(6) Fraser, I. M., Strother A., Tilton, B. E. (1976) Fed. Proc. 33, 244.
(7) Baty, J. D., Price Evans, D. A., Robinson, P. A. (1975) Biomed. Mass Spect. 2, 304.
(8) Goodwin, C. G., Rollo, I. M. (1955) The Chemotherapy of malaria, piroplasmosis, trypanosomiasis and leishmaniasis. In Hutner, S. H., Lwoff, R. A. (ed.) Biochemistry and physiology of Protozoa, vol. II. Academic Press Inc., New York.
(9) Clark, A. M., Hufford, C. D., McChesney, J. D. (1981) Antimicro. Agents Chemotherapy 19, 337–341.
(10) Baker, J. K., McChesney, J. D., Hufford, C. D., Clark, A. M. J. Chromat., in press.

Metabolism and Distribution of Primaquine in Monkeys

John K. Baker[1], John A. Bedford[2], Alice M. Clark[3], and James D. McChesney[3]

Received: July 6, 1983; accepted October 23, 1983

Abstract Rhesus monkeys were administered primaquine diphosphate (6.0 to 10.5 mg/kg, I.V.), and plasma samples were analyzed by high performance liquid chromatography for the presence of the unchanged drug and the major metabolite, 8-(3-carboxy-l-methylpropylamino)-6-methoxyquinoline (II). Primaquine had an unusually high affinity for tissue compartments which produced a rapid initial drop in plasma concentration. Within 15 minutes, the plasma concentration of II far exceeded that of primaquine. 35 to 83 % of the primaquine dose was converted to II; moreover, metabolite II possessed much lower affinity for the tissue compartments than primaquine itself.

Primaquine (I) is used for the prophylaxis of malaria in endemic areas. Though it probably is the most effective drug for this use, it has a fairly low therapeutic index, and its use is frequently associated with hemolytic anemia and methemoglobin formation. It is also thought that the toxicity of primaquine results from biotransformation of the drug. Only trace quantities of unchanged primaquine have been found in urine, and 6-methoxy-8-aminoquinoline was the only metabolite that has been positively identified. However, this metabolite represented only 4 % of the dose (2). More recently, it has been shown that the major metabolite of primaquine by fungi (3) and by rats (4, 5) is the oxidative deamination product (II), 8-(3-carboxy-l-methylpropylamino)-6-methoxyquinoline.

The objective of the present study was to determine the extent of conversion of primaquine diphosphate to II in Rhesus monkey as a common animal model for malaria studies.

These studies with Rhesus monkeys might more accurately reflect the pharmacokinetics and metabolism of primaquine in man than the previous studies with rats (4, 5).

Materials and Methods

Primaquine diphosphate was utilized as obtained from Aldrich (Milwaukee, WI), and the metabolite II was obtained from the fermentation of primaquine as previously reported (3). This material had spectral and chromatographic properties identical to synthetic II.

The analysis of the plasma samples for primaquine diphosphate and II was accomplished using high performance liquid chromatography with a C-18 reversed phase column and ultraviolet detection as previously reported (4). The identification of primaquine and II was based on comparisons of retention times and the absorbance ratios of 254 nm and 280 nm with a dual UV detector system of the test samples, the two reference standards, and plasma blanks.

In the metabolism studies, the Rhesus monkey *(M. Mulatta)* was first sedated with ketamine HCl (12 mg/kg); then an I.V. drip (30 drops/min) of lactated ringers solution was administered to the restrained animal. After I. V. administration of the test drug, 10 ml blood samples were collected at 5, 10, 15, 30 minutes and thereafter at 1, 2, 4, 8, and 24 hours using an indwelling catheter. The primaquine diphosphate was administered as an aqueous solution. Because of the low water solubility of II, this material was first converted to the water soluble salt by the addition of an equivalent amount of sodium hydroxide immediately before administration.

Results

Following I. V. administration of primaquine diphosphate, an extremely rapid fall in plasma concentration of the drug was observed in the three monkeys that were tested (Fig. 1). Furthermore, concentration of the metabolite in plasma after 15 minutes was far greater than that of the drug (Fig. 2). The concentration of the metabolite remained high and at a fairly constant level during the 2 to 8

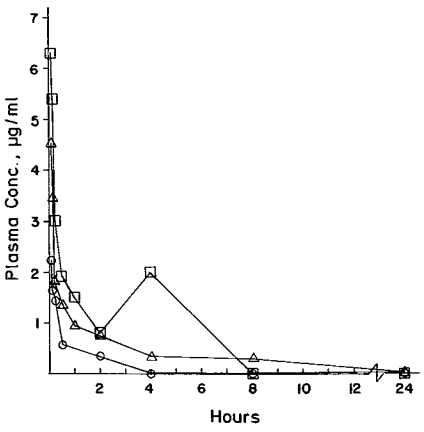

Figure 1: Plasma concentration of primaquine diphosphate following IV administration: O, monkey #183 given 10.5 mg/kg primaquine diphosphate; □ monkey #413 given 10.5 mg/kg primaquine diphosphate; △, monkey #331 given 6.0 mg/kg primaquine diphosphate.

