Br. J. clin. Pharmac. (1984), **17**, 441–446

Pharmacokinetics of primaquine in man: identification of the carboxylic acid derivative as a major plasma metabolite

G. W. MIHALY, S. A. WARD, G. EDWARDS, M. L'E ORME & A. M. BRECKENRIDGE
Department of Pharmacology and Therapeutics, The University of Liverpool, P.O. Box 147, Liverpool L69 3BX

1 A method is described for the simultaneous determination of the carboxylic acid and *N*-acetyl-derivatives of primaquine, in plasma and urine.

2 After oral administration of 45 mg primaquine, to five healthy volunteers, absorption was rapid, with peak primaquine levels of 153.3 ± 23.5 ng/ml at 3 ± 1 h, followed by an elimination half-life of 7.1 ± 1.6 h, systemic clearance of 21.1 ± 7.1 l/h, volume of distribution of 205 ± 37 l and cumulative urinary excretion of 1.3 ± 0.9% of the dose.

3 Primaquine underwent rapid conversion to the carboxylic acid metabolite of primaquine, which achieved peak levels of 1427 ± 307 ng/ml at 7 ± 4 h. Levels of this metabolite were sustained in excess of 1000 ng/ml for the 24 h study period, and no carboxyprimaquine was recovered in urine.

4 *N*-acetyl primaquine was not detected in plasma or urine.

5 Following [^{14}C]-primaquine administration to one subject, plasma radioactivity levels rapidly exceeded primaquine concentrations. Plasma radioactivity was accounted for mainly as carboxyprimaquine. Though 64% of the dose was recovered over 143 h, as [^{14}C]-radioactivity in urine, only 3.6% was due to primaquine. As neither carboxyprimaquine nor *N*-acetylprimaquine were detected in urine, the remaining radioactivity was due to unidentified metabolites.

Keywords primaquine pharmacokinetics metabolism carboxyprimaquine malaria

Introduction

Primaquine is the drug of choice against the exo-erythrocytic stages of *Plasmodium vivax* and *Plasmodium ovale* (Strube, 1975). In spite of considerable clinical experience with the toxicology of primaquine, there is little information regarding the mode of action and disposition of this drug. Drug metabolism studies have been undertaken in dogs (Strother *et al.*, 1981), rodents (Baker *et al.*, 1982) and microbes (Hufford *et al.*, 1983). However the metabolic fate of primaquine in man has not been reported.

The present study reports the pharmacokinetics of primaquine in man. Using a newly developed high performance liquid chromatographic (h.p.l.c.) method, the formation of a carboxylic acid derivative (carboxyprimaquine, Figure 1), as the principal plasma metabolite in man, is described.

Methods

Chemicals

Primaquine diphosphate was supplied by Aldrich Chemicals Ltd, Gillingham, Dorset, U.K., 8-(3-amino-1-methylpropylamino)-6-methoxyquinoline was a gift from the Walter Reed Army Medical Research Centre, Washington D.C., U.S.A., carboxyprimaquine and the *N*-acetyl derivative (*N*-acetylprimaquine) (Figure 1), were kind gifts from Professor J. McChesney, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, U.S.A. The hydroxylated analogues of primaquine; 5-hydroxyl-, 6-desmethylene, and 5,6-dihydroxyprimaquine, were also gifts from the Walter Reed Army Medical Research Centre. Indomethacin was obtained from Merck Sharp and Dohme Research Laboratories, Hertford-

