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CHARACTERIZATION OF METRONIDAZOLE METABOLISM BY HUMAN LIVER MICROSOMES

STEFFEN LOFT,*† S. VICTORIA OTTON,‡§ MARTIN S. LENNARD,‡ GEOFFREY T. TUCKER‡ and HENRIK E. POULSEN*

*Department of Pharmacology, University of Copenhagen, Denmark and ‡Department of Medicine and Pharmacology, University of Sheffield, Royal Hallamshire Hospital, Sheffield, U.K.

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Abstract—The metabolism of metronidazole was studied in microsomes isolated from livers of human kidney donors. The formation of the major *in vivo* metabolite, hydroxymetronidazole, proceeded according to biphasic kinetics, suggesting the involvement of at least two enzymatic sites. The affinity constant (K_m) of the high affinity site ranged from 140 to 320 μM and metabolism at this site contributed more than 75% of the intrinsic clearance. Thus, at therapeutic doses of metronidazole most of the hydroxylation *in vivo* should be associated with this site. Antipyrine, cimetidine, α-naphthoflavone, caffeine, theophylline, mephenytoin, tolbutamide, quinidine, acetone and nifedipine were poor inhibitors of the formation of hydroxymetronidazole by human liver microsomes. Propranolol (500 μM) inhibited the hydroxylation rate by 70%. Phenacetin inhibited metronidazole hydroxylation with a competitive inhibition constant (K_i) of 4–5 μM. However, metronidazole did not inhibit the O-deethylation of phenacetin. It is concluded that cytochromes P450 IA2, IIC9, IIC10, IID6, IIE1 and IIIA3 do not contribute significantly to the high affinity hydroxylation of metronidazole in man.

The antimicrobial drug, metronidazole, has been proposed as a probe for the study of cytochrome P450 activity *in vivo* [1–3]. In man as well as the rat, the clearance of metronidazole can be measured using a single sample of saliva and the contribution of each elimination pathway can be assigned from the assay of collected urine [1–3]. In man hydroxylation of the methyl group of metronidazole accounts for more than 50% of a dose and this reaction is inducible by treatment with phenobarbitone [2, 4]. The minor pathways of metronidazole elimination are oxidation of the alcohol moiety, glucuronidation and renal clearance of the unchanged compound [2]. Previous data obtained in man and rat suggest that different cytochromes P450 metabolize metronidazole and the established probe drug, antipyrine [1–5]. Thus, the simultaneous use of these two drugs in a cocktail for the non-invasive study of drug metabolism capacity is suggested [2]. However, the *in vitro* kinetics of metronidazole metabolism have not been studied in man and the specific cytochromes P450 involved have not been identified. Substrates of the same enzyme will act as mutually competitive inhibitors and substrates and/or inhibitors of specific cytochromes P450 may be used to map their involvement in a particular reaction [6–9].

In the present study we investigated the kinetics of metronidazole metabolism by microsomes prepared from the livers of kidney transplant donors and the inhibitory effects of selected substrates and inhibitors of specific cytochromes P450 [10]. These were caffeine, theophylline, phenacetin and α-naphthoflavone (P450IA2) [11, 12], mephenytoin (P450IIC8-10) [13], tolbutamide (P450IIC10) [14], quinidine (P450IID6) [15], acetone (P450IIE1) [16] and nifedipine (P450IIIA3) [17].

MATERIALS AND METHODS

Chemicals. Reference compounds for the analysis of metronidazole and its metabolites were kindly donated by Rhône-Poulenc Pharma (Vitry, France) and Dumex Ltd (Copenhagen, Denmark). The hydrochloride salts of propranolol and quinidine were purchased from the Sigma Chemical Co. (Poole, U.K.) Phenacetin and paracetamol were obtained from Hopkins and Williams Ltd (Chadwell Heath, U.K.) Cimetidine was from Smith, Kline and French Ltd (Welwyn Garden City, U.K.) Tolbutamide was purchased from the Upjohn Company (Kalamazoo, MI). Antipyrine and theophylline were from BDH Chemicals (Poole, U.K.). Nifedipine was obtained from Bayer UK Ltd (Newbury, U.K.). Laboratory reagent grade acetone was purchased from May and Baker Ltd (Dagenham, U.K.) Racemic mephenytoin was from Sandoz (Dorval, PQ, Canada). Caffeine was obtained from Sigma (Milwaukee, WI). α-Naphthoflavone was purchased from Aldrich (Steinheim, F.R.G.). Glucose-6-phosphate (G6P)‖, NADP and glucose-6-phosphate dehydrogenase (G6PD), grade II, were purchased from Boehringer Mannheim (Lewes, U.K.).

