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# INHIBITION OF DRUG METABOLISM BY THE ANTIMALARIAL DRUGS CHLOROQUINE AND PRIMAQUINE IN THE RAT

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**Abstract**—The effect of the antimalarial drugs chloroquine (CQ) and primaquine (PQ) on rat liver microsomal drug metabolism has been studied *in vitro* and *in vivo*. After acute administration, PQ increased pentobarbitone sleeping time in a dose-related manner [control, 94.0 ± 9.4 min; 10 mg/kg, 137.0 ± 2.4 min; 20 mg/kg, 197.0 ± 7.5 min; 50 mg/kg, 269.0 ± 2.9 min (means ± S.E.M.)], prolonged zoxazolamine paralysis time (control, 140.0 ± 10.0 min; 50 mg/kg, 341.5 ± 25.6 min) and decreased antipyrine blood clearance from 2.17 ± 0.19 to 0.86 ± 0.12 ml/min. CQ showed no effect on pentobarbitone sleeping time or zoxazolamine paralysis time, but decreased antipyrine clearance from 2.17 ± 0.19 to 1.11 ± 0.18 ml/min. Both drugs inhibited aminopyrine *N*-demethylase activity, although the concentration required to produce 50% inhibition was much greater for CQ (10 mM) than for PQ (approximately 0.1 mM). Lineweaver–Burk plots showed that CQ inhibited competitively whereas PQ inhibition was apparently non-competitive. Ethoxyresorufin *O*-deethylase activity decreased by about 40 and 50% in the presence of CQ and PQ respectively (250 nM, equimolar with substrate). There was no evidence of induction following chronic administration of CQ and PQ (50 mg/kg/day for 4 days). There was an apparent decrease in cytochrome P-450 content and aminopyrine *N*-demethylase activity was decreased. These results demonstrate that PQ and CQ inhibit hepatic drug metabolism both *in vitro* and *in vivo* and that PQ appears to be the more potent inhibitor.

Chloroquine (CQ) [7-chloro-4-(diethylamino-1-methylamino)-quinolone] is the most widely used drug in the treatment of the asexual erythrocytic form of the plasmodial life-cycle, whereas primaquine (PQ) [8-(4-amino-1-methylbutylamino)-6-methoxy-quinolone] eliminates the exoerythrocytic forms. In animal studies, CQ has been reported to inhibit the activity of imidazole-*N*-methyltransferase, alcohol dehydrogenase, succinic dehydrogenase, *p*-nitroanisole *O*-demethylase and glucuronyl transferase [1–4] and PQ to inhibit the metabolism of CQ [5]. In man, there is some evidence to suggest that CQ reduces the clearance of the pesticides DDT and DDE [6]. We have investigated in detail the effects of CQ and PQ on drug metabolism in the rat *in vitro* and *in vivo*.

## MATERIALS AND METHODS

*Chemicals.* Drugs and chemicals used were: CQ diphosphate and PQ diphosphate (Sigma), [*N*-methyl-$^{14}$C]antipyrine (Radiochemical Centre, Amersham, U.K.), pentobarbitone sodium ['Sagatal' (May & Baker, Dagenham, U.K.)], zoxazolamine, aminopyrine, semicarbazide hydrochloride, NADPH (Sigma) and ethoxyresorufin (ERR) (Pierce). Resorufin was a gift from ICI. All other reagents were obtained from BDH Ltd.

*Animals.* Adult male rats of the Wistar strain, weighing 200–400 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.

*Pentobarbitone sleeping time.* Groups of five rats were injected intraperitoneally (i.p.) with pentobarbitone sodium (40 mg/kg, 20 mg/ml) 30 min after a single i.p. injection of CQ or PQ (10, 20 or 50 mg/kg in 0.9% saline) or saline (control). In chronic studies, groups of five rats were given i.p. injections of CQ or PQ (10, 20 or 50 mg/kg/day) or saline (controls) in divided doses for 4 days. Pentobarbitone sleeping time was determined on the sixth day. The sleeping time was defined as the time taken between loss and return of the righting reflex.