[1] Department of Medicinal Chemistry
[2] Research Institute of Pharmaceutical Sciences
[3] Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677

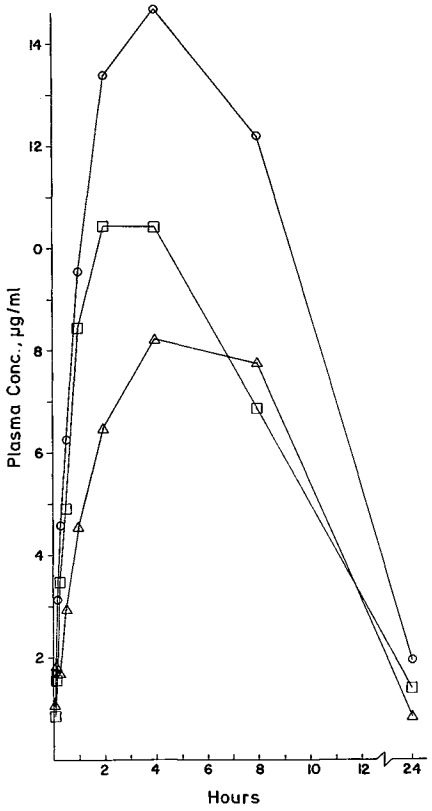

Figure 2: Plasma concentration of the carboxylic acid metabolite II: O, monkey #183 given 10.5 mg/kg primaquine diphosphate; □, monkey #413 given 10.5 mg/kg primaquine disphosphate; △, monkey #331 given 6.0 mg/kg primaquine diphosphate.

hour period, and it remained at readily detectable levels for 24 hours. These results are similar to those observed with rats (4, 5) except that monkeys produced a much higher concentration of the metabolite II.

The monkeys were also given II directly (Fig. 3, top curve) in order to determine the relationship between dose and the area under the curve (AUC) for the metabolite. From a comparison of the AUC (as measured by the trapezoidal method) for II following administration of primaquine diphosphate and the AUC for II following administration of II, the extent of conversion of primaquine to the metabolite was calculated (Tab. I). The dose level did not appear to have a large effect on the conversion (6.0 mg/kg gave 43 % conversion; 10.5 mg/kg gave 35 % conversion), but these results were obtained in two different animals. However, it was observed that the younger, lighter weight subject gave a higher yield (83 %) of the metabolite.

Table I: Extent of conversion of primaquine diphosphate to the carboxylic acid metabolite (II).

Monkey	Weight	Dose of primaquine disphosphate	% conversion to II
#413	13.5 kg	10.5 mg/kg	35 %
#183	6.2 kg	10.5 mg/kg	83 %
#331	12.0 kg	6.0 mg/kg	43 %

The plasma concentration of primaquine diphosphate (Fig. 3, bottom curve) was analyzed using a non-weighted linear least squares regression of the feathered data and it was found to conform to a two compartment model of the following form:

$C = Ae^{-\alpha t} + Be^{-\beta t}$

where:	which allows:
$A = 4.84$ µg/ml	$k_{21} = 0.94\ hr^{-1}$
$\alpha = 4.93\ hr^{-1}$	$k_{12} = 3.30\ hr^{-1}$
$B = 0.95$ µg/ml	$k_{el} = k_3 + k_4 = 0.85\ hr^{-1}$
$\beta = 0.16\ hr^{-1}$	$V_d = 7.92$ liters

The plasma concentration-time curve of II following I. V. administration of II (Fig. 3, top curve) also conformed to a two compartment model, but the extent of distribution to the tissue compartment was markedly reduced.