Source of human liver. Small samples of human liver were obtained from six kidney transplant donors (HL1-HL6) with the approval of the Ethics

† To whom correspondence should be addressed at: Department of Pharmacology, University of Copenhagen, Juliane Mariesvej 20, DK-2100 Copenhagen Ø, Denmark.

§ Current address: Addiction Research Foundation, 33 Russell St., Toronto, Ontario, Canada.

‖ Abbreviations used: K_m, affinity constant; V_{max}, maximal velocity; K_i, competitive inhibition constant; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase.

Table 1. Characteristics of liver donors

Liver no.	Age (years)	Sex	Cause of death	Drug history	
				Pre-admission	In hospital
HL1	29	Male	Not known	Not known	Not known
HL2	49	Male	Road traffic accident	None	Ampicillin, cloxacillin, frusemide, mannitol, metronidazole, pancuronium, phenoperidine, phenytoin
HL3*	58	Male	Posterior fossa bleed and pneumonia	None	Chlorpromazine, dexamethasone, dobutamine, dopamine, frusemide, heparin, hydralazine, phenoxybenzamine, phentolamine
HL4	46	Male	Cerebrovascular incident	Cimetidine, indomethacin, prochlorperazine	Bretylium, chloropromazine, dexamethasone, frusemide, heparin, insulin, lignocaine, phenoxybenzamine, phentolamine
HL5	54	Male	Not known	Not known	Not known
HL6	Not known	Male	Road traffic accident	None	Alfetanil, atracurium, cefuroxime, dopamin, frusemide, metronidazole, midazolam, morphine, propofol, ranitidine, verapamil

* Characterized *in vitro* as a poor hydroxylator of debrisoquine and sparteine.

Committee of the Royal Hallamshire Hospital and the local Coroner. Clinical details of the donors are listed in Table 1. Based on independent *in vitro* analyses, the donor of liver HL3 was phenotypically a poor metabolizer of sparteine and debrisoquine.

Preparation of microsomes. Liver microsomes were prepared by differential centrifugation as described previously [18]. The microsomal protein content was measured by the method of Lowry *et al.* [19] using bovine serum albumin (fraction V) as the standard.

Incubation conditions. Preliminary experiments established that the hydroxylation of metronidazole depended on the presence of a NADPH-generating system and that this reaction was linear with time for at least 120 min and with respect to the content of microsomal protein up to 3 mg/mL. The acetic acid metabolite, which is formed by oxidation of the alcohol moiety of metronidazole, was detected in trace amounts only.

The incubation mixture (0.5 mL) comprised metronidazole dissolved in 0.1 mL 1.15% KCl, 0.1 mL (w/v) microsomal suspension (equivalent 0.75 mg protein), 0.1 mL 1.15% (w/v) KCl and 0.2 mL 0.2 M phosphate buffer (pH 7.4) containing an NADPH-generating system. The latter comprised 2 μmol G6P, 0.2 μmol NADP, 1 μmol $MgCl_2$ and 0.2 units G6PD. The mixtures were incubated at 37° under air in a shaking bath for 90 min. The reaction was stopped by adding 0.1 mL $ZnSO_4$ on ice followed by centrifugation at 900 *g* for 10 min. The supernatant was analysed for parent compound and metabolites.

Enzyme kinetics. Kinetic studies were performed in microsomal preparations from each of six human livers using 10–11 metronidazole concentrations ranging from 25 μM to 30 mM. Less than 3% of the substrate was consumed during the incubation.

Inhibition of metronidazole hydroxylation. The inhibition of metronidazole hydroxylation was studied with microsomes from HL1, HL3 and HL6 at a substrate concentration of 100 μM in duplicate experiments. Water-soluble compounds tested for inhibition of metronidazole hydroxylation were added in 0.1 mL 1.15% w/v KCl. These were caffeine (500 μM), theophylline (500 μM), phenacetin (10 μM), quinidine (500 μM), tolbutamide (500 μM), acetone (5 mM), propranolol (500 μM), cimetidine (500 μM) and antipyrine (500 μM and 5 mM). Water insoluble test compounds were added in 5 μL of DMSO:water (1:1, v/v). These were α-naphthoflavone (20 μM), mephenytoin (200 μM) and nifedipine (200 μM). Control incubation mixtures contained an equal amount of DMSO, which *per se* inhibited the rate of metronidazole hydroxylation by approximately 30%.