*Zoxazolamine paralysis time.* Rats were injected i.p. with zoxazolamine (60 mg/kg, 30 mg/ml) 30 min after a single i.p injection of CQ or PQ (50 mg/kg, 25 mg/ml). In chronic dosing studies, rats were given i.p injections of CQ or PQ (50 mg/kg, 25 mg/ml) or saline (controls) in divided doses for 4 days. Zoxazolamine paralysis time was determined on the sixth day. The paralysis time was defined as the time taken between loss and return of the righting reflex and movement of the limbs.

*Elimination of* [$^{14}$C]*antipyrine.* Rats were anaesthetised with pentobarbitone sodium (60 mg/kg, 0.1 ml/100 g.b.w.). The carotid artery and femoral

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vein were cannulated using polyethylene tubing (PE 50). Heparinised saline (200 units) was injected to prevent coagulation of the blood samples collected from the carotid artery.

CQ or PQ (50 mg/kg, 25 mg/ml) was injected i.p 30 min before the administration of [$^{14}$C]antipyrine (15 mg/kg, 15 mg/ml, 5 $\mu$Ci/ml, 0.1 ml/100 g.b.w.) via the femoral vein. Blood samples were collected at 0, 10, 30, 60, 90, 120, 150 and 180 min. 0.3 ml of saline was returned to the blood system via the femoral vein to compensate for the blood sample taken. The concentration of [$^{14}$C]antipyrine in whole blood was measured by the method of Bakke *et al.* [7] which is based on a simple solvent extraction procedure at an alkaline pH.

In vitro *studies.* Rats were killed by cervical dislocation, the livers rapidly removed and homogenised in ice-cold M/15 phosphate buffer (pH 7.4) containing 0.15 M KCl using a Teflon in glass homogeniser. The 25% homogenate was centrifuged at 13,000 *g* for 20 min at 4°. The resulting supernatant was decanted without disturbing the pellet and centrifuged at 105,000 *g* for 60 min at 4°. The microsomal pellet was resuspended in 0.2 ml phosphate buffer. Microsomal protein and cytochrome P-450 were determined respectively by the methods of Lowry *et al.* [8] and Omura and Sato [9]. The *N*-demethylation of aminopyrine was carried out with the following reaction mixture: aminopyrine (0.75–2.5 mM), semicarbazide (9.37 mM), CQ or PQ (0.001–10 mM), microsomes (0.5 ml of 4 mg/ml suspension) and NADPH (0.6 mM). Formaldehyde production was measured with the Nash reagent [10] and absorbance determined at 415 nm. In some studies the effect of two fixed concentrations of CQ (3 and 10 mM) and PQ (0.01 and 0.1 mM) on the kinetics of aminopyrine *N*-demethylation (0.25, 0.75, 1.5 and 2.5 mM aminopyrine) was investigated.

ERR *O*-deethylase activity was determined by the method of Burke and Meyer [11]. Rats were pretreated with $\beta$-napthoflavone (BNF) (75 mg/kg/3 days). The incubation mixture contained ERR (250 nM), microsomal protein (0.01–0.02 mg/ml) and NADPH (0.25 mM) in 0.1 M phosphate buffer (pH 7.8). The reaction was monitored in a cuvette for 3 min at 30°.

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

## RESULTS

Acute administration of PQ prolonged pentobarbitone-induced sleeping time (Fig. 1) in a dose-related manner [control, 94.0 ± 9.4 min; 10 mg/kg, 137.0 ± 2.4 min; 20 mg/kg, 197.0 ± 7.5 min; 50 mg/kg, 269 ± 2.9 min (means ± S.E.M.)] and prolonged zoxazolamine paralysis time (Fig. 2) (control, 140.0 ± 10.0 min; 50 mg/kg, 341.5 ± 25.6 min). In contrast, acute administration of CQ had no effect on either pentobarbitone sleeping time or zoxazolamine paralysis time.

Both PQ and CQ (50 mg/kg) caused significant changes in the pharmacokinetics of antipyrine (Table 1). Following PQ pretreatment, the antipyrine half-life was increased from 100.8 ± 12.9 (control, mean ± S.E.M.) to 190.3 ± 14.4 min, blood clearance ($Cl_B$) was decreased from 2.17 ± 0.19 to 0.86 ± 0.12 ml/min and there was a small change in the volume of distribution (Vd). Comparable changes in both the half-life (107% increase) and clearance (49% decrease) were seen following CQ administration. There was no change in the Vd.

