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EFFECT OF CHLOROQUINE AND PRIMAQUINE ON ANTIPYRINE METABOLISM

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1 The effects of two antimalarial drugs, chloroquine and primaquine on antipyrine kinetics and metabolism have been studied in volunteers.

2 Chloroquine (250 mg) given 2 h before antipyrine (600 mg orally) had no effect on salivary kinetics of antipyrine or on the urinary recovery of metabolites. Primaquine (45 mg) given 2 h before antipyrine (300 mg orally), increased antipyrine half-life (calculated from 0–24 h) from 12.7 ± 3.2 (mean ± s.d.) to 25.3 ± 3.9 h and decreased clearance from 3.01 ± 0.67 to 1.32 ± 0.32 l h^{-1}. There was no change in the apparent volume of distribution. Antipyrine half life changed with time in the presence of primaquine and when calculated between 24 and 48 h had returned to control.

3 After primaquine, the metabolic clearance (calculated from 0–24 h) of antipyrine to its three main metabolites, 3-hydroxymethylantipyrine, 4-hydroxyantipyrine and norantipyrine was significantly reduced. There was no selective effect on a particular metabolic pathway.

4 There was no change in 6β-hydroxycortisol excretion (expressed as a ratio of total 17-hydroxycorticosteroids) in the period 0–48 h following primaquine administration.

5 The inhibition of hepatic metabolism by primaquine but not the structurally related chloroquine may be an example of a structure activity phenomenon and could be of clinical significance.

Keywords chloroquine primaquine antipyrine pharmacokinetics

Introduction

We have previously shown (Back *et al.*, 1983) that the antimalarial drugs primaquine (PQ) and chloroquine (CQ) inhibit hepatic drug metabolism both *in vitro* and *in vivo* in rats, and PQ is the more potent inhibitor. We have extended this study by comparing the effects of these drugs on antipyrine kinetics and the clearance of antipyrine to its three main metabolites (3-hydroxymethylantipyrine, 4-hydroxyantipyrine and norantipyrine) in volunteers.

Methods

The subjects studied were six healthy males (aged 23–35; weight, 62–70 kg). They were all non-smokers and were taking no other drugs. The study was approved by the Mersey Regional Hospital Ethical Committee and the nature of the study explained to each subject.

Experimental design:

There were two studies.

In study 1, six subjects received antipyrine (600 mg) by mouth on two occasions separated by a 1 week interval. Two hours before the second dose, each subject took CQ phosphate (250 mg) orally.

In study 2, five subjects received antipyrine (300 mg) by mouth on two occasions separated by a 1 week interval. Two hours before the first dose, each subject took PQ (45 mg) orally. Since the dose of PQ was much less than that of CQ (in study 1), the dose of antipyrine was reduced by half in this study. Danhof *et al.* (1979a) have previously shown the kinetics of antipyrine to be independent of dose.

Antipyrine capsules were prepared by the Pharmacy of the Royal Liverpool Hospital.

Saliva and urine sampling

Saliva samples were taken immediately before and at 2, 4, 6, 8, 12, 24, 30 and 48 h after antipyrine. Urine was collected from 0–24 and 24–48 h and into containers with sodium metabisulphite (about 1 mg ml^{-1} final concentration) added to stabilize antipyrine metabolites.

Assay of antipyrine and its metabolites

Saliva antipyrine was measured by high performance liquid chromatography (h.p.l.c.) substantially according to the method of Danhof *et al.* (1979b). A reverse-phase column (Lichrosorb RP-2; Alltech, Carnforth, England) and a mobile phase of meth-

anol: phosphate buffer (0.05 M, pH 6.5) 40:60 was used. The coefficient of variation of the assay was 7.8% at a concentration of 11.3 $\mu g\ ml^{-1}$. Urinary antipyrine and its metabolites were measured by the method of Danhof *et al.* (1979b). The coefficients of variation for the individual metabolites were: antipyrine, 5.6% at 4.3 $\mu g\ ml^{-1}$; 3-hydroxymethylantipyrine, 8.9% at 15.1 $\mu g\ ml^{-1}$; 4-hydroxyantipyrine, 4.2% at 69.1 $\mu g\ ml^{-1}$, and norantipyrine 10.4% at 26.6 $\mu g\ ml^{-1}$.