$C = Ae^{-\alpha t} + Be^{-\beta t}$

where:	which allows
$A = 15.6$ µg/ml	$k_{35} = 0.36\ hr^{-1}$
$\alpha = 2.30\ hr^{-1}$	$k_{53} = 1.91\ hr^{-1}$
$B = 69.9$ µg/ml	$k_6 = 0.18\ hr^{-1}$
$\beta = 0.146\ hr^{-1}$	$V_d = 0.96$ liters

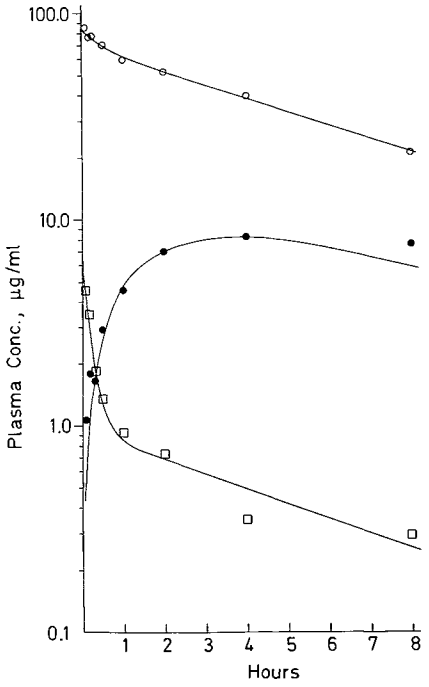

Figure 3: Plasma concentration of primaquine diphosphate and II following I. V. administration: O, conc. of II following administration of II (6.35 mg/kg) to monkey #331; ●, conc. of II following administration of primaquine diphosphate (6.0 mg/kg) to monkey #331; □, conc. of primaquine diphosphate following administration of primaquine diphosphate (6.0 mg/kg) to monkey #331. The solid lines are computer generated using the models described in the text.

In order to determine the rate of conversion of primaquine diphosphate to metabolite II (Fig. 3, middle curve), two different methods were used. For monkey #331 it was found that 43 % (Tab. I) of the primaquine diphosphate had been converted to metabolite II. Since $k_{el} = 0.85\ hr^{-1}$ for primaquine, one can estimate for $k_3 = 0.37\ hr^{-1}$ (i. e. 43 % of $0.85\ hr^{-1}$). A second approach to the estimation of k_3 was to compare the biexponential models for the plasma concentration of II following the administration of II and following the administration of primaquine diphosphate. It was assumed that $\beta = 0.146\ hr^{-1}$ in both cases, and that the formation of the metabolite followed first order kinetics. The first part of the biexponential was then obtained by a least squares regression analysis of the feathered data to give:

$C = 20.9\ (e^{-0.146t} - e^{-0.449t})$

Using this approach, one arrives at an estimate of $k_3 = 0.45\ hr^{-1}$ which is within the experimental error of the previous estimate. The value for k_4 (0.40 hr^{-1}) (Fig. 4) was then obtained by subtracting k_3 from the total metabolism and excretion rate constant for primaquine diphosphate ($k_{el} = 0.85\ hr^{-1}$).

From a combination of the pharmacokinetic data in these three experiments with monkey # 331, the overall model for primaquine and metabolite II was constructed (Fig. 4). This model suggests that primaquine was taken up into the tissues very quickly ($t_{1/2}$ = 13 min). Moreover, the distribution of primaquine into the tissues (k_{12}/k_{21} = 3.51) was markedly higher than that observed for the metabolite (k_{35}/k_{53} = 0.19). In addition to this difference in the distribution of primaquine and

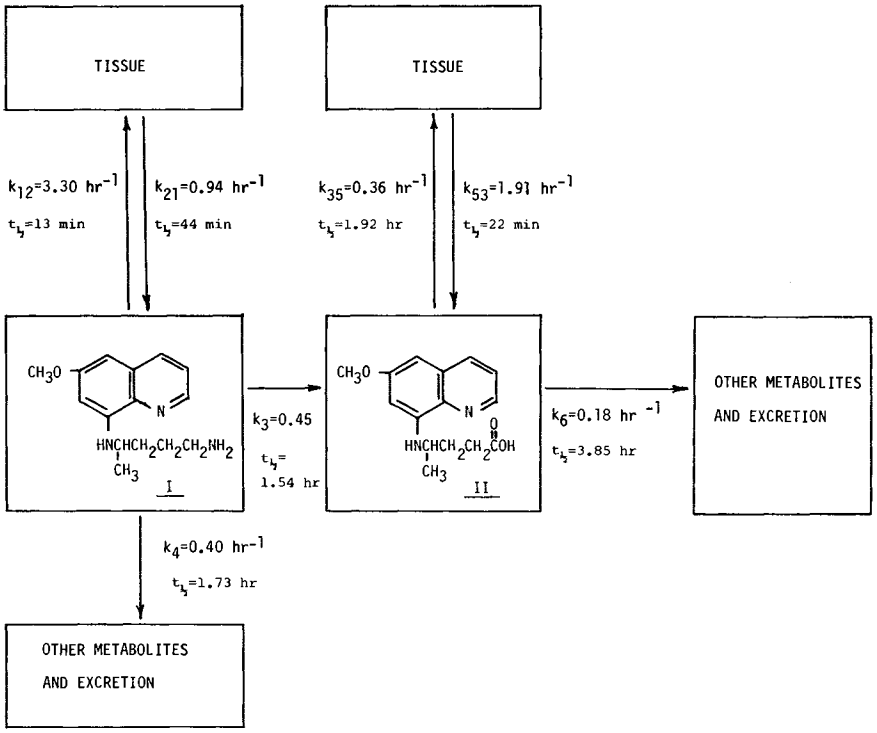

Figure 4: Pharmacokinetics of primaquine diphosphate and II in monkey #331.

