Pergamon

*Biochemical Pharmacology*, Vol. 48, No. 9, pp. 1779–1783, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00322-X

# CHARACTERISATION OF PRAZIQUANTEL METABOLISM BY RAT LIVER MICROSOMES USING CYTOCHROME P450 INHIBITORS

COLLEN M. MASIMIREMBWA and JULIA A. HASLER*

Department of Biochemistry, University of Zimbabwe, Mt Pleasant, Harare, Zimbabwe

(*Received* 20 *January* 1994; *accepted* 4 *July* 1994)

**Abstract**—The metabolism of praziquantel (PZQ) was studied in microsomes isolated from livers of differently pretreated rats and in the presence of various inhibitors of cytochrome P450 (P450) isoforms. Microsomes from phenobarbitone (PB)-pretreated rats metabolised PZQ to its major metabolite 4OH-praziquantel (4OH-PZQ) at a greater rate than those from 20-methylcholanthrene (MC) and saline (SA) pretreated rats. The $V_{max}$ for the PB microsomes was 600 nmol 4OH-PZQ formed/mg/min $\times 10^{-3}$ compared to 91.4 nmol/mg/min $\times 10^{-3}$ for MC and 238 nmol/mg/min $\times 10^{-3}$ for SA microsomes. These results indicate that PZQ is metabolised by PB-inducible isoforms of P450. Inhibitor studies were conducted with microsomes from SA-pretreated animals. In these studies, caffeine, disulfuram, and tolbutamide were poor inhibitors of the metabolism of PZQ to 4OH-PZQ, with $I_{50}$ values not determinable. Quinidine and quinine inhibited the hydroxylation of PZQ but with high $K_i$ values. 17$\alpha$-Ethynylestradiol, cimetidine and diphenylhydramine were effective inhibitors of the formation of 4OH-PZQ, with 17$\alpha$-ethynylestradiol being the most potent with a $K_i$ of $0.5 \pm 0.05$ $\mu$M. From the known specificities of these P450 inhibitors, it is therefore concluded that cytochromes P450 1A2, 2E1, 2C9–10, and 2D6 probably do not contribute significantly to the metabolism of PZQ to its major metabolite in rats. It is likely that cytochromes P450 2B1 and 3A, both inducible by PB, are predominantly responsible for the formation of 4OH-PZQ.

*Key words:* praziquantel; praziquantel metabolism; 4-hydroxypraziquantel; cytochrome P450 inhibitors

PZQ,† 2-cyclohexylcarbonyl (1,2,3,6,7,11b) hexahydro-4*H*-pyrazin (2,1-a) isoquino-lin-4-one, is a broadly effective trematode and cestocide [1]. After oral administration, the drug is absorbed rapidly, followed by rapid and extensive metabolism [2]. Praziquantel is subjected to pronounced first-pass metabolism and the major metabolite in serum of humans and animals has been identified as the 4-hydroxycyclohexyl-carbonyl analogue of PZQ (4OH-PZQ) [1, 2]. A number of studies has been done on the pharmacokinetics of PZQ in rats [3, 4] and in humans [3, 5]. The mechanism of action [3, 6] and toxicology [7] of PZQ have also been studied. Other investigations have been directed towards identifying the metabolites of PZQ [8, 9], and a few studies have been carried out on the metabolism of PZQ *in vitro* [10].

Cytochrome P450 is a family of haem proteins which plays a major role in the metabolism of a wide range of xenobiotic and endogenous compounds [11]. Cytochrome P450 exists as multiple isoforms which respond differently to specific inducers [12, 13] and inhibitors [13, 14]. Within each mammalian species there are estimated to be over 20 different cytochrome P450 gene products that have been characterised with regard to their properties and catalytic specificities [14, 15]. Comparison of rat cytochrome P450 isoforms with those found in humans has also been done with a view to improving the extrapolation of findings from animal studies to humans [16]. There are many useful approaches available for determining which cytochrome P450 isoforms make major contributions to the metabolism of a particular drug. These include assessing the effect of specific inhibitors and antibodies on the metabolism of a drug in microsomal preparations. Another approach is to use microsomal preparations from animals that have been pretreated with inducers of specific forms of cytochrome P450 [12, 14].