Metronidazole-phenacetin coinhibition. The inhibition of metronidazole hydroxylation by phenacetin and paracetamol was studied with microsomes from HL1, HL3 and HL6 using substrate concentrations of 50, 100 and 200 μM and inhibitor concentrations of 0, 10, 20 and 30 μM. The effect of metronidazole (0, 50, 100 and 200 μM) on the O-deethylation of phenacetin 20 μM was studied on two occasions with microsomes from HL1 using 0.4 mg of microsomal protein/mL incubation mixture and a 10 min incubation time. Preliminary studies had established that after incubation with 1.5 mg of protein/mL for 90 min most of the substrate was O-deethylated to paracetamol by a process requiring the presence of a NADPH-generating system.

Analysis of metronidazole and metabolites. Aliquots (200 μL) of the supernatant of the incubation mixture were mixed with acetonitrile (300 μL) containing the internal standard (1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole). After centrifugation, 250 μL of the supernatant was transferred to a microvial and left to evaporate overnight until approximately 60 μL remained. Fifteen microlitres

of the residue was injected onto a 15 cm Spherisorb ODS 5 μ HPLC column [5]. The eluent was phosphate buffer (pH 4.5)/methanol/acetonitrile 90/4/6 (v/v/v) monitored at 320 nm. The limit of assay for hydroxymetronidazole was 20 nM, corresponding to a hydroxylation rate of 0.15 pmol/min/mg protein. The inter-day coefficient of variation for 90 duplicate samples averaged 5% in the relevant concentration range studied.

Analysis of phenacetin and paracetamol. Aliquots (100 μL) of the supernatant of the incubation mixture were mixed with an equal volume of 2 N perchloric acid containing antipyrine as internal standard. Ten to twenty microlitres of the supernatant after centrifugation was injected onto the HPLC column. For phenacetin analysis a 15 cm Nucleosil ODS 5 μ column was eluted with methanol/water 45/55 (v/v) and the effluent was monitored at 254 nm. For measurement of paracetamol a 25 cm μBondapak ODS 10 μ column was eluted with phosphate buffer (pH 4.4)/methanol 92/8 (v/v) and the conductivity of the effluent was monitored with a LCA15 electrochemical detector set at 0.6 V with a 1 μA full range deflection. The assay limit of paracetamol was 20 nM, corresponding to a de-ethylation rate of 5 pmol/min/mg protein. The inter-day coefficient of variation for 42 duplicate samples averaged 7% in the concentration range studied.

Data analysis. The rate of appearance of hydroxymetronidazole was fitted by one and two site Michaelis–Menten equations using the BMDP AR program (Berkeley, CA) for non-linear regression. The residual sums of squares were compared between the two kinetic models using the F-test. Similarly, data on the effect of phenacetin on the hydroxylation of metronidazole were fitted by an equation for competitive inhibition. All data were weighted by the reciprocal of the rate of metabolism. P-values less than 0.05 were considered to indicate statistical significance.

RESULTS

Enzyme kinetics

For microsomes from all six livers the relationship between the rate of metronidazole hydroxylation and substrate concentration was best described by a two-site Michaelis–Menten equation ($P < 0.05$; Fig. 1; Table 2). The affinity constant (K_m) of the high affinity site varied approximately two-fold and there was a more than 10-fold variation in the corresponding maximal velocity (V_{max}) (Table 2). The K_m values of the low affinity site varied 16-fold and were in the millimolar range. Corresponding V_{max} values varied 4-fold (Table 2). At a substrate concentration of 100 μM the low affinity site contributed 20, 16 and 18% of the hydroxylation rate of metronidazole in microsomes from livers HL1, HL3, and HL6, respectively.

Inhibition of metronidazole hydroxylation

In microsomes from the three livers investigated, cimetidine and antipyrine, even at concentrations equivalent to their published K_m values or competitive inhibition constants (K_i) with respect to other substrates, inhibited the rate of metronidazole hydroxylation by 30% or less (Table 3). Propranolol (500 μM) inhibited the hydroxylation rate of metronidazole by 70% (Table 3).

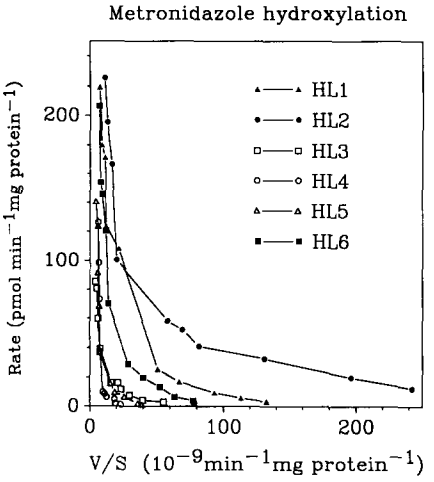

Fig. 1. Eadie–Hofstee plots of the kinetics of metronidazole hydroxylation by microsomes from six different human livers.

