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MEFLOQUINE METABOLISM BY HUMAN LIVER MICROSOMES

EFFECT OF OTHER ANTIMALARIAL DRUGS

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Abstract—A number of drugs have been studied for their effect on the metabolism of the antimalarial drug mefloquine by human liver microsomes (N = 6) *in vitro*. The only metabolite generated was identified as carboxymefloquine by co-chromatography with the authentic standard. Ketoconazole caused marked inhibition of carboxymefloquine formation with IC_{50} and K_i values of 7.5 and 11.2 μM, respectively. The inhibition by ketoconazole, a known inhibitor of cytochrome P450 isozymes, and the dependency of metabolite formation on the presence of NADPH indicated that cytochrome P450 isozyme(s) catalysed metabolite production. Of compounds actually or likely to be coadministered with mefloquine to malaria patients only primaquine and quinine produced marked inhibition (IC_{50}, 17.5 and 122 μM; K_i, 8.6 and 28.5 μM, respectively). However, despite these *in vitro* data with primaquine, clinical studies have failed to show any significant effect of single dose primaquine on the pharmacokinetics of mefloquine. With quinine, because peak plasma concentrations are very close to the K_i value, there is likely to be inhibition of mefloquine metabolism in patients receiving both drugs. Sulfadoxine, artemether, artesunate and tetracycline did not significantly inhibit carboxymefloquine formation.

A single oral dose of mefloquine (WR 142,490, (+)erythro-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol) has proved to be a relatively effective treatment against multi-drug resistant falciparum malaria in recent years. However, resistance to mefloquine is emerging [1, 2] particularly in parts of Thailand. There is therefore considerable interest in using mefloquine with other antimalarial drugs both concurrently and sequentially. Multiple drug administration raises the question of potential pharmacokinetic/metabolic drug interactions with consequent implications for clinical usage.

Despite numerous pharmacokinetic studies of mefloquine [3] there is comparatively little information available on the metabolism of mefloquine in humans. The main circulating metabolite is carboxymefloquine with concentrations exceeding those of the parent compound by more than 3-fold [4–6]. This metabolite appears to be devoid of any significant antimalarial activity [7]. Most metabolic data have been obtained from rodents [8–11] with the carboxylic acid metabolite being identified in rat faeces. It is not known if cytochrome P450 isozyme(s) are responsible for removal of the piperidine ring and formation of carboxymefloquine.

In the present work we have screened a number of antimalarial and other drugs for their propensity to inhibit the metabolism of mefloquine by human liver microsomes *in vitro*. Previous studies have shown that primaquine inhibits hepatic microsomal cytochrome P450-dependent enzymes both *in vitro* and *in vivo* [12–19] and therefore inhibition of mefloquine metabolism would be one pointer towards P450 involvement in the formation of the carboxy metabolite. The other drugs studied were quinine, sulfadoxine, tetracycline, artemether and artesunate, all actually or potentially used in combination with mefloquine. For comparative purposes, the antifungal agent, ketoconazole, which is well established as a potent inhibitor of cytochrome P450 enzymes was also studied.

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MATERIALS AND METHODS

Drugs and chemicals. Drugs and chemicals used were: mefloquine hydrochloride, [^{14}C]mefloquine (sp. act. 138 μCi/mg; radiochemical purity > 97%) and carboxymefloquine which were gifts from the Walter Reed Army Medical Research Centre (Washington, DC, U.S.A.). NADPH (B-nicotinamide adenine dinucleotide-3′-phosphate, reduced form), tetracycline, primaquine diphosphate, quinine hydrochloride and sulfadoxine were obtained from the Sigma Chemical Co. (Poole, U.K). Ketoconazole was a gift from Janssen (Beerse, Belgium). Solvents were of HPLC-grade and obtained from the Fison Co. (Loughborough, U.K.). All other reagents were of analytical grade and supplied by BDH (Poole, U.K.).

Human liver samples. Histologically normal livers were obtained from kidney transplant donors soon after clinical death (four males, two females; aged 21–66 years). Ethical approval for the study was granted and consent to removal of the liver samples was obtained from the donors' relatives. Livers were stored as 10–20-g portions at −80° until required.