In pharmacokinetic studies, pretreatment with phenobarbitone resulted in lowered maximum serum PZQ concentrations and reduced its bioavailability in rats [17], whilst pretreatment with the antiepileptics phenytoin and carbamazepine had a similar effect in humans [18]. Pretreatment with dexamethasone also decreased PZQ bioavailability in humans [19]. Induction of the forms of cytochrome P450 that metabolise PZQ is the most likely explanation of the above observed effects. In contrast, in rats, cimetidine, ketoconazole and miconazole each increased the bioavailability of PZQ *in vivo* and inhibited microsomal metabolism of PZQ *in vitro* [20]. These agents are known to inhibit cytochrome P450 isoforms [21], and their effect on PZQ pharmacokinetics and metabolism *in vitro* is probably due to the inhibition of the isoforms of cytochrome P450 that metabolise PZQ. In this study we have characterised PZQ metabolism in hepatic microsomes prepared from rats pretreated with classic inducers and using inhibitors of specific

* Corresponding author. FAX (263) 4 333407.

† Abbreviations: PZQ, praziquantel; 4OH-PZQ, 4-hydroxypraziquantel; PB, phenobarbitone; MC, 20-methylcholanthrene; SA, saline; P450, cytochrome P450.

isoforms of cytochrome P450. The aim of the study was to identify cytochrome P450 isoforms that participate in the metabolism of PZQ to 4OH-PZQ.

## MATERIALS AND METHODS

*Chemicals.* PZQ was a generous gift from Bayer (Bayerwerk, Germany) and 4OH-PZQ a generous gift from Merck (Darmstadt, Germany). Ethyl acetate was purchased from Merck (Darmstadt, Germany) and HPLC-grade acetonitrile was from Caledon Lab Ltd (Georgetown, Ontario, Canada). Sodium phenobarbitone was a generous gift from CAPS Ltd (Harare, Zimbabwe). All biochemicals, 20-methylcholanthrene, and the specific inhibitors of cytochrome P450 isoforms were purchased from Sigma (St Louis, MO, U.S.A.).

*Animal pretreatment.* Male Sprague–Dawley rats, 8 weeks of age and weighing 150–200 g, were obtained from the University of Zimbabwe Animal House. Rats received i.p. injections of either 50 mg/kg sodium PB dissolved in 0.9% NaCl or 40 mg/kg of MC dissolved in olive oil for 4 successive days. Control animals received equivalent volumes of 0.9% NaCl (SA). During pretreatment, the animals were allowed free access to food and water. On the morning of the 5th day, PB, MC and SA pretreated rats were sacrificed and livers removed for preparation of PB, MC and SA microsomes.

*Microsomal metabolism of praziquantel.* Microsomes were prepared by ultracentrifugation [22]. The microsomes were then stored at −80° in 0.1 mM Tris–HCl buffer, pH 7.4, containing 1.0 mM EDTA, and 20% glycerol. The protein concentration was determined by the Lowry method [23] using BSA as standard. Preliminary experiments using PB microsomes established that the hydroxylation of PZQ to 4OH-PZQ depended on the presence of an NADPH-generating system and that this reaction was linear with time for at least 30 min. Subsequent incubations were therefore for 15 min at 37°. The incubation mixture (1.0 mL) comprised PZQ dissolved in 5 μL of DMSO, 500 μL of a 2 mg/mL microsomal suspension, 240 μL of 50 mM Tris–HCl pH 7.4 buffer and an NADPH-generating system. The latter comprised 200 mM DL-isocitric acid, 0.3 mM NADP, 10 mM MgCl and 1.0 unit of isocitric dehydrogenase. In experiments with inhibitors, different concentrations dissolved in 5 μL of either water or DMSO were added to the incubation mixture. The reaction was initiated by adding the isocitric dehydrogenase and allowing the mixture to incubate at 37° under air in a shaking incubator for 15 min. The reaction was terminated by adding 2 vol ice-cold ethyl acetate and vortex mixing. The incubation medium was immediately extracted for 4OH-PZQ with ethyl acetate.