Both drugs inhibited aminopyrine *N*-demethylase activity *in vitro* (Fig. 3). PQ was a more potent inhibitor with 40% inhibition at 0.1 mM compared to 10% inhibition at this concentration of CQ. The

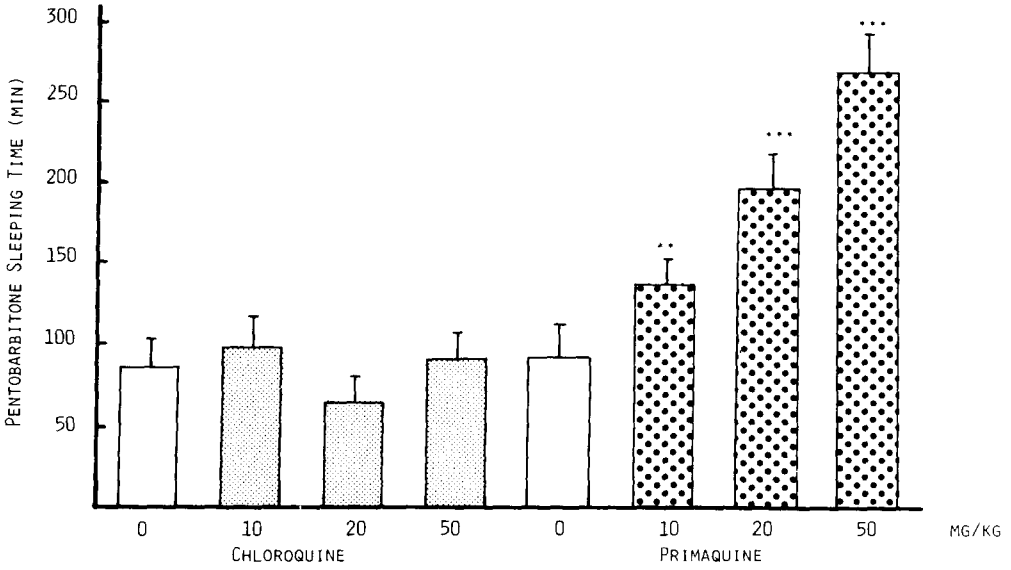


Fig. 1. Effect of CQ and PQ on pentobarbitone sleeping time. Pentobarbitone sodium (40 mg/kg) was injected i.p. 30 min after the i.p. injection of CQ or PQ. Values are means ± S.E.M. of five rats. $^{**}P < 0.01$ and $^{***}P < 0.001$.

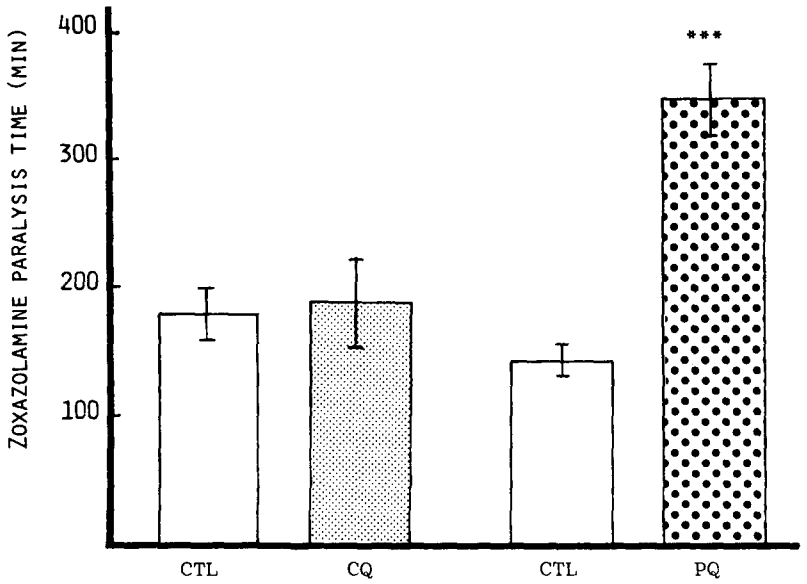


Fig. 2. Effect of CQ and PQ on zoxazolamine paralysis time. Zoxazolamine (60 mg/kg) was injected i.p. 30 min after the i.p. injection of CQ or PQ. Values are means ± S.E.M. of five rats. ***$P < 0.001$.