Preparation of liver microsomes. Washed microsomes were prepared by the classical differential

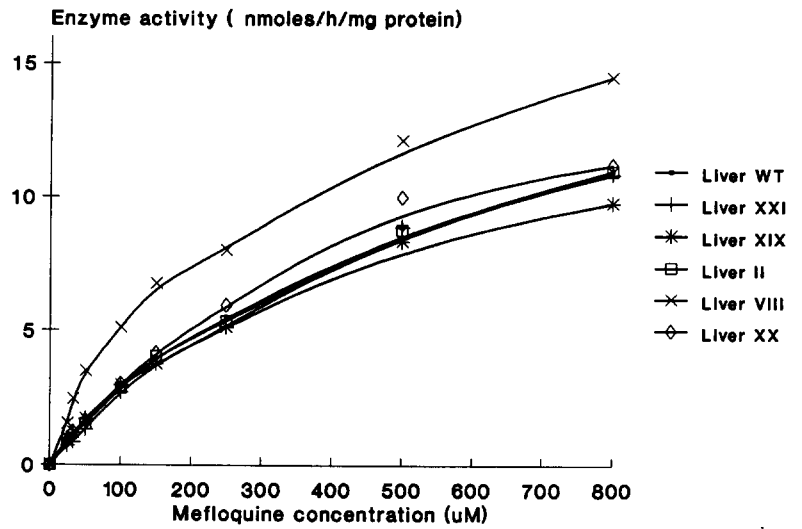

Fig. 1. Plot of velocity (nmol/hr/mg protein) vs mefloquine concentration for the formation of carboxymefloquine in microsomes from six human livers.

centrifugation technique [20]. Microsomal protein content was determined by the method of Lowry *et al.* [21].

Kinetic and inhibition studies. Incubations containing [^{14}C]mefloquine (0.1 μCi), mefloquine (100 μM), microsomal protein (2 mg) and 0.067 M phosphate buffer (pH 7.4) to a final volume of 2.5 mL were performed in Sovirel tubes (15 mL capacity) at 37° with agitation. Microsomal reactions were started by the addition of 1 mM NADPH. Mefloquine and the putative inhibitors were dissolved in methanol which was evaporated to dryness before addition of the other reaction constituents. The range of inhibitor concentrations used for calculation of IC_{50} values was 2.5–500 μM. The reaction was terminated after 3 hr by the addition of 2 mL 0.1 M glycine buffer (pH 3.4).

Radiolabelled unmetabolized mefloquine and its metabolite, carboxymefloquine were extracted into 6 mL of ethylacetate by mechanical tumbling for 20 min. The organic layer was separated by centrifugation (2500 *g* for 10 min) and evaporated to dryness in a Buchler vortex evaporator. Extraction of total radioactivity was greater than 95%. The residue was reconstituted in methanol (50 μL) and an aliquot (20 μl) was analysed by radiometric HPLC (slight modifications from previously published method of Riviere *et al.* [22]).

Carboxymefloquine was identified according to the retention time of the authentic standard (retention time of carboxymefloquine, 2.2 min; retention time of mefloquine, 8.5 min). Separation was performed on a Techopak 10 C-18 (30 cm × 0.39 cm) column protected by an inline guard column. The system was operated at room temperature in an isocratic mode with the mobile phase of 68% methanol and 32% water containing 0.01 M octanesulfonic acid (adjusted to pH 3.4 with phosphoric acid) at a flow rate of 2.2 mL/min.

Initial velocity conditions for enzyme activity were established employing a mefloquine concentration of 100 μM which was linear over 3 hr and up to 5 mg microsomal protein. The coefficient of variation of the rate of metabolite formation between different incubations (N = 6) of the same liver was < 8%.

The kinetic parameters (V_{max} and K_m) of formation of carboxymefloquine (mefloquine concentration of 25–800 μM) were determined in the absence and presence of some of the inhibitors using an iterative programme (ENZPACK) based on non-linear least squares regression analysis to fit the experimental data to the Michaelis–Menten equation. The apparent K_i was determined by substituting K_m and V_{max} values in the appropriate equation [23] and by the graphical method of Dixon [24].

RESULTS

Microsomal protein content was 16.2 ± 2.4 mg/g of liver (mean ± SD, N = 6), and the enzyme activity for carboxymefloquine formation at a substrate concentration of 100 μM was 2.85 ± 0.74 nmol/hr/mg protein. This metabolite was the only one evident in the *in vitro* incubations and was not seen in the absence of NADPH. Enzyme activity was highest in microsomes prepared from a liver obtained from a donor who had been receiving phenobarbitone for more than 20 years (Liver VIII, Fig. 1).