Antipyrine was obtained from BDH Chemicals Ltd, Poole. 4-hydroxyantipyrine and norantipyrine were a gift from Dr D. McKillip, ICI, Alderley Edge and 3-hydroxymethylantipyrine a gift from Professor D. Breimer, Leiden, Netherlands.

Conjugated metabolites were hydrolysed with β-glucuronidase/sulphatase (*Helix pomatia*, Type H-1, Sigma; 184 units ml^{-1} urine).

Assay of primaquine

The concentration of primaquine in saliva was measured by h.p.l.c. (Ward *et al.*, 1983). Saliva (0.5–1 ml) was made alkaline with 880 ammonia (2 ml) and extracted twice with a mixture of hexane: ethylacetate (9:1; v/v; 5 ml). The combined organic phases were evaporated to dryness under nitrogen and the residues reconstituted in methanol (50 μl).

Chromatography was carried out using a reverse phase column (Partisil 10 ODS III; H.p.l.c. Technology, Macclesfield). The mobile phase consisted of water, acetonitrile and methanol (60:30:10; v/v/v) containing 0.5 mM octane sulphonic acid, buffered to pH 3.5 with orthophosphoric acid at a flow rate of 1.5 $ml\ min^{-1}$.

Assay of 6β-hydroxycortisol

Urinary 6β-hydroxycortisol (6 βOHC) was measured by radioimmunoassay (Park, 1978) and urinary 17-hydroxycorticosteroids (17-OHCS) by the colorimetric assay of Sanghvi *et al.* (1973).

Pharmacokinetic analysis

Antipyrine half-life was calculated from the elimination rate constant obtained by least squares regression analysis of saliva drug concentrations. The apparent volume of distribution (V_d) was calculated by dividing the dose by the saliva concentration at zero time.

Antipyrine clearance (CL) was calculated from the dose divided by the area under the saliva drug concentration-time curve (AUC) extrapolated to infinity.

The clearance of antipyrine associated with the formation of a metabolite (CL_m) was calculated from

$$CL_m = CL \times \%\ \text{dose m} \times 10^{-2}$$

where CL is the total saliva antipyrine clearance and % dose m, the amounts of individual metabolites excreted in urine over 0–48 h as a percentage of the dose.

Statistical analysis

For statistical analysis a paired Student's *t*-test was used.

Results

CQ had no significant effect on antipyrine saliva kinetics (Table 1) or on the urinary recovery of metabolites. In contrast, PQ produced significant changes in antipyrine elimination half-life (0–24 h) and clearance (Table 2). The half-life was increased from 12.7 ± 3.2 to 25.2 ± 3.9 h (mean ± s.d.), and clearance decreased from 3.01 ± 0.67 to 1.32 ± 0.32 $l.h^{-1}$; there was no change in its apparent volume of distribution. With reference to Figure 1, it is evident that the kinetics of antipyrine change with time following PQ and thus although the half-life was increased when calculated for 0–24 h, it was virtually the same as the control (12.7 ± 3.2 h) when calculated over the period 24–48 h.

The mean concentrations of PQ in saliva are shown

Table 1 The effect of chloroquine (CQ; 250 mg) on saliva pharmacokinetic parameters of antipyrine (600 mg; 0–24 h).

Subject	$t_{½}$ *(h)*		*CL (l h^{-1})*		V_d *(l)*	
	Control	*CQ*	*Control*	*CQ*	*Control*	*CQ*
1	14.2	14.3	1.35	1.52	27.7	31.4
2	17.3	15.0	1.77	1.77	44.3	38.5
3	7.9	7.9	3.92	2.83	44.5	32.2
4	11.0	15.4	2.85	2.50	45.5	55.4
5	9.5	9.2	2.98	2.85	40.8	37.9
6	15.2	8.7	1.63	2.55	35.9	32.0
Mean	12.5	11.7	2.42	2.34	39.8	37.9
s.d.	3.6	3.5	0.99	0.56	6.9	9.1

Table 2 The effect of primaquine (PQ; 45 mg) on saliva pharmacokinetic parameters of antipyrine (300 mg; 0–24 h).