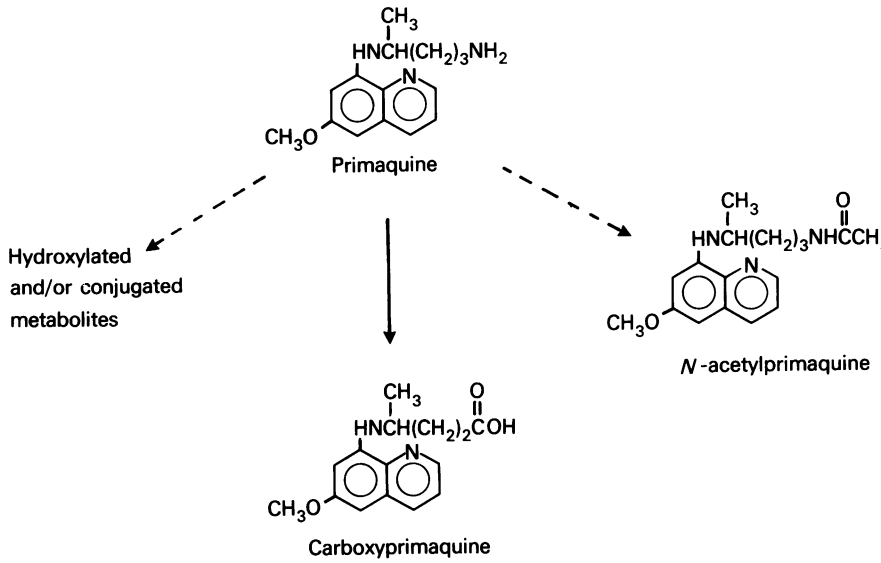

Figure 1 Metabolic fate of primaquine in man showing known pathways (→) and proposed but unidentified routes of metabolism (- - -).

shire, U.K. and [^{14}C]-primaquine (sp. act. 1.55 mCi/mmol) was synthesised by New England Nuclear, Boston, Mass, U.S.A. Solvents were of h.p.l.c. grade and obtained from Fisons, Loughborough, U.K. All other reagents were of analytical grade and supplied by British Drug Houses (B.D.H. Chemicals Ltd), Poole, Dorset, U.K.

Chromatography

All assays were carried out on a Spectra Physics Liquid Chromatograph. The system consisted of an SP 8700 Solvent Delivery system with an SP 8750 organiser module equipped with a Rheodyne valve injection system. Chromatographic separation was carried out on a 'Rad-Pak' phenyl reversed-phase column housed in a 'Z-module' (Waters Assoc., Hartford, Cheshire, U.K.) Detection was by U.V. absorption at 254 nm.

Assay methods

(a) *Primaquine* Primaquine levels were measured by the method of *Ward et al.* (1983) with minor modifications. Samples were chromatographed on a 'Rad-Pak' phenyl column, eluted with a mobile phase of methanol: water (50:50 v/v) containing 1% triethylamine (buffered with orthophosphoric acid to pH = 7.0) and flowing at 3 ml/min. These modifications resulted in more rapid chromatography and a reduction in peak tailing.

(b) *Carboxyprimaquine and* N-*acetylprimaquine* The plasma and urine levels of carboxyprimaquine and *N*-acetylprimaquine were measured using the following sample treatment and chromatography conditions. To plasma or urine samples (0.5 ml) containing indomethacin as internal standard (2.5 μg/25 μl, as an aqueous solution), was added an equal volume of phosphate buffer (NaH_2PO_4; 0.1 M; buffered to pH 2.6 with orthophosphoric acid) followed by vortex mixing for 30 s. This mixture was extracted with a combination of hexane and ethyl acetate (9:1 v/v, total vol 5 ml), by mechanical tumbling for 10 min. After centrifugation (1000 *g* for 10 min) and separation, the organic phase was evaporated to dryness at 37°C under a stream of nitrogen. The residue was reconstituted in methanol (100 μl) and 25 μl was injected on to the chromatograph. Chromatographic separation was achieved on a 'Rad-Pak' phenyl column eluted with methanol:water (65:35) containing 1% triethylamine (buffered to pH 6.0) flowing at 3 ml/min.

Chromatography of plasma extracts of samples collected from the volunteers in this study, consistently gave two sharp peaks corresponding to authentic carboxyprimaquine and indomethacin at retention times of 3.2 and 5.2 min respectively. *N*-acetylprimaquine was chromatographed with baseline separation at a retention time of 4.1 min. Concentrations of metabolite were quantitated by relating peak

height ratios of metabolite to internal standard in chromatograms of samples, to peak height ratios obtained from known standards. The assay has a limit of sensitivity of 75 ng/ml for carboxyprimaquine and 10 ng/ml for *N*-acetylprimaquine as measured by a peak corresponding to four times the size of baseline noise at 0.01 AUFS. The within day and day to day coefficients of variation for the carboxyprimaquine assay were 2.9% and 5.0% respectively. There was no interference from primaquine, the hydroxylated analogues (predicted as metabolites, Strother *et al.*, 1981) nor from the commonly used antimalarials chloroquine and pyrimethamine.