Table 1 shows mean IC_{50} values and enzyme kinetic parameters determined in the absence and presence of alleged inhibitors. Graphical plots for determination of IC_{50} values are presented in Fig. 2. The most potent inhibition was evident with the 1-substituted imidazole antimycotic compound, ketoconazole, and the antimalarial primaquine with IC_{50} values of 7.5 and 17.5 μM, respectively; while for quinine it was 122 μM. Other compounds showed little or no inhibition of the enzyme activity as judged by IC_{50} values of more than 500 μM.

The K_m value for carboxymefloquine formation

Table 1. Characteristics of inhibition of carboxymefloquine formation by various drugs

Inhibitors	K_m (μM)	V_{max} (nmol/hr/mg protein)	IC_{50} (μM)	K_i (μM)	Nature of inhibition
Control	236 ± 46	9.9 ± 1.2	—	—	—
Ketoconazole	243 ± 27*	3.0 ± 0.9*	7.5	11.2	Non-competitive
Primaquine	268 ± 49*	3.3 ± 1.1*	17.5	8.6	Non-competitive
Quinine	331 ± 73†	7.5 ± 2.2†	122	28.5	Mixed
Sulfadoxine	—	—	>500	—	—
Artemether	—	—	>500	—	—
Artesunate	—	—	>500	—	—
Tetracycline	—	—	>500	—	—

Results are expressed as means ± SD (N = 6).
* At the inhibitor concentration of 10 μM.
† At the inhibitor concentration of 25 μM.

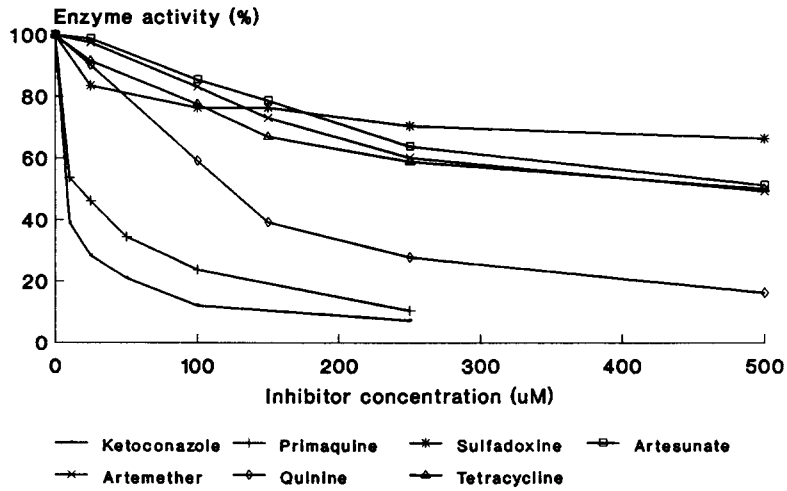

Fig. 2. Effect of ketoconazole, primaquine, sulfadoxine, artesunate, arthemether, quinine and tetracycline on the formation of carboxymefloquine by human liver microsomes. Results are expressed as percentage enzyme activity with the formation of carboxymefloquine in control incubation taken as 100%. Each point represents the mean data from six livers.

determined by regression analysis from Lineweaver–Burk plots with mean data from six livers was 236 ± 46 μM and the V_{max} value was 9.9 ± 1.2 nmol/hr/mg microsomal protein. Ketoconazole and primaquine produced non-competitive inhibition (decrease in V_{max}, no change in K_m) with the respective K_i values being 11.2 and 8.6 μM, while quinine produced mixed inhibition (a greater than 5% change in V_{max} and K_m) with a K_i value of 28.5 μM (Fig. 3).

DISCUSSION

This study has provided some evidence for the involvement of isozyme(s) of cytochrome P450 in the formation of carboxymefloquine, i.e. no metabolite formation when NADPH was omitted from the incubation and marked inhibition of metabolism in the presence of ketoconazole. The antimycotic drug ketoconazole gave non-competitive inhibition (K_i = 11.2 μM) and this is consistent with previous findings of inhibition of the oxidation of many other substrates both *in vitro* and *in vivo* [14, 25–31].