Table 1. The effect of CQ and PQ on antipyrine pharmacokinetics

| Pharmacokinetic parameter | Saline | CQ | PQ |
|---|---|---|---|
| $t_{1/2}$ (min) | 100.8 ± 12.9 | 208.2 ± 19.5** | 190.3 ± 14.4** |
| Vd (ml) | 302.2 ± 20.6 | 313.0 ± 22.5 | 238.8 ± 21.8* |
| $Cl_B$ (ml/min) | 2.17 ± 0.19 | 1.11 ± 0.18** | 0.86 ± 0.12*** |

[$^{14}$C]Antipyrine (15 mg/kg, 5 $\mu$Ci/kg) was injected i.v. 30 min after the i.p. injection of CQ or PQ (50 mg/kg). Values are means ± S.E.M. of five rats.
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ (significantly different from controls).

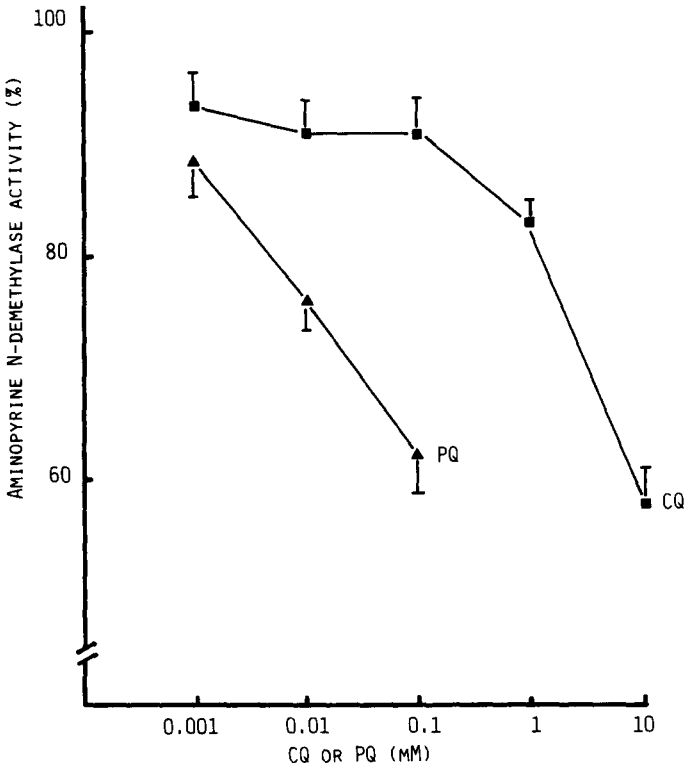


Fig. 3. Effect of CQ and PQ on aminopyrine *N*-demethylase activity. The results are the means ± S.E.M. of four experiments with four different rat liver microsomal preparations.