(c) *Plasma radioactivity* Plasma and urine levels of [^{14}C]-radioactivity were measured by scintillation counting using an Intertechnique SL33 liquid-scintillation counter.

Volunteer study

Five healthy male volunteers (24–45 years) who were taking no other drugs each received oral primaquine (I.C.I. pharmaceuticals; 6 × 7.5 mg tablets = 45 mg as primaquine base) after an overnight fast. Venous blood samples were taken pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h. Blood was centrifuged (1000 *g* for 15 min) and the separated plasma stored at −20°C until analysis. Urine was collected for 1 h pre-dose and from 0 to 24 h. Urine volume and pH were recorded and an aliquot frozen at −20°C. In addition to the standard dose one volunteer (RB) received a tracer dose of [^{14}C]-primaquine (8.25 μCi, 2.4 mg) by mouth. In this volunteer plasma was collected over 120 h and urine collections were continued serially for 6 days. Plasma and urine samples were assayed for primaquine, carboxyprimaquine, and *N*-acetylprimaquine by h.p.l.c. and for [^{14}C] by liquid scintillation counting. All aspects of these protocols were approved by the Mersey Regional Health Authority Ethics Committee and Ethics Committee of the World Health Organisation. Permission for the administration of [^{14}C]-primaquine was obtained from the D.H.S.S. Radioisotopes panel.

Pharmacokinetic calculations

The peak plasma concentration (C_{max}) and the time at which it was reached (t_{max}) were obtained graphically. The terminal phase elimination rate constant (λ_z) was determined by least squares regression analysis of the post absorption and distribution plasma concentration time data and the terminal phase elimination half-life ($t_{½}$) from ratio of $0.693/\lambda_z$. The area under the plasma concentration-time curve from time = 0 to time = t' was calculated by the trapezoidal rule (Gibaldi & Perrier, 1975) and from t' to infinity by the ratio $C_{t'}/\lambda_z$, where $C_{t'}$ was the plasma concentration at time = t'. The area under the curve from t = 0 to infinity (AUC) was then obtained from the sum of these areas. Oral clearance (CL_o) was calculated from the ratio of dose/AUC and systemic clearance (CL), from the ratio CL_o/F, where F is the bioavailability and equals 0.74 (unpublished observation). The volume of distribution was then calculated from the expression;

$$V_z = \frac{CL \times t_{½}}{0.693}$$

Results

The mean plasma concentration-time profiles for primaquine and carboxyprimaquine are shown in Figure 2 and the pertinent pharmacokinetic estimates listed in Table 1. After oral dosage, primaquine underwent rapid absorption, reaching peak levels of 153.3 ± 23.5 (mean ± s.d.) ng/ml, between 2 and 3 h. Thereafter, drug levels declined rapidly and monoexponentially, with a terminal phase elimination half-life of 7.1 ± 1.6 h and an oral clearance of 28.5 ± 9.5 l/h. Renal clearance of unchanged primaquine represented a trivial route of elimination, as only 1.3 ± 0.9% of the dose was recovered in urine.

Primaquine undergoes rapid sidechain metabolism to carboxyprimaquine. Peak levels of this metabolite of 1427 ± 307 ng/ml, were ten fold higher than parent drug levels and were reached between 3 and 12 h post dose. These metabolite levels were maintained in excess of 1000 ng/ml for the duration of the study period. However, carboxyprimaquine was not detected in urine, suggesting that this compound is subjected to further metabolism prior to excretion. Furthermore, concentrations of *N*-acetylprimaquine were rarely seen to exceed the minimum detectable level of (10 ng/ml) in this study.