Primaquine was also a potent inhibitor of mefloquine metabolism *in vitro* (K_i = 8.6 μM) and again this is consistent with previous work showing that primaquine as a nitrogen heterocycle binds to the heme site of cytochrome P450. However, despite the *in vitro* inhibition, clinical studies have failed to show any significant effect of coadministered primaquine on the pharmacokinetics of mefloquine [32]. An *in vivo* interaction is very much dependent on plasma (or more importantly, hepatic) concentration of drug. After oral administration of 750 mg mefloquine, peak plasma concentrations are approximately 1500–2000 ng/mL (5 μM) at 4–18 hr [33]. After oral administration of 45 mg primaquine, peak plasma levels of 150–200 ng/mL (0.4 μM) are reached within 2 to 3 hr [3]. The concentration of primaquine attained is thus lower than the K_i value for inhibition of mefloquine metabolism. Resetar *et*

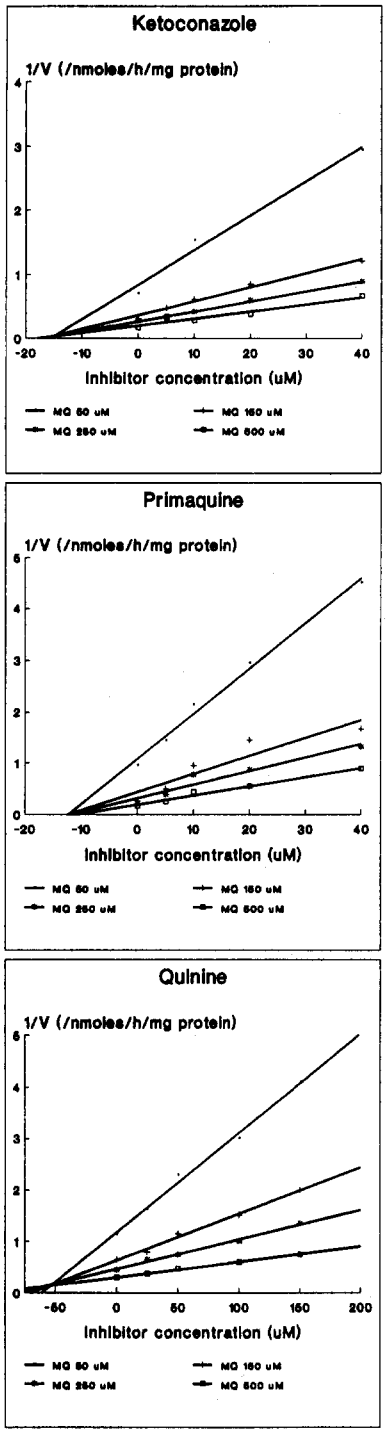

Fig. 3. Dixon plots (reciprocal of velocity, $1/v$, vs inhibitor concentration) showing inhibition of mefloquine metabolism by ketoconazole, primaquine and quinine.

al. [34] have calculated the theoretical percentage inhibition obtainable *in vivo* at physiological (peak plasma) concentrations of a putative inhibitor and substrate using the equation

$$i = 100 \times [I]/(K_i(1 + [S]/K_m) + [I]).$$

Using the same equation (where i = per cent inhibition), primaquine will produce less than 5% inhibition. Another important *in vivo* consideration is that the half-life of primaquine is very much shorter (6–8 hr) than that of mefloquine (15–20 days). In contrast to primaquine, at a peak concentration of 6 μM ketoconazole [35], the calculated inhibition *in vivo* will be 35%.

Quinine inhibited carboxymefloquine formation, while sulfadoxine, artemether, artesunate and tetracycline were found to be very weak inhibitors. Peak concentration of quinine after oral administration of 600 mg quinine sulphate is approximately 20 μM [36] and this translates into a calculated inhibition *in vivo* of approximately 40%. The inference is that in patients receiving both mefloquine and quinine higher blood concentrations of mefloquine will be seen. Sulfadoxine is a drug of particular interest because it is used in combination with mefloquine and pyrimethamine in the preparation MSP (Fansimef®). In a previous clinical study [37], it was shown that when mefloquine was administered as the combination of MSP (mefloquine + sulfadoxine + pyrimethamine) the half-life of mefloquine was slightly prolonged. We suggested the possibility of inhibition of mefloquine metabolism by sulfadoxine. This now seems unlikely.

