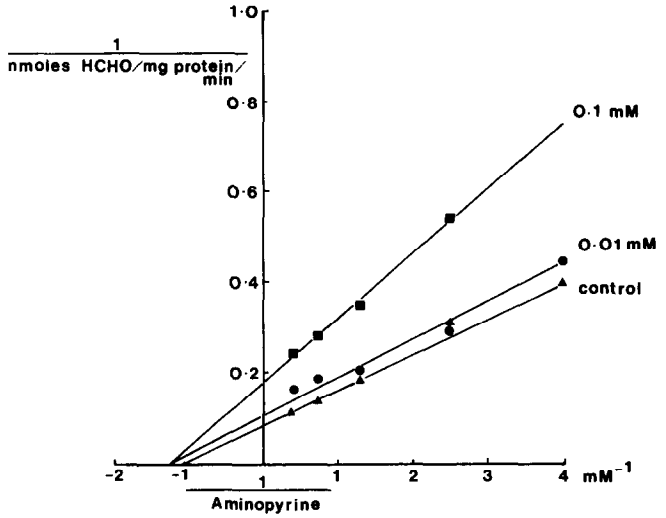

Fig. 2. Lineweaver–Burk plots for aminopyrine N-demethylation at various fixed mefloquine concentrations. Each point is the mean of 4 experiments.

34% inhibition respectively, compared to 2% and 12% inhibition at these concentrations of MQ. The concentration of inhibitor causing 50% inhibition (IC_{50}) was 0.2 mM for MQ. Due to colour interference with the assay by concentrations of PQ greater than 0.1 mM an IC_{50} value is difficult to accurately determine. However, at lower concentrations PQ resulted in greater inhibition than MQ. Previous studies [3] have shown that PQ apparently inhibits non-competitively, characterised by a decrease in V_{max} and no significant change in K_m. Kinetic studies with MQ have shown that it too appears to inhibit by non-competitive inhibition (Fig. 2).

Ether-extractable radioactivity from control microsomal incubations with EE_2 was resolved into four peaks by reversed-phase h.p.l.c.; these corresponded to unmetabolised EE_2, 2-hydroxyethinyloestradiol (2-$OHEE_2$), 16-hydroxyethinyloestradiol (16-$OHEE_2$), and a diffuse heterogeneous peak (Fig. 3). 2-$OHEE_2$ was the principal metabolite of EE_2 in microsomal incubations. MQ caused significant inhibition of EE_2 metabolism *in vitro* (Table 1). At the lower concentration of MQ (0.1 mM) the percentage of EE_2 was significantly increased. MQ (0.5 mM) showed a marked inhibition of EE_2 metabolism the percentage of 2-$OHEE_2$ being reduced from a control value of 49.3 ± 10.8 to 5.1 ± 3.1 (Fig. 3). The metabolic profile was very similar to that produced in the presence of PQ (0.5 mM) (Table 1).

After administration of tolbutamide, MQ significantly reduced excretion of the metabolite, hydroxytolbutamide, in urine. Tolbutamide was not detectable in urine. MQ particularly reduced hydroxytolbutamide excretion for the first 8 hr after administration from a control value of 54.4 ± 3.1%

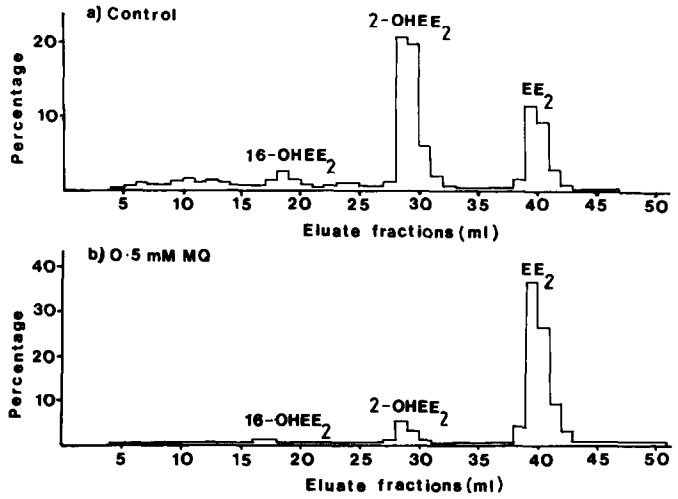

Fig. 3. Reversed-phase high performance liquid chromatograms of ether-extractable metabolites of [^{3}H]EE_2. (a) Control; (b) in the presence of 0.5 mM Mefloquine. The solid lines show profiles of radioactivity. The position of authentic unlabelled standards are indicated.

Table 1. The effect of mefloquine on ethinyloestradiol (EE_2) metabolism *in vitro*. H.p.l.c. analysis performed on ether-extractable ^3H-labelled material from microsomal incubations

	Control†	0.1 mM MQ	0.5 mM MQ	0.5 mM PQ
Heterogenous peak	4.8 ± 4.0	3.9 ± 1.3	***0	***0
16-OHEE$_2$	4.0 ± 1.8	4.1 ± 1.1	0.5 ± 0.8	0.4 ± 0.6
2-OHEE$_2$	49.3 ± 10.8	44.8 ± 3.1	***5.1 ± 3.1	***1.5 ± 0.4
EE$_2$	19.6 ± 4.5	***33.3 ± 1.7	***83.0 ± 3.2	***86.3 ± 4.6

Results are Mean ± S.D. of 4 experiments in each group.
*** $P \leq 0.001$, significantly different from controls.
† Control incubations contained DMSO.

to 9.3 ± 3.4%. PQ only reduced excretion of hydroxytolbutamide from 54.4 ± 3.1% to 32.2 ± 14.1% over the same period (Fig. 4).