In one subject (RB), a tracer dose of [^{14}C]-primaquine was coadministered with the oral dose. The pharmacokinetics of primaquine were comparable in this subject to the other four volunteers. However, in this subject the plasma concentration-time profile of [^{14}C]-radioactivity differed markedly from that for primaquine (Figure 3). Peak levels of ^{14}C were attained rapidly but exceeded primaquine levels by ten to twenty fold. Only 1.9% of the total area under the plasma radioactivity-time curve was accounted for by primaquine, whereas the carboxy-metabolite contributed 55% of the area

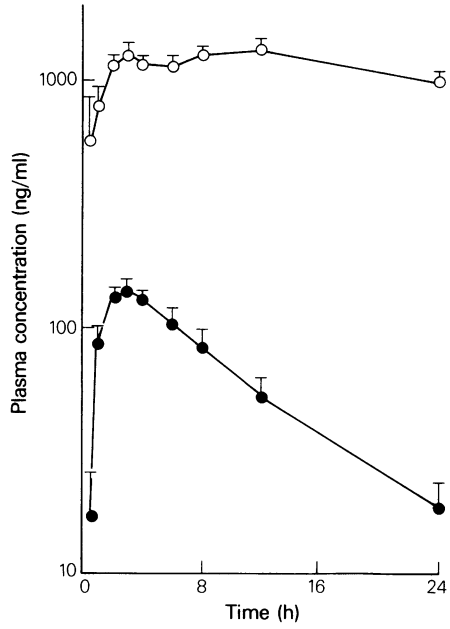

Figure 2 Mean plasma concentrations (± s.e. mean) of primaquine (●——●) and carboxyprimaquine (O——O) after oral dosage (45 mg) to five healthy subjects.

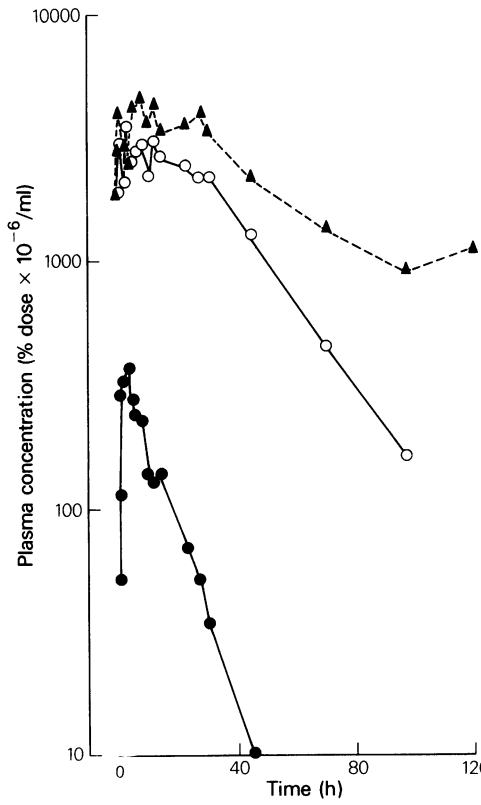

Figure 3 Plasma concentrations of primaquine (●——●), carboxyprimaquine (O——O) and ^{14}C radioactivity (Δ- - -Δ) in subject RB following oral dosage of tracer [^{14}C]-primaquine (2.4 mg, 8.25 μCi) together with the standard dose (45 mg).

Table 1 Summary of pharmacokinetic parameters for primaquine and its carboxy metabolite after oral dosage with primaquine (45 mg). Time to peak plasma concentration (t_{max}); peak plasma concentration (C_{max}); terminal phase elimination half-life ($t_{½}$); oral clearance (CL_0); systemic clearance (CL); volume of distribution (V_z) and amount excreted in the urine to 24 h ($Ae(24)$).

Pharmacokinetic parameter	*Subjects*					
	SW	*GE*	*AB*	*MO*	*RB*	*Mean ± s.d.*
a) *Primaquine (PQ)*						
t_{max} (h)	3	2	3	2	3	3 ± 1
C_{max} (ng/ml)	131.2	132.8	180.4	146.0	176.0	153.3 ± 23.5
$t_{½}$ (h)	5.8	6.5	7.8	5.9	9.6	7.1 ± 1.6
CL_0 (l/h)	41.6	28.6	18.5	33.4	20.3	28.5 ± 9.5
CL* (l/h)	30.8	21.2	13.7	24.7	15.0	21.1 ± 7.1
V_z* (l)	257	198	154	209	208	205 ± 37
$Ae(24)$ (% of dose)	1.4	0.5	0.4	1.9	2.4	1.3 ± 0.9
b) *Carboxyprimaquine ($PQCO_2H$)*						
t_{max} (h)	8	2	8	12	3	7 ± 4
C_{max} (ng/ml)	1078	1386	1199	1808	1666	1427 ± 307
$Ae(24)$ (% of dose)	0	0	0	0	0	0