Clinically important pharmacokinetic interactions have also been shown when tetracycline was coadministered with quinine and mefloquine (Karbwang, unpublished observations). In both studies, plasma levels of quinine or mefloquine were elevated significantly. However, the results from the present study again do not support the role of inhibition of drug-metabolizing enzymes by tetracycline. As tetracycline is excreted into bile and faeces in significant amounts, it may be of interest to investigate further whether there is competition for biliary excretion.

In conclusion, the results of the present study have indicated that some drugs can inhibit the biotransformation of mefloquine by human liver. The concurrent administration of quinine and ketoconazole with mefloquine could lead to clinically important pharmacokinetic interactions.

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REFERENCES

1. Boudreau EF, Webster HK, Pavanand K and Thosingha L, Type II mefloquine resistance in Thailand. *Lancet* **ii**: 1335, 1982.
2. Webster HK, Thaithong S, Pavaranand K, Yong Vainitehit K, Pinswasdi C and Boudreau EF, Cloning and characterization of mefloquine-resistant *Plasmodium falciparum* from Thailand. *Am J Trop Med Hyg* **34**: 1022–1027, 1985.
3. Karbwang J and White NJ, Clinical importance of antimalarial pharmacokinetics. *Asia Pac J Pharmacol* **3**: 181–189, 1988.
4. DeSouza JM, Heizmann P and Schwartz DE, Single dose kinetics of mefloquine in Brazilian male subjects. *Bull WHO* **65**: 353–356, 1987.
5. Franssen G, Rouviex B, Lebras J, Bauchet I and