Subject	$t_{½}$ *(h)*		*CL (l h^{-1})*		V_d *(l)*	
	Control	*PQ*	*Control*	*PQ*	*Control*	*PQ*
1	12.9	24.4	2.56	1.11	47.0	40.5
2	15.8	31.8	2.55	1.04	57.9	49.0
3	15.9	21.3	2.97	1.79	68.0	54.3
4	8.8	23.6	4.16	1.53	52.4	52.2
5	10.1	25.3	2.82	1.13	41.2	41.3
Mean	12.7	25.3***	3.01	1.32***	53.3	47.5
s.d.	3.2	3.9	0.67	0.32	10.3	6.3

***$P < 0.001$; significantly different from control

in Table 3. The mean peak concentration (8.45 ± 5.17 ng ml^{-1}) was seen at 4 h (range 2–8 h) post administration which was equivalent to 2 h post antipyrine administration (since antipyrine was given 2 h after PQ). PQ half-life was 4.8 ± 1.6 h indicating rapid elimination. By 24 h, less than 5% of the dose would be present in the body.

The metabolic clearance of antipyrine to the three major metabolites (3-hydroxymethylantipyrine, 4-hydroxyantipyrine and norantipyrine) was not altered by CQ pretreatment (Figure 2). However, after PQ administration the clearance to all three metabolites was markedly impaired (Figure 3) as follows:— 3-hydroxymethylantipyrine (control, 0.36 ± 0.05; PQ, 0.13 ± 0.04 l h^{-1}; mean ± s.d.), 4-hydroxyantipyrine (control, 0.91 ± 0.33; PQ, 0.27 ± 0.10) and norantipyrine (control, 0.43 ± 0.18; PQ, 0.19 ± 0.07). In this study, the mean percentage of the dose recovered in urine as unchanged drug and metabolites from 0–24 h was 44.0 ± 5.7 in the control study and 27.5 ± 3.4 (mean ± s.d.) following PQ (Figure 4). The difference was highly significant ($P < 0.001$). From 0–48 h, the mean percentage recovered was 58.6 ± 4.1 in the control study and 50.4 ± 8.6 following PQ ($P < 0.05$).

There were significant differences in recovery of 3-hydroxymethylantipyrine ($P < 0.01$), 4-hydroxyantipyrine ($P < 0.001$) and norantipyrine ($P < 0.05$) at 24 h, whereas at 48 h only 4-hydroxyantipyrine was significantly different from control ($P < 0.02$). Antipyrine was significantly higher in 0–24 h ($P < 0.01$) and 0–48 h ($P < 0.02$) urine following PQ.

There was no correlation between the percentage decrease in antipyrine clearance following PQ (range 40.4–63.2%) and the initial value of clearance.

There was no significant change in 6β-OHC excretion when expressed as a ratio of total 17-OHCS (Table 4).

Table 3 Saliva primaquine concentrations (ng ml^{-1}) and half-life after oral administration of 45 mg to five subjects. Results are mean ± s.d.

Time (h)		*Concentration (ng ml^{-1})*
2		7.66 ± 5.91
4		8.45 ± 5.17
6		7.24 ± 3.33
8		5.60 ± 2.95
10		3.50 ± 3.67
14		1.92 ± 1.80
26		n.d.
	Half-life	4.84 ± 1.57