*Sample extraction and analysis.* Each 1.0 mL microsomal incubation medium was extracted for the PZQ metabolite (4OH-PZQ) three times with ethyl acetate (2 mL). The three ethyl acetate extracts were pooled and reduced to dryness by blowing air over the extract in tubes placed in a water-bath at 60°. The dry sample was reconstituted in 200 μL of the mobile phase. Fifty microlitres of this sample was injected on to the HPLC for the determination of 4OH-PZQ. A Varian 9000 HPLC was used with a RP-18 (5 μm, 150 × 4.6 mm) column; the mobile phase was acetonitrile:water (30:70 v/v), at a flow rate of 0.8 mL/min with a column temperature of 30°. The recovery of 0.75 μg/mL 4OH-PZQ added to a microsomal suspension was 98% with a coefficient of variation of 3.3% and N = 7.

*Enzyme kinetics.* Data were plotted using the ENZFIT program for Lineweaver–Burke plots to determine the $K_m$ and $V_{max}$ values for PZQ metabolism in PB, MC and SA microsomes. Dixon plots were generated from the inhibition studies to determine the $K_i$ values of the four most potent inhibitors of PZQ metabolism to 4OH-PZQ.

*Data analysis.* A paired two-tailed Student's *t*-test was used to compare the differences in the Michaelis–Menten parameters for PZQ hydroxylation to 4OH-PZQ in microsomes from differently pretreated rats.

Table 1. Michaelis–Menten parameters for formation of 4-hydroxypraziquantel from praziquantel in rat liver microsomes

| Microsomes | $K_m$ (μM) | $V_{max}$ (nmol/mg/min × $10^{-3}$) |
|---|---|---|
| PB | 90.2 ± 23.1 | 600 ± 143 |
| MC | 32.5 ± 26.2* | 92 ± 21† |
| SA | 31.4 ± 5.9† | 238 ± 12† |

For each set of microsomes, N = 3.
Significantly different from the PB microsomes, at: *P < 0.05, †P < 0.005.

## RESULTS

### *Michaelis–Menten kinetics*

$K_m$ and $V_{max}$ values, calculated from Lineweaver–Burke plots for the metabolism of PZQ to 4OH-PZQ mediated by microsomes from PB, MC and SA pretreated rats, are shown in Table 1. Figure 1 shows the plots of rate of 4OH-praziquantel production with varying concentrations of praziquantel by microsomes from differently pretreated

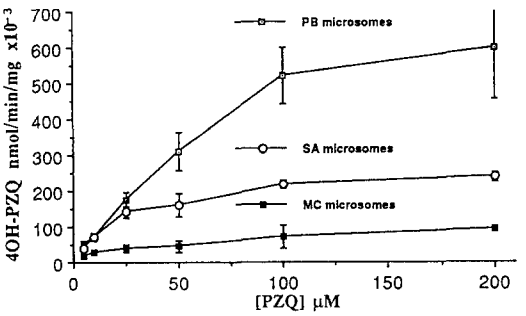


Fig. 1. Plots of rate of 4OH-praziquantel production vs varying concentrations of praziquantel by microsomes from SA-, MC- and PB-pretreated rats. For each differently pretreated group of rats, N = 3.

Table 2. Inhibition of praziquantel hydroxylation by specific substrates/inhibitors of cytochrome P450s

| P450 | Substrate/ inhibitor | 4OH-PZQ formation (% of control) at [$I$] = 1 mM | $I_{50}$ ($\mu$M) | Approximate* $K_m$ and $K_i$ ($\mu$M) |
|---|---|---|---|---|
| 1A2 | caffeine | 60.4 ± 4.7 | — | 500–1000† |
| 2B1 | diphenhydramine | 18.1 ± 1.2 | 250 | — |
| 2C9-10 | tolbutamide | 58.3 ± 1.2 | — | 224.4 |
| 2D6 | quinidine | 13.1 ± 7.2 | 196.4 | 20.1 |
| | quinine | 31.1 ± 4.4 | 446.4 | 3.5 |
| 2E1 | disulfiram | 60.8 ± 5.4 | — | — |
| 3A | cimetidine | 33.6 ± 8.3 | 339 | 25–100§ |
| | 17$\alpha$-ethynyl-estradiol | 13.4 ± 1.1‡ | 2.9 | — |

N = 3.
* $K_i$ and $K_m$ from Refs [24 and 25].
† Human data; ‡ at [$I$] = 25 $\mu$M.

rats. The $K_m$ for PB microsomes was greater than that for MC and SA microsomes, the differences being statistically significant. The $V_{max}$ values obtained were statistically significantly different from each other, in the order PB > SA > MC microsomes.