metabolite II in slowly perfused tissue, a striking difference in the distribution to highly perfused tissue was also observed. The volume of distribution for primaquine diphosphate (7.92 liters) was markedly higher than the volume of distribution for metabolite II (0.96 liters). In previous studies with ^{14}C-labeled primaquine given to rats (5), very high concentrations were found in lung and liver tissue within seconds of administration of an I. V. dose.

Several factors appeared to contribute to the high plasma levels of II as compared to primaquine itself. First, the rate of formation of II (k_3 = 0.45 hr^{-1}) was considerably faster than the rate of its elimination (k_6 = 0.18 hr^{-1}). Secondly, II was not taken up by the tissue to any significant extent in comparison to primaquine. Thirdly, a large fraction of the dose (35 % to 83 %) is converted to this metabolite.

Discussion

Previous studies on the distribution of xenobiotics in isolated lung tissue have shown that compounds containing a large aromatic ring structure linked by an alkyl chain to a basic nitrogen (pKa > 8.5) were rapidly ($t_{½}$ = 3 min) taken up into the tissue (6). Our earlier studies (5) with ^{14}C-labeled primaquine in rats have also shown that this drug is selectively and rapidly ($t_{½}$ < 3 min) concentrated in lung and liver tissue. The present study with monkeys also shows that primaquine is rapidly taken up into highly perfused tissues ($t_{½}$ ≤ 13 min). The primary amino group of primaquine is rather basic (pKa = 10.39) (7), while the nitrogens of the aromatic ring system would not be positively charged under physiological conditions (quinoline nitrogen pKa = 3.20, aniline nitrogen pKa ≃ −1.5). Thus the properties of primaquine under physiological conditions fit the characteristics of the model developed by Orton (6) for the rapid accumulation of drugs by lung tissue. The carboxylic acid metabolite (II) on the other hand, possesses an ionized carboxyl group on the alkyl chain which may account for its negligible accumulation in tissues of monkeys and rats (5).

The extent of the conversion of primaquine to metabolite II (35 % to 83 %) in monkeys was higher than that observed previously (5) with rats (22 %). There was some indication that the younger monkey could more efficiently convert primaquine to II. In future studies with humans, subject age should be a significant variable in the experimental design. In preliminary human studies (1.3 mg/kg primaquine free base, oral admin.) a plasma concentration of 4.8 μg/ml for II was observed 2 hours after the dose, which is at least ten times higher than the dose normalized concentration of II for rats given primaquine orally (4), but approximately equal to the levels observed in the present study with monkeys.

When ^{14}C-labeled primaquine was given by intravenous administration to rats (5), nearly half of the dose was excreted in the feces, and enterohepatic recirculation of the drug occurred. In monkeys, the loss of primaquine to 'other metabolites and excretion' (Fig. 4, k_4 = 0.40 hr^{-1}) likely represents loss to the feces rather than excretion of unchanged drug in the urine or metabolic conversion to metabolites other than II.

Acknowledgements

The authors thank Ken Nichols for excellent technical assistance on the project. The human plasma samples were kindly provided by Dr. Paul E. Carson. This investigation received the financial support of the UNDP/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases. The project was also supported in part by the Research Institute of Pharmaceutical Sciences.

References

1. Beutler, E. (1969) Pharmacol. Rev. 21, 73–103.
2. Baty, J. D., D. A. P. Evans, P. A. Robinson (1975) Biomed. Mass Spectrometry 2, 304–307.
3. Clark, A. M., C. D. Hufford, J. D. McChesney (1981) Antimicrob. Agents Chemother. 19, 337–341.
4. Baker, J. K., J. D. McChesney, C. D. Hufford, A. M. Clark (1982) J. Chromatogr. 230, 69–77.
5. Clark, A. M., J. K. Baker, J. D. McChesney (1983) J. Pharm. Sci., in press.
6. Orton, T. C., M. W. Andersen, R. D. Pickett, T. E. Eling and T. R. Fouts (1973) J. Pharmacol. Exp. Ther. 186, 482–497.
7. Hufford, C. D., J. D. McChesney, and J. K. Baker (1983) J. Heterocyclic Chem. 20, 273–275.