n.d. Not detectable

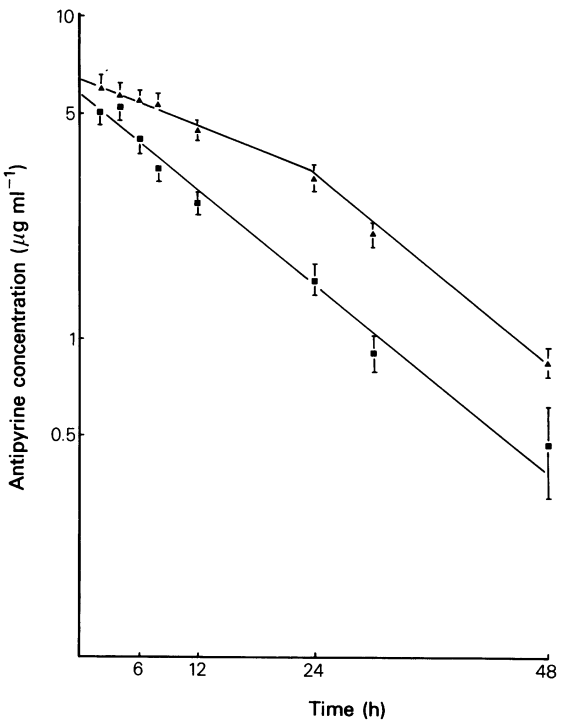

Figure 1 Saliva concentrations of antipyrine following oral administration of 300 mg. ■ — ■, control; ▲—▲, 2 h after primaquine (45 mg). Although following primaquine the concentration-time profile is apparently curvilinear, for ease of presentation kinetic parameters have been calculated for 0–24 and 24–48 h; hence the break in the curve at 24 h.

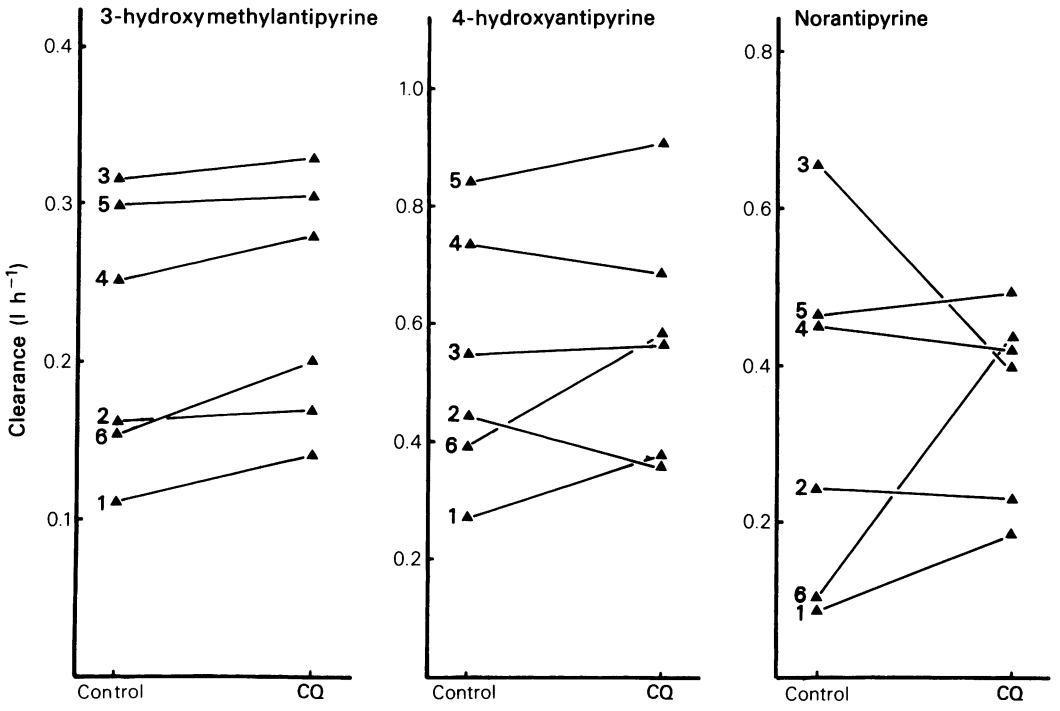

Figure 2 The effect of chloroquine (CQ) on the clearance of antipyrine to metabolites. Subject numbers are shown by the control values.