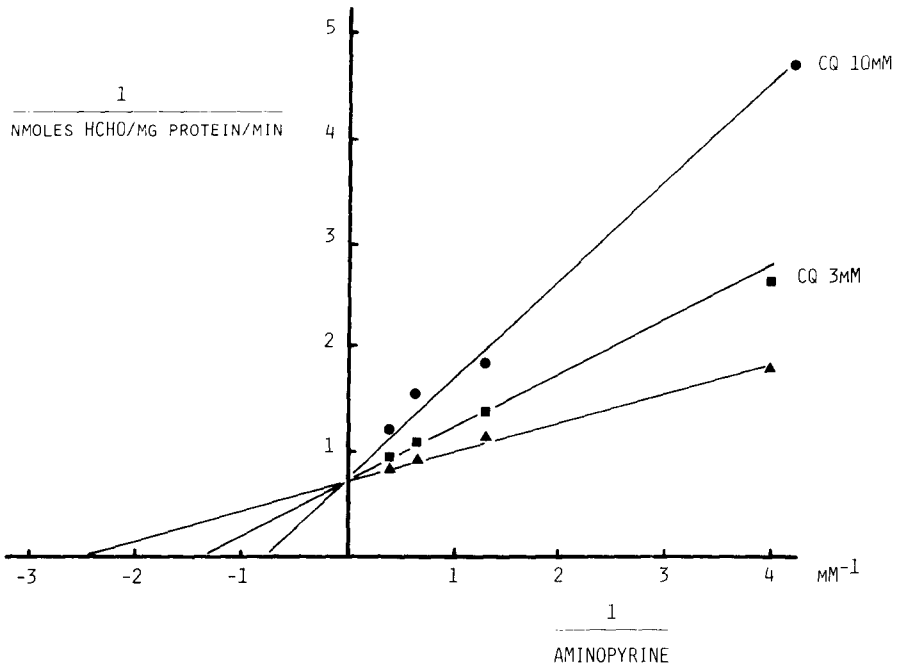


Fig. 4. Lineweaver–Burk plots for aminopyrine *N*-demethylation at various fixed CQ concentrations. Each point is the mean of four experiments.

concentration of inhibitor causing 50% inhibition ($IC_{50}$) was difficult to accurately assess for PQ because of colour interference with the assay (PQ in solution is coloured), but approximated to 0.1 mM. In contrast, the $IC_{50}$ for CQ was approximately 10 mM. These results indicated a different mechanism of inhibition of aminopyrine *N*-demethylase. This was further investigated in kinetic studies. Lineweaver–Burk plots (Figs 4 and 5) gave evidence that CQ exhibited competitive inhibition (no change in $V_{max}$, increase in $K_m$) at both concentrations studied (3 and 10 mM), whereas PQ apparently inhibited non-competitively (decrease in $V_{max}$, no significant change in $K_m$). Figure 6 shows the effect of PQ and CQ on ERR *O*-deethylase activity in BNF-treated rats. BNF treatment caused a marked increase in activity from 11.54 ± 1.4 pmoles/min/mg protein (controls) to 3.14 ± 0.21 nmoles/min/mg protein (BNF-treated). ERR *O*-deethylase activity decreased by 40 and 50% in the presence of CQ and PQ (250 nM, i.e. equimolar with substrate) respectively. Inhibition by PQ was consistently greater than CQ over a 100-fold range of inhibitor concentrations.

Chronic treatment of rats with either PQ or CQ

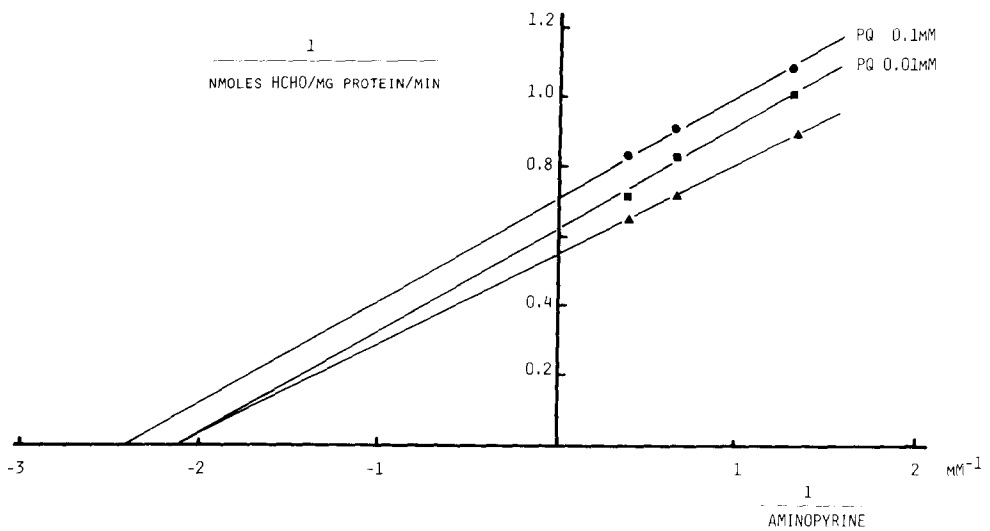


Fig. 5. Lineweaver–Burk plots for aminopyrine *N*-demethylation at various fixed PQ concentrations. Each point is the mean of four experiments.