*Inhibition of praziquantel hydroxylation*

A range of inhibitor concentrations was used to generate plots of percentage rate of 4-hydroxylation vs inhibitor concentration for eight compounds that inhibit cytochrome P450 (plots not shown). Table 2 shows the $I_{50}$ values obtained from these plots for compounds which inhibited PZQ hydroxylation in microsomes from untreated animals by more than 50% in the concentration ranges used. For the less potent inhibitors, only the percentage PZQ hydroxylation compared to the controls at the highest inhibitor concentration of 1.0 mM was recorded. From these plots, caffeine, tolbutamide and disulfiram were found to be poor inhibitors of PZQ hydroxylation whilst 17$\alpha$-ethynylestradiol was the most potent inhibitor.

The four inhibitors which inhibited PZQ hydroxylation by more than 50% were further investigated by determining their $K_i$ values and the mode of inhibition. Dixon plots for the inhibitory effects of quinidine and diphenhydramine (Fig. 2) and 17$\alpha$-ethynylestradiol and cimetidine (Fig. 3) were used to calculate $K_i$ values and assign mode of inhibition (Table 3).

## DISCUSSION

The results of our study show that PZQ is metabolised to its major metabolite, 4OH-PZQ, to a much greater extent by microsomes from PB-pretreated rats than by microsomes from MC- and SA-pretreated rats. The finding that PB induction enhances the metabolism of PZQ is in agreement with our *in vivo* study in which PZQ bioavailability and maximum serum levels were greatly decreased in PB-pretreated rats as compared to MC-pretreated rats and the SA control rats [17]. The $K_m$ was unexpectedly higher for the PB microsomes as

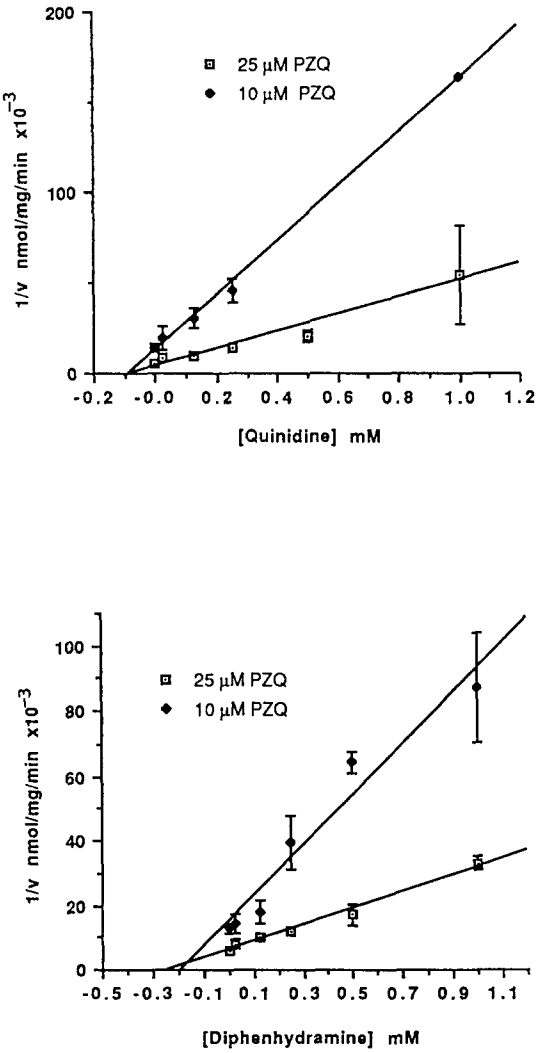


Fig. 2. Dixon plots illustrating the inhibition of praziquantel hydroxylation by quinidine and diphenhydramine in microsomes from livers of saline pretreated rats. For each study, N = 3.