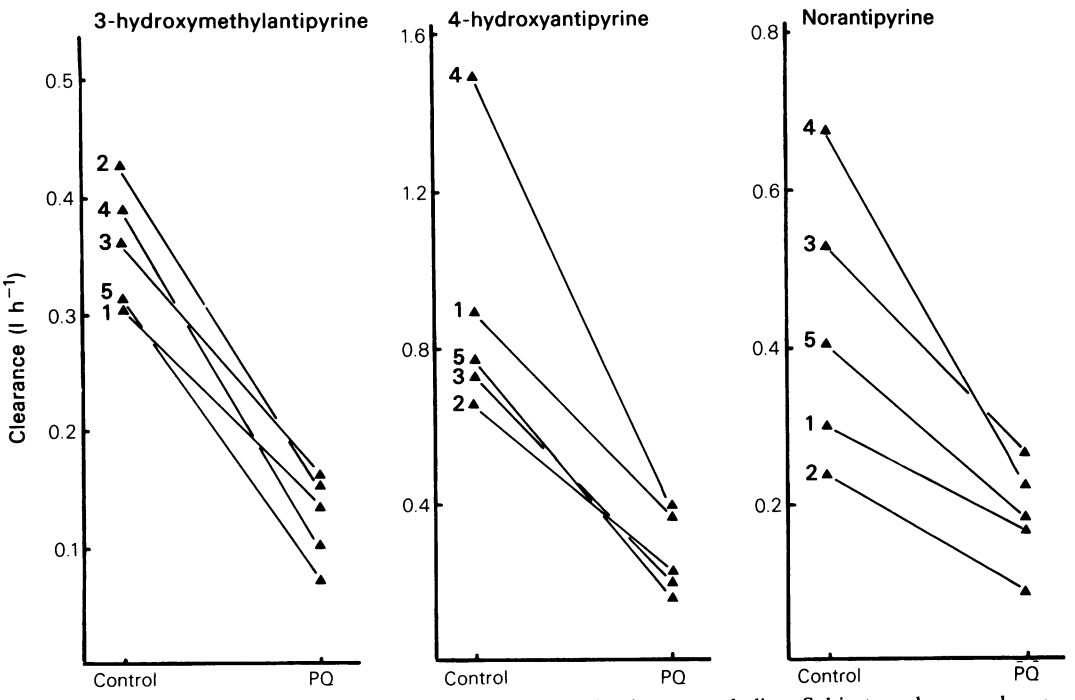

Figure 3 The effect of primaquine (PQ) on the clearance of antipyrine to metabolites. Subject numbers are shown by the control values.