* calculated using bioavailability = 0.74 (unpublished observation)

(Figure 3). The carboxyprimaquine concentrations declined to below detectable levels by 72 to 96 h, at which time 1.3×10^{-3}% of the dose was present per ml of plasma as [^{14}C]-radioactivity indicating the persistence of other metabolic product(s) of primaquine. Nonetheless, 64% of the radiolabel dose was recovered in urine by 143 h, though mainly as metabolites other than carboxyprimaquine or *N*-acetylprimaquine. Only 3.6% of the dose was recovered as the parent compound.

Discussion

Although used as an antimalarial for over 40 years, there is a dearth of information regarding the mode of action and disposition of primaquine in animals and man. The antimalarial activity and toxicity of primaquine, has been attributed to one, or more, of its metabolites (Fraser *et al.*, 1976; Fletcher *et al.*, 1977). Studies in microbes (Hufford *et al.*, 1983) have identified the carboxylic acid and *N*-acetylated metabolites of primaquine. By contrast, in rodents, only the carboxylic acid metabolite has been found (Baker *et al.*, 1982). In addition several phenolic analogues have been proposed as metabolites of primaquine in the dog (Strother *et al.*, 1981). At present, there are no published reports of the metabolic fate of primaquine in man.

The plasma kinetics and urinary excretion of primaquine have been recently investigated (Greaves *et al.*, 1980). After oral dosage (45 mg) primaquine was shown to reach peak drug levels within 1–2 h, undergo a clearance from plasma of 25 l/h and extensive tissue distribution ($V_z \simeq 200$ l). These parameters were calculated assuming a bioavailability of 100%. However, in preliminary studies by us in two subjects, who were administered intravenous and oral primaquine, the bioavailability was shown to be 74% (unpublished observations). Renal elimination of unchanged primaquine accounted for less than 1% of the administered dose.

The findings of the present study, on the pharmacokinetics of primaquine, are in broad agreement with those of Greaves *et al.* (1980) (Figure 2, Table 1). In addition, using our estimate of bioavailability of 74%, systemic clearance of primaquine can be calculated to be 21.1 ± 7.1 l/h which represents less than a quarter of liver blood flow (90 l/h) and indicates primaquine to be a 'low to intermediate clearance' compound. This calculated value of systemic clearance allows the volume of distribution to be determined, which at 205 ± 37 l, reflects extensive tissue distribution of the drug.

The present study has also shown the carboxy derivative of primaquine, as the principal metabolite in human plasma. Primaquine underwent rapid and extensive conversion to carboxyprimaquine which persisted at elevated levels in plasma for the duration of the 24 h study period. At 24 h, the metabolite levels in plasma were fifty fold higher than the parent drug. As the area under the curves for carboxyprimaquine was considerably greater than that for primaquine, the clearance of this metabolite must necessarily have been considerably lower than that for primaquine. Furthermore by contrast to primaquine, which undergoes extensive tissue distribution, the more polar carboxyprimaquine would be expected to be more restricted to plasma water and hence have a much lower volume of distribution. A more detailed pharmacokinetic study of this metabolite *per se* could not be undertaken in the present study. As only negligible levels of the carboxyprimaquine were recovered in urine, the metabolite probably undergoes further metabolism prior to excretion.

An analogous process of metabolism has been reported for propranolol where side chain deamination and oxidation of the terminal carbon to the carboxylic acid produces the metabolite naphthoxylactic acid (Walle *et al.*, 1979). This compound is present in plasma in considerably higher levels than propranolol, but unlike carboxyprimaquine, naphthoxylactic acid is eliminated from plasma at a comparable rate to propranolol and about 14% of the dose appears as this metabolite in urine.