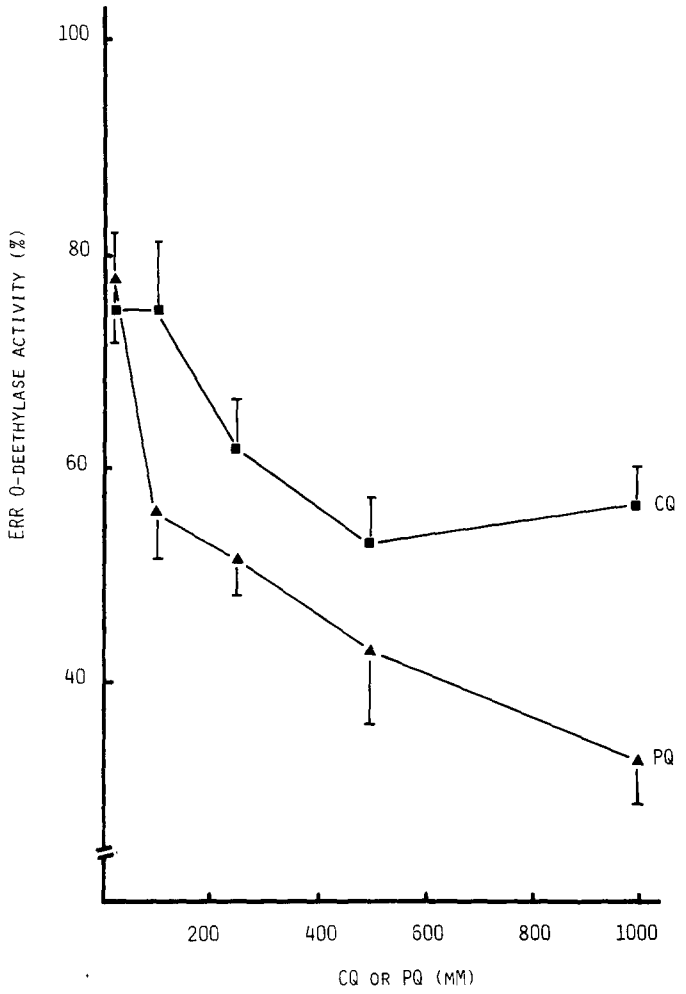


Fig. 6. Effect of CQ and PQ on ERR *O*-deethylase activity. The results are the means ± S.E.M. of experiments with four different rat liver microsomal preparations.

Table 2. The effect of CQ and PQ (50 mg/kg/day for 4 days) on hepatic enzyme activity

| | Saline | CQ | PQ |
|---|---|---|---|
| Microsomal protein (mg/g liver) | 15.03 ± 0.21 | 15.38 ± 0.41 | 15.66 ± 0.78 |
| Cytochrome P-450 (nmoles/mg protein) | 0.95 ± 0.03 | 0.71 ± 0.04** | 0.62 ± 0.06*** |
| Aminopyrine *N*-demethylase activity (nmoles/min/mg protein) | 6.80 ± 0.32 | 4.55 ± 0.45** | 4.10 ± 0.41** |
| ERR *O*-deethylase activity (pmoles/min/mg protein) | 11.54 ± 1.40 | 14.89 ± 1.72 | 9.49 ± 0.96 |
| Pentobarbitone sleeping time (min) | 95.8 ± 15.2 | 90.8 ± 3.9 | 117.3 ± 15.7 |
| Zoxazolamine paralysis time (min) | 185.0 ± 15.1 | 255.8 ± 17.7** | 218.8 ± 28.5 |

Values are given as the means ± S.E.M. of four experiments.
**$P < 0.01$ and ***$P < 0.001$ (significantly different from saline controls).

(50 mg/kg/day for 4 days) gave no change in the pentobarbitone-induced sleeping time compared to a control group (Table 2). Similarly, there was no significant difference in the zoxazolamine-induced paralysis time following PQ administration, although CQ caused an increase from 185.0 ± 15.1 to 255.8 ± 17.7 min (Table 2).

Table 2 further shows the effect of chronic drug treatment on hepatic enzyme parameters. Cytochrome P-450 content was decreased by CQ (by 25%) and PQ (by 35%) although there was no change in microsomal protein. Aminopyrine *N*-demethylase activity was decreased from a control value of 6.80 ± 0.32 to 4.55 ± 0.45 (CQ) and 4.10 ± 0.41 (PQ) nmoles/min/mg protein. There was no significant difference in ERR *O*-deethylase activity.