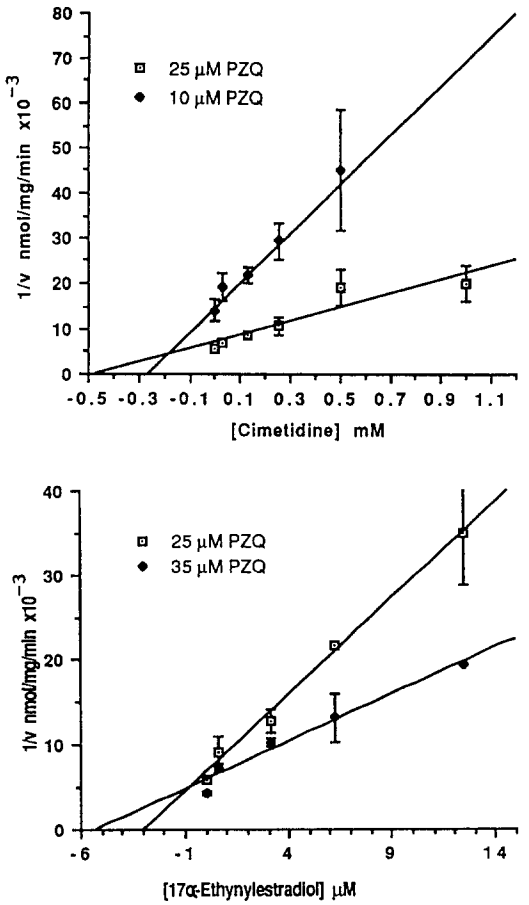


Fig. 3. Dixon plots illustrating the inhibition of praziquantel hydroxylation by cimetidine and by 17$\alpha$-ethynylestradiol in microsomes from livers of saline pretreated rats. For each study, N = 3.

compared to MC and SA microsomes, and this could imply that there is an inducible (at least by PB) microsomal P450 which has a lower affinity for PZQ. It should be noted here that CYP2B and CYP3A forms are inducible by PB and steroids [13].

Specific inhibitors were used in an attempt to identify the forms of P450 that contribute to PZQ metabolism. Caffeine, tolbutamide and disulfiram in concentrations in excess of their $K_m$ and/or $K_i$ values with respect to prototype substrates in humans did not significantly inhibit PZQ hydroxylation. This suggests that cytochromes P450 1A2, 2C isoforms and 2E1 for which the compounds are markers, respectively [14, 24], are not involved to any great extent in the hydroxylation of PZQ to its major metabolite 4OH-PZQ. Quinidine and quinine inhibited PZQ metabolism with $K_i$ values of 126 and 110 $\mu$M, respectively. As these are in excess of their typical $K_i$ values, this suggests minor participation of cytochrome P450 2D1 for which quinidine and quinine are inhibitors [25], with the human orthologue being 2D6. It should, however, be taken into account that quinidine might be inhibiting cytochrome P450 3A isoforms for which it is a substrate [15].

Table 3. $K_i$ values and modes of inhibition of praziquantel hydroxylation in saline pretreated rat liver microsomes for some species-specific inhibitors of cytochrome P450 (N = 3)

| Substrate/inhibitor | $K_i$ ($\mu$M) | Type of inhibition |
|---|---|---|
| quinidine | 110.0 ± 33 | non-competitive |
| diphenhydramine | 96.7 ± 37.9 | competitive |
| quinine | 120.0 ± 14 | competitive |
| cimetidine | 253.0 ± 42 | competitive |
| 17$\alpha$-ethynylestradiol | 0.5 ± 0.05 | competitive |

Diphenhydramine, cimetidine and 17$\alpha$-ethynylestradiol significantly inhibit PZQ hydroxylation. Diphenhydramine is a specific inhibitor of cytochrome P450 2B1 whilst cimetidine and 17$\alpha$-ethynylestradiol are inhibitors for 3A isoforms of cytochrome P450 [12, 14]. 17$\alpha$-Ethynylestradiol is known to destroy selectively CYP3A forms by metabolite intermediate complexation [26], and has also been shown to be a substrate of the same enzyme [27]. The 17$\alpha$-ethynylestradiol could therefore be interacting with CYP3A forms through a biphasic mechanism of inhibition: the observed competitive inhibition (Fig. 3) being its competition with PZQ for the CYP3A active site and the dramatic reduction in the production of 4OH-PZQ, with increasing inhibitor concentration being due to the mechanism-based inactivation of the CYP3A forms.