The h.p.l.c. assay developed for carboxyprimaquine enabled the simultaneous determination of *N*-acetylprimaquine, previously identified in microbial metabolism studies (Hufford *et al.*, 1983), but not detectable in studies on rodents (Baker *et al.*, 1982). In the present studies in man, the *N*-acetylated compound rarely exceeded the minimum detectable level of 10 ng/ml in human plasma and urine.

Following [^{14}C]-primaquine administration to subject RB, the area under the plasma level-time profile of the carboxyprimaquine constituted 55% of the total area due to plasma radioactivity. This observation suggests that the carboxylic acid derivative is the principal metabolite in plasma. This is particularly apparent over the first 24 h, during which metabolite levels closely follow plasma [^{14}C]-radioactivity (Figure 3). Thereafter [^{14}C] levels in plasma persist, in spite of falling carboxyprimaquine levels, again suggesting further biotransformation of this metabolite. Over a 143 h urine collection period, 3.6% of the administered dose was recovered as primaquine and none as carboxyprimaquine.

Urinary recovery of [^{14}C]-radioactivity over this period accounted for 64% of the dose, mainly as unidentified metabolites.

The present study has shown that after oral dosage primaquine is readily absorbed and undergoes rapid metabolism to the carboxylic acid derivative that persists at elevated levels in plasma. This compound represents the principal plasma metabolite and is ultimately subjected to further biotransformation prior to excretion. The association of this metabolite with pharmacodynamic and toxicological effects of primaquine is yet to be examined.

The authors are grateful to Mrs Pearl Williams for typing this manuscript. This work was supported by the National Health and Medical Research Council of Australia (GWM), the Merseyside Regional Health Authority (SAW) and financial support from the UNDP/World Bank/World Health Organisation Special Programme for Research and Training in Tropical Diseases.

References

Baker, J. K., McChesney, J. D., Hufford, C. D. & Clark, A. M. (1982). High-performance liquid chromatographic analysis of the metabolism of primaquine and the identification of a new mammalian metabolite. *J. Chromatogr.*, **230**, 69–77.

Fletcher, K. A., Canning, M. V. & Gilles, H. M. (1977). Metabolites of primaquine and their effects on erythrocytes. *Trans. Roy. Soc. trop. Med. Hyg.*, **71**, 111.

Fraser, I. M., Strother, A. & Tilton, B. E. (1976). Methaemaglobin formation in normal and G.6.PD deficient human erythrocytes by primaquine and a mouse liver microsomes system. *Fed. Proc.*, **35**, 244.

Gibaldi, M. & Perrier, D. (1975). Pharmacokinetics In *Drugs and the pharmaceutical sciences*, ed. Swarbrick, J., Volume 1 pp 293–296. New York: Marcel Dekker Inc.

Greaves, J., Evans, D. A. P., Gilles, H. M., Fletcher, K. A., Bunnag, D. & Harinasuta, T. (1980). Plasma kinetics and urinary excretion of primaquine in man. *Br. J. clin. Pharmac.*, **10**, 399–405.

Hufford, C. D., Clark, A. M., Quinones, I. N., Baker, J. K. & McChesney, J. D. (1983). Microbial metabolism studies on the major microbial and mammalian metabolite of primaquine. *J. pharm. Sci.*, **72**, 92–94.

Strother, A., Fraser, I. M., Allahyari, R. & Tilton, B. E. (1981). The metabolism of 8-aminoquinoline antimalarial agents. *Bulletin of W.H.O.*, **59**, 413–425.

Strube, R. E. (1975). The search for new antimalarial drugs. *J. trop. Med. Hyg.*, **78**, 171–186.

Walle, T., Conradi, E. C., Walle, K., Fagan, T. & Gaffney, T. (1979). Naphthoxylactic acid after single and long term doses of propranolol. *Clin. Pharmac. Ther.*, **26**, 548–554.

Ward, S. A., Edwards, G., Orme, M. L'E & Breckenridge, A. M. (1983). Determination of primaquine in biological fluids by reversed-phase high performance liquid chromatography. *J. Chromatogr.*, **305**, 239–243.

(Received September 5, 1983, accepted November 19, 1983)