DISCUSSION

The metabolism of mefloquine has been studied in several species [5, 7, 12, 13]. Five metabolites have been isolated and the structure of two identified as 2,8-bis-trifluoromethyl-quinolone-4-methanol and a carboxylic metabolite [5]. Following administration of MQ, the ratio of the carboxy metabolite to MQ was high in mice, low in rats, and intermediate in man [5]. Elimination of MQ is reported to be slow with the faeces being the main route of excretion; there is also evidence of significant enterohepatic recirculation of MQ and/or its metabolites [7].

The present study has shown that MQ inhibits hepatic microsomal enzymes both *in vitro* and *in vivo*. In comparison with PQ, MQ does not appear to be more potent *in vitro* but is *in vivo*. It should be remembered that inhibition *in vivo* will be a reflection not only of binding to the enzyme but also of actual concentrations of drug present in the liver and more particularly in the vicinity of the enzyme. Insufficient information is available on the pharmacokinetics of PQ and MQ in the rat to compare elimination characteristics.

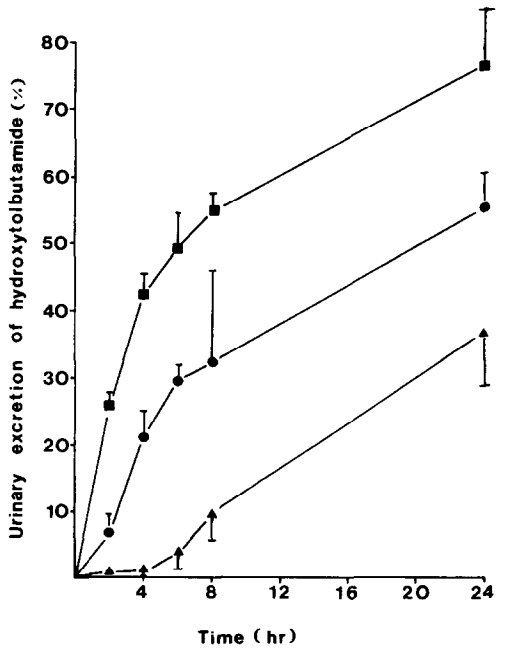

Fig. 4. The cumulative urinary excretion of hydroxytolbutamide in control rats (■—■), and rats given either primaquine (●—●) or mefloquine (▲—▲). For details see text. Values are mean ± S.D. of 4 rats in each group.

The range of substrates chosen in this study to assess the inhibitory effects of the two antimalarials would seem to indicate a fairly widespread and non-selective effect on cytochrome P-450(s). Ethinyloestradiol (EE_2), the oestrogenic component of most combined oral contraceptives, undergoes extensive hydroxylation in the rat and the principal metabolite is 2-hydroxy EE_2; formation of this metabolite is by a P-450-dependent enzyme [14, 15]. It has however, been suggested that steroids are oxidised by enzymes which are distinct from those responsible for the oxidation of drugs [16–18]. Therefore, the inhibition of metabolism of the steroid EE_2, and also aminopyrine and tolbutamide by MQ and PQ suggests that these drugs inhibit more than one microsomal enzyme system.

Since we know that PQ inhibits drug metabolism in man [4] it would seem probable, by extrapolation of the present results, that MQ will produce similar effects.

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REFERENCES

1. M. W. Davidson, B. G. Griggs, D. W. Boykin and W. D. Wilson, *Nature, Lond.* **254**, 632 (1975).
2. R. E. Howells, *Br. Med. Bull.* **38**, 193 (1982).
3. D. J. Back, H. S. Purba, C. Staiger, M. L'E Orme and A. M. Breckenridge, *Biochem. Pharmac.* **32**, 257 (1983).
4. D. J. Back, H. S. Purba, B. K. Park, S. A. Ward and M. L'E Orme, *Br. J. clin. Pharmac.* **16**, 497 (1983).
5. *Bull. WHO.* **61**, 169 (1983).
6. R. E. Desjardins, C. J. Canfield, J. D. Haynes and J. D. Chulay, *Antimicrob. Ag. Chemother.* **16**, 710 (1979).
7. J. Y. Mu, Z. H. Israeli and P. G. Dayton, *Drug. Metab. Dispos.* **3**, 198 (1975).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).

9. T. Nash, *Biochem. J.* **55**, 416 (1953).
10. J. L. Maggs, P. S. Grabowski, M. E. Rose and B. K. Park, *Xenobiotica* **12**, 657 (1982).
11. R. L. Nation, G. W. Peng and W. L. Chiou, *J. Chromatogr.* **146**, 121 (1978).
12. R. S. Rozman, N. A. Molek and R. Koby, *Drug Metab. Dispos.* **6**, 654 (1978).
13. R. Von Jauch, E. Greisser and G. Osterhelt, *Arzneim-Forsch (Drug Res.)* **30**, 60 (1980).
14. H. M. Bolt, H. Kappus and H. Remmer, *Xenobiotica* **3**, 773 (1973).
15. K. T. Shiverick and M. Notelovitz, *Biochem. Pharmac.* **32**, 2399 (1983).
16. H. M. Bolt and H. Kassel, *Xenobiotica* **6**, 33 (1976).
17. D. D. Breimer, *Clin. Pharmacokin.* **8**, 371 (1983).
18. P. Jenner, B. Testa and F. J. Di Carlo, in *Drug Metabolism and Disposition*, pp. 12–21. Elsevier, New York (1983).