The significant inhibition of PZQ hydroxylation by these compounds implies an involvement of the 2B and 3A isoforms of cytochrome P450 in the metabolism of PZQ. Two forms of cytochrome P450 2B (2B1 and 2B2) are induced in rat liver by phenobarbital but only 2B2 is expressed in the untreated animal [16]. Although one 2B (2B6) gene has been found in humans, expression of this gene has not been demonstrated [28]. The finding that 2B isoforms might play an important role in PZQ hydroxylation in rats therefore is likely to be of no significance for human metabolism of praziquantel.

Cytochrome P450 3A isoforms, however, are found in both species (3A1 and 3A2 in rat and 3A3, 4, 5 and 7 in humans) and are induced by a number of the same compounds including phenobarbital [13, 16, 29]. Although it is usually not reliable to extrapolate animal model findings to humans, it has been shown that the rat and human CYP3A forms exhibit comparable specificities and have similar mechanisms of regulation [30]. The suggestion that praziquantel is metabolised by 2B and 3A isoforms of cytochrome P450 could explain the decreased bioavailability of PZQ and its lowered maximum serum levels in rats pretreated with PB [17], in humans pretreated with dexamethasone [19] and in phenytoin and carbamazepine pretreated humans [18], compounds which are now known to induce CYP3A forms [31].

In the evaluation of the safety of drugs and other

chemicals there is an interest in determining their potential for induction or inhibition of the enzymes of drug metabolism. Such interactions may alter the pharmacological effects of concurrent medication, modify the toxicity or carcinogenicity of xenobiotics, or alter the levels of important endogenous compounds [29]. Knowledge of the specific forms of cytochrome P450 that are predominantly involved in the metabolism of PZQ assists in making predictions about drug interactions due to induction or inhibition. From our studies, we would anticipate that agents and factors affecting the cytochrome P450 3A status of an individual could be expected to alter PZQ metabolism. Further studies on the metabolism of PZQ using antibodies and heterologous expression systems for individual cytochrome P450 isoforms would provide conclusive evidence of the specific cytochrome P450 isoforms involved.

*Acknowledgements*—The authors gratefully acknowledge the financial support of the International Program in Chemical Sciences, Uppsala University, Sweden, and the Research Board, University of Zimbabwe.