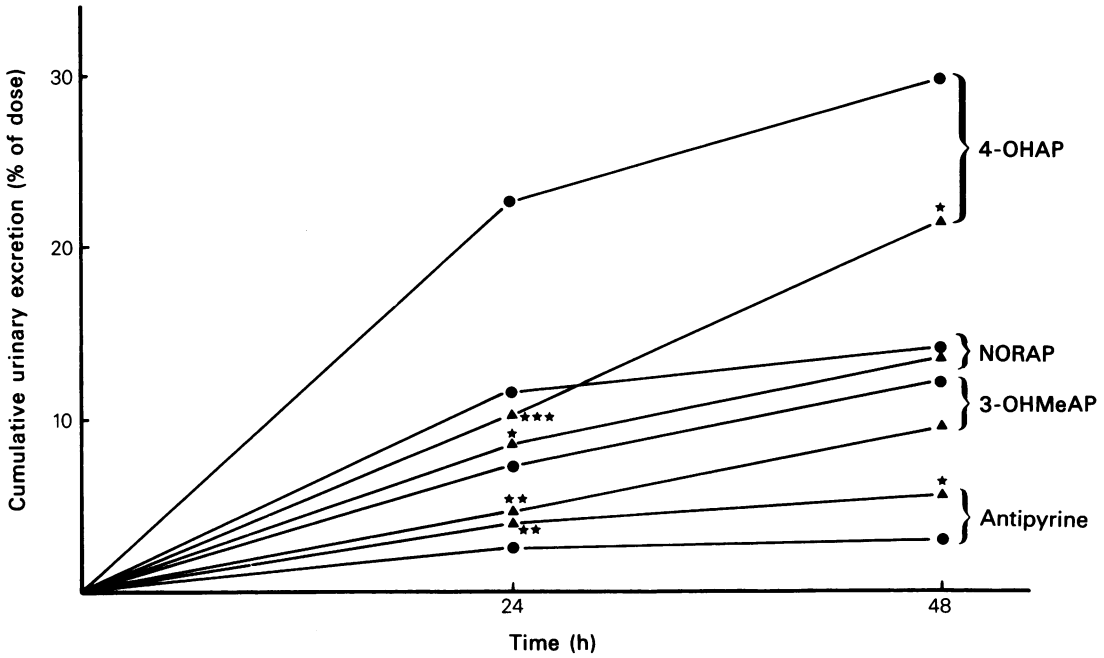

Figure 4 The cumulative urinary excretion of antipyrine and metabolites. ●—●, control; ▲—▲, primaquine treatment. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; significantly different from controls

Discussion

The results of this study confirm the observations in rats that PQ inhibits hepatic drug metabolism (Back *et al.*, 1983). We have shown also that CQ is without effect on antipyrine metabolism in man. There is therefore, as in the rat, a very marked difference in the metabolic effects of the two aminoquinolones. We have previously suggested (Back *et al.*, 1983) that the most probable explanation is a structure-activity phenomenon similar to that highlighted for the imidazole group of compounds (Wilkinson *et al.*, 1972, 1974, 1977; Rogerson *et al.*, 1977).

The approximately two-fold increase in the 'initial' (0–24 h) half-life of antipyrine by PQ is a reflection of the amount of inhibitor present and the inhibitor constant. When the concentration of inhibitor (PQ) falls (Table 3) the kinetics of antipyrine return to control (Figure 1). The half-life and clearance thus change with time.

Table 4 The effect of chloroquine (CQ) and primaquine (PQ) on the excretion of 6β-hydroxycortisol (6β-OHC) and 17-hydroxycorticosteroids (17-OHCS). Results are expressed as the ratio of 6β-OHC to 17-OHCS and are given as the mean ± s.d.

Time (h)	*Control*	*CQ*	*PQ*
0–24	4.8 ± 1.1 (6)	4.7 ± 1.2 (6)	5.9 ± 1.9 (5)
24–48	5.1 ± 2.2 (5)	7.0 ± 1.9 (5)	3.9 ± 0.4 (5)

Despite the evidence that the metabolism of antipyrine to its major metabolites, 3-hydroxymethylantipyrine, 4-hydroxyantipyrine and norantipyrine is mediated by different forms of cytochrome P-450 (Huffman *et al.*, 1973; Boobis *et al.*, 1981; Danhof *et al.*, 1982) we were unable to show any selectivity in the inhibition of the pathways of metabolism by PQ. A similar lack of selectivity has been reported by Bax *et al.* (1981) for the inhibition of antipyrine metabolism by propranolol.

A number of compounds administered either as a single dose or chronically, (reviewed by Park, 1982) have previously been shown to inhibit antipyrine metabolism in man (as judged by a percentage increase in half-life) to varying extents, e.g. allupurinol (167%, Vesell *et al.*, 1970), aminopyrine (75%, Vesell *et al.*, 1976), cimetidine (35%, Serlin *et al.*, 1979), delta-9-tetrahydrocannabinol (22%, Benowitz & Jones, 1977), oral contraceptive steroids (57%, Homeida *et al.*, 1978), levodopa plus L-α-methyl dopa hydrazine, (8% Vesell *et al.*, 1971) metoprolol and propranolol (21% and 64% respectively, Bax *et al.*, 1981). The percentage inhibition (100%, 0–24 h) of antipyrine half-life by a single dose of PQ represents a major effect on metabolism.

In contrast to cimetidine which reduced the excretion of 6β-OHC (expressed as a ratio of total 17-OHCS) from 3.65 ± 1.00 to 2.66 ± 0.72 (Serlin *et al.*, 1979), PQ in the present study had no apparent effect.

Park (1981) had discussed the scope and limitations of 6β-OHC as an index of mixed function oxygenase activity and the present study further indicates that measurement of 6β-OHC (which is only a minor metabolite of cortisol) may not be a good *in vivo* index of enzyme inhibition.

Since antimalarial drugs are in widespread use, any inhibition of the metabolism of other drugs could be important in altering the pharmacological response to give possible adverse effects.

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