Verdier F, Divided dose kinetics of mefloquine in man. *Br J Clin Pharmacol* **28**: 179–184, 1989.
6. Schwartz DE, Weber W, Richard-Lenoble D and Gentilini M, Kinetic studies of mefloquine and one of its metabolites Ro 21-5104 in the dog and in man. *Acta Tropica* **37**: 238–242, 1980.
7. Hakanson A, Lingren AL and Bjorkman A, Comparison of the activity *in vitro* of mefloquine and two metabolites against *Plasmodium falciparum*. *Trans Roy Soc Trop Med Hyg* **84**: 503–504, 1990.
8. Coleman MD, Fleckenstein L, Shipley LA and Heiffer MH, Disposition of the antimalarial mefloquine in the isolated perfused rat liver. *Biochem Pharmacol* **37**: 235–239, 1988.
9. Mu JY, Israili ZH and Dayton PG, Studies of the disposition and metabolism of mefloquine HCL (WR 142 490) a quinoline methanol antimalarial in the rat. *Drug Metab Dispos* **3**: 198–210, 1975.
10. Rozman RS, Molek NA and Koby R, The absorption distribution and excretion in mice of the antimalarial mefloquine erythro-2,8-bis(trifluoromethyl)-(2-piperidyl)-4-quinolinemethanol hydrochloride. *Drug Metab Dispos* **6**: 654–658, 1978.
11. von Jauch R, Greisser E and Oesterhelt G, Metabolismus von Ro 21-5998 (mefloquine) bei der Ratte. *Arzneimittel Forsch* **30**: 60–66, 1990.
12. Back DJ, Purba HS, Park BK, Ward SA and Orme ML'E, Effect of chloroquine and primaquine on antipyrine metabolism. *Br J Clin Pharmacol* **16**: 497–502, 1983.
13. Back DJ, Purba HS, Staiger C, Orme ML'E and Breckenridge AM, Inhibition of drug metabolism by the antimalarial drugs chloroquine and primaquine in the rat. *Biochem Pharmacol* **32**: 257–263, 1983.
14. Back DJ, Tjia JF, Kawbwang J and Colbert J, In vitro inhibition studies of tolbutamide hydroxylase activity of human liver microsomes by azoles, sulphonamides and quinolines. *Br J Clin Pharmacol* **26**: 23–29, 1988.
15. Mihaly GW, Ward SA, Nicholl DD, Edwards G and Breckenridge AM, The effects of primaquine stereoisomers and metabolites on drug metabolism in the isolated perfused rat liver and *in vitro* rat liver microsomes. *Biochem Pharmacol* **34**: 331–336, 1985.
16. Murray M, The *in vitro* effects of quinoline derivatives on cytochrome P-450 and aminopyrine *N*-demethylase activity in rat hepatic microsomes. *Biochem Pharmacol* **33**: 3277–3281, 1984.
17. Murray M and Farrell GC, Effect of primaquine on hepatic microsomal haemoproteins and drug oxidation. *Toxicology* **42**: 205–217, 1986.
18. Riviere JH and Back DJ, Effect of mefloquine on hepatic drug metabolism in the rat: comparative study with primaquine. *Biochem Pharmacol* **34**: 567–571, 1985.
19. Riviere JH and Back DJ, Inhibition of ethinyloestradiol and tolbutamide metabolism by quinoline derivatives *in vitro*. *Chem Biol Interact* **59**: 301–308, 1986.
20. Purba HS, Maggs JL, Orme ML'E, Back DJ and Park BK, The metabolism of 17α-ethinyloestradiol by human liver microsomes: formation of catechol and chemically reactive metabolites. *Br J Clin Pharmacol* **23**: 447–453, 1987.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
22. Riviere JH, Back DJ, Breckenridge AM and Howells RE, The pharmacokinetics of mefloquine in man: lack of effect of mefloquine on antipyrine metabolism. *Br J Clin Pharmacol* **20**: 469–474, 1985.
23. Webb JL, Determination of mechanisms and constants of inhibition. In: *Enzyme and Metabolic Inhibitors*, Vol. 1, pp. 141–191. New York, Academic Press, 1963.
24. Dixon M, The determination of enzyme inhibitor constants. *Biochem J* **55**: 170–171, 1953.
25. Back DJ and Tjia JF, Inhibition of tolbutamide metabolism by substituted imidazole drugs *in vivo*: evidence for a structure–activity relationship. *Br J Pharmacol* **85**: 121–126, 1985.
26. Back DJ, Stevenson P and Tjia JF, Comparative effects of two antimycotic agents ketoconazole and terbinafine on the metabolism of tolbutamide, ethinyloestradiol, cyclosporin and ethoxycoumarin by human liver microsomes *in vitro*. *Br J Clin Pharmacol* **28**: 166–170, 1989.
27. Brown MW, Maldonado AL, Meredith CG and Speeg KV, Effect of ketoconazole on hepatic oxidative drug metabolism. *Clin Pharmacol Ther* **37**: 290–297, 1985.
28. Cockburn I, Cyclosporin A: A clinical evaluation of drug interactions. *Trans Proc* **18** (Suppl 5): 50–55, 1986.
29. First MR, Schroeder TJ, Alexander JW, Stephen GW, Weiskittel PW, Myre SA and Pesce AJ, Cyclosporin dose reduction by ketoconazole administration in renal transplant recipients. *Transplantation* **51**: 365–370, 1991.
30. Lavrijson K, Van Houdt J, Thijs D, Mueldermans W and Heykants J, Interaction of miconazole, ketoconazole and itraconazole with rat liver microsomes. *Xenobiotica* **17**: 45–57, 1987.
31. Meredith CG, Maldonado AL and Speeg KV, The effect of ketoconazole on hepatic oxidative drug metabolism in the rat *in vivo* and *in vitro*. *Drug Metab Dispos* **13**: 156–162, 1985.
32. Karbwang J, Na Bangchang K, Thanavibul A, Back DJ and Bunnag D, The pharmacokinetics of mefloquine in the presence of primaquine. *Eur J Clin Pharmacol*, in press.
33. Karbwang J and White NJ, Clinical pharmacokinetics of mefloquine. *Clin Pharmacokinet* **19**: 264-279, 1990.
34. Resetar A, Minick D and Spector T, Glucuronidation of 3'-azido-3'-deoxythymidine catalysed by human liver UDP-glucuronosyltransferase. Significance of nucleoside hydrophobicity and inhibition by xenobiotics. *Biochem Pharmacol* **42**: 559–568, 1991.
35. Sonino N, The use of ketoconazole as an inhibitor of steroid production. *N Engl J Med* **317**: 812–818, 1987.
36. White NJ, Clinical pharmacokinetics of antimalarial drugs. *Clin Pharmacokinet* **10**: 187–215, 1985.
37. Karbwang J, Bunnag D, Breckenridge AM and Back DJ, The pharmacokinetics of mefloquine when given alone or in combination with sulfadoxine and pyrimethamine in Thai male and female subjects. *Eur J Clin Pharmacol* **32**: 173–177, 1987.