## REFERENCES

1. Andrews P, Thomas H, Pohlke R and Seubert J, Praziquantel. *Med Res Rev* **3**: 147–200, 1983.
2. Groll E, Praziquantel. *Adv Pharmacol Chemother* **20**: 219–238, 1984.
3. Andrews P, Praziquantel: Mechanisms of anti-schistosomal activity. *Pharmacol Ther* **29**: 129–156, 1985.
4. Kokwaro GO and Taylor G, Effect of experimentally induced hepatic cirrhosis on the pharmacokinetics of orally administered praziquantel in rats. *Eur J Drug Metab Pharmacokinet* **15**: 199–202, 1990.
5. Jung H, Vazquez ML, Sanchez M, Penagos P and Sotelo J, Clinical Pharmacokinetics of praziquantel. *Proc West Pharmacol Soc* **34**: 334–340, 1991.
6. Harnett W, The antihelminthic action of praziquantel. *Parasitol Today* **4**: 144–146, 1988.
7. Frohberg H, Results of toxicological studies on praziquantel. *Arzneimittelforsch/Drug Res* **34**: 1137–1144, 1984.
8. Ali MH, Fetterolf DD, Abramson FP and Cohn VH, Metabolism studies of the antischistosomal drug praziquantel using Tanden Mass Spectrometry: qualitative identification of 17 hydroxylated metabolites from mouse urine. *Biomed Environ Mass Spectrom* **19**: 179–185, 1990.
9. Diekmann HW and Buhring KU, The fate of praziquantel in the organism III. Metabolism in rat, beagle dog and rhesus monkey. *Eur J Drug Metab Pharmacokinet* **2**: 107–111, 1976.
10. Hogemann A, Kiec-Kononowicz K, Westhoff F and Blascke G, Microsomal oxidation of praziquantel. *Arzneimittelforsch/Drug Res* **40**: 1159–1162, 1990.
11. Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsulus IC, Gotoh O, Okuda K and Nebert DW, The P450 superfamily: update on new sequencies, gene mapping, accession numbers, early trivial names of enzymes and nomenclature. *DNA Cell Biol* **12**: 1–51, 1993.
12. Murray M and Gordon FR, Selectivity in the inhibition of mammalian cytochromes P450 by chemical agents. *Pharmacol Rev* **42**: 85–100, 1990.
13. Okey AB, Enzyme induction in the cytochrome P450 enzymes. *Pharmacol Ther* **45**: 241–298, 1990.
14. Guengerich FP, Characterization of human cytochrome P450 enzymes. *FASEB J* **6**: 745–748, 1992.
15. Guengerich FR, Enzymatic oxidation of xenobiotic chemicals. *CRC Crit Rev Biochem Mol Biol* **25**: 97–153, 1990.
16. Soucek P and Gut I, Cytochrome P450 in rats: structures, functions, properties and relevant human forms. *Xenobiotica* **22**: 83–103, 1992.
17. Masimirembwa CM, Naik YS and Hasler JA, Effect of phenobarbitone and 3-methylcholanthrene pretreatment on the pharmacokinetics of praziquantel in rats. *Eur J Drug Metab Pharmacokinet* **18**: 261–264, 1993.
18. Bittencourt PRM, Gracia CM, Martins R, Fernandes AG, Diekmann HW and Jung W, Phenytoin and carbamazepine decrease oral bioavailability of praziquantel. *Neurology* **42**: 492–496, 1992.
19. Vazquez ML, Jung H and Sotelo J, Plasma levels of praziquantel decrease when dexamethasone is given simultaneously. *Neurology* **37**: 1561–1562, 1987.
20. Diekmann HW and Overbosch D, Inhibitory effects of cimetidine, ketoconazole and miconazole on the metabolism of praziquantel. *Acta Leiden* **57**: 217–228, 1989.
21. Murray M, Mechanism of the inhibition of cytochrome P450 mediated drug oxidation by therapeutic agents. *Drug Metab Rev* **18**: 55–81, 1987.
22. van der Hoeven TA, Isolation of microsomes by polyethylene glycol 600 fractionation of the post-mitochondrial fraction. *Anal Biochem* **115**: 398–402, 1981.
23. Lowry OH, Rosebrough WJ, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
24. Loft S, Otton SV, Lennard MS, Tucker GT and Poulsen HE, Characterisation of metronidazole metabolism by human liver microsomes. *Biochem Pharmacol* **41**: 1127–1134, 1991.
25. Strobl G, Heinrich B, Wolff T and Guengerich FP, Inhibition of Bufuralol-1'hydroxylation in rat and human liver: *in vitro* studies and inhibitor modelling. In: *Cytochrome P450: Biochemistry and Biophysics, Proceedings of the Sixth International Conference on Biochemistry and Biophysics of Cytochrome P450* (Eds. Taylor and Francis), pp. 407–410. London, 1989.
26. Murray M, P450 Enzymes inhibition mechanisms, genetic regulation and effects of liver disease. *Clin Pharmacokinet* **23**: 132–146, 1992.
27. Guengerich FP, Oxidation of 17$\alpha$-ethynylestradiol by human liver cytochrome P450. *Mol Pharmacol* **33**: 500–508, 1988.
28. Wrighton SA and Stevens JC, The human hepatic cytochromes P450 involved in drug metabolism. *CRC Crit Rev Toxicol* **22**: 1–21, 1992.
29. Boobis AR, Sesardic D, Murray BP, Edwards RJ, Singleton AM, Rich KJ, Murray, Torre DL, Segura J, Pelkonen O, Pasanen M, Kobayashi S, Zhi-Guang T and Davies DS, Species variation in the response of the cytochrome P450-dependent monooxygenase system to inducers and inhibitors. *Xenobiotica* **20**: 1139–1161, 1990.
30. Smith DA, Species differences in metabolism and pharmacokinetics: are we close to an understanding? *Drug Metab Rev* **23**: 355–373, 1991.
31. Barry M and Feely J, Enzyme induction and inhibition. *Pharmacol Ther* **48**: 71–94, 1990.