## DISCUSSION

The present results show that CQ and PQ inhibit hepatic microsomal drug metabolism although there are clearly defined differences between the two compounds in their inhibitory activity. Following a single dose, PQ prolonged pentobarbitone sleeping time and zoxazolamine paralysis time although CQ was without effect. Both drugs inhibited antipyrine metabolism *in vivo* and aminopyrine and ERR metabolism *in vitro*. These findings therefore confirm the somewhat sporadic reports in the literature of enzyme inhibition by CQ and PQ both *in vitro* [1–4] and *in vivo* [5, 6].

Similar results have previously been obtained with ethoxyquin (EQ) (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinolone) by Parke *et al.* [12] and Kahl and Netter [13]; a single dose of EQ causing inhibition of hexobarbital metabolism *in vivo* and ethylmorphine *N*-demethylation and biphenyl 1–4 hydroxylation *in vitro* [12]. It was shown that the inhibitory action of EQ was preferentially directed towards the cytochrome P-450 mediated reactions induced by phenobarbitone while P-448 monooxygenases were less sensitive to EQ [13].

Another related compound, oxine (8-hydroxyquinolone) strongly inhibits microsomal cytochrome P-450 reductase activity [14]. Similarly, oxine 5-sulfonic acid inhibited rabbit liver aminopyrine *N*-demethylase activity in a dose-dependent manner [15].

Inhibition of both pentobarbitone sleeping time and zoxazolamine paralysis time by PQ implies an effect on both P-450 and P-448 monooxygenases which was further confirmed by inhibition of aminopyrine *N*-demethylation and ERR *O*-deethylation. Similarly CQ showed inhibitory activity against both substrates *in vitro*.

The prolongation of the elimination half-life and the decreased $Cl_B$ of antipyrine by PQ is in accordance with all the findings both *in vitro* and *in vivo*. The small decrease in the Vd is difficult to explain but is similar to that previously reported to be produced by cimetidine [16]. Despite the apparent lack of an effect of CQ on pentobarbitone and zoxazolamine metabolism there was a significant increase in the antipyrine half-life and a decrease in $Cl_B$. Whether or not different enzymes are involved in the metabolism of pentobarbitone and antipyrine is not known (although both P-448- and P-450-dependent enzymes are involved in the metabolism of the latter [17]). The affinity of the substrate for the enzyme may also be important, particularly since in the pharmacokinetic study three drugs were present, i.e. pentobarbitone (anaesthetic, 60 mg/kg), antipyrine (15 mg/kg) and CQ (50 mg/kg).

PQ is a much more potent inhibitor of aminopyrine *N*-demethylation than CQ. There was an approximately 100-fold difference in inhibitor concentration required to produce 50% inhibition (PQ, 0.1 mM; CQ, 10 mM). The formal mechanism of inhibition by CQ was found to be competitive; the PQ data although more difficult to interpret gave evidence of non-competitive inhibition. The exact mechanisms of inhibition are clearly unresolved and await further study.

Again, PQ is a more potent inhibitor of ERR *O*-deethylase activity, although the difference in concentration producing a similar inhibition of enzyme activity was not so great as for aminopyrine *N*-demethylation.

The reason for the difference in the degree of enzyme inhibition produced by the two aminoquinolones is not clear. The most probable explanation is a structure–activity phenomenon similar to that which has been so well highlighted for the imidazole group of compounds [18–21].

In a previous study [22] it was shown that the fungistatic drug clotrimazole inhibited enzyme activity *in vitro* but when administered for 3 days *in vivo* induced hepatic microsomal cytochrome P-450 content, monooxygenase activity and epoxide hydratase activity. It was therefore concluded that during continuous clotrimazole administration both inhibition and induction of monooxygenase activity can occur. However, we found no evidence of induction following 4 days administration (study on the sixth day) of PQ and CQ. In fact, there was an apparent decrease in P-450 content and aminopyrine *N*-demethylase activity.

We conclude that the antimalarial drugs PQ and CQ inhibit drug metabolism both *in vitro* and *in vivo* and that such inhibition may lead to pharmacokinetic interactions *in vivo*.

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