Even at concentrations in excess of their K_m values or K_i values with respect to prototype substrates, none of the investigated substrates or inhibitors of cytochromes P450 of family II and III had marked effects on the hydroxylation of metronidazole (Table 4).

Metronidazole–phenacetin coinhibition

Caffeine, theophylline and α-naphthoflavone, which are both substrates and inhibitors of cytochrome P450IA2, had minimal effects on the formation rate of hydroxymetronidazole. However, another substrate of cytochrome P450IA2, phenacetin, in a concentration within the range of published K_m values, inhibited the rate of metronidazole hydroxylation by approximately 25% (Table 4). Thus, the effect of phenacetin was investigated more thoroughly (Fig. 2). Most of the added phenacetin was metabolized during the 90 min incubation using the high microsomal protein content necessary for the determination of the metronidazole hydroxylation rate. Accordingly, the log average concentration of phenacetin over the incubation period was defined as the inhibitor concentration [26]. With microsomes from livers HL1 and HL3 the $K_i \pm$ SD values of phenacetin with respect to hydroxylation of metronidazole were 4.3 ± 1.8 μM and 5.2 ± 1.6 μM, respectively, using a one site enzyme model. Identical K_i values were obtained using a two site enzyme model with competitive inhibition of the high affinity site. Dixon (Fig. 2) and Cornish-Bowden (not shown) plots were not completely compatible with competitive inhibition. However, this may have been obscured by the contribution of the low affinity site and/or uncertainty about the effective phenacetin concentration. When the initial concentrations of phenacetin were used

Table 2. Michaelis–Menten parameters (with SD) of metronidazole hydroxylation in human liver microsomes

Liver no.	High affinity site		Low affinity site		
	K_{m1} (μM)	V_{max1} pmol/min/mg protein	K_{m2} (mM)	V_{max2} pmol/min/mg protein	Contribution to CL_{int} (%)
HL1	239 ± 199	25 ± 11	14 ± 6	279 ± 38	15
HL2	166 ± 41	49 ± 6	38 ± 18	517 ± 160	4.5
HL3*	268 ± 104	13 ± 3	26 ± 10	174 ± 39	12
HL4	140 ± 73	3.2 ± 0.8	96 ± 35	717 ± 221	24
HL5	221 ± 161	8.7 ± 3.4	64 ± 28	433 ± 135	15
HL6	321 ± 77	23 ± 4	29 ± 6	345 ± 37	14

Incubation of 1.5 mg microsomal protein/mL with metronidazole 25–30000 μM (n = 10–11) for 90 min
* Characterized *in vitro* as a poor hydroxylator of debrisoquine and sparteine.
$CL_{int} = V_{max1}/K_{m1} + V_{max2}/K_{m2}$: intrinsic clearance.

Table 3. Effect of various substrates and inhibitors of cytochromes P450 on the rate of metronidazole hydroxylation by human liver microsomes

Substrate/ inhibitor	K_m or K_i (μM)	Inhibitor concentration (μM)	Hydroxylation rate (% of control)*		
			HL1	HL3	HL6
Antipyrine [20]	1000–7000	500	94	88	96
Antipyrine [20]	1000–7000	5000	ND	68	83
Cimetidine [8, 21]	25–1000	500	84	80	76
Propranolol [18, 21, 22]	5–40	500	22	47	12

* Incubation of 1.5 mg microsomal protein/mL with metronidazole 100 μM for 90 min; values are the mean of duplicate experiments.
ND, not determined.

Table 4. Inhibition of metronidazole hydroxylation by specific substrates/inhibitors of human cytochromes P450

P450 gene	Trivial name	Substrate/ inhibitor	Approximate K_m or K_i (μM)	Inhibitor concentration (μM)	Hydroxylation rate (% of control)*		
					HL1	HL3	HL6
IA2	$P450_{PA}$	Phenacetin [6, 11, 22]	4–40	10	76	74	64
		Caffeine [11, 12, 23]	500–1000	500	99	101	86
		Theophylline [9, 24]	500–1000	500	107	71	99
		α-Naphthoflavone [11, 12, 23]	1–5	20	81	73	101
IIC8–10	$P450_{MP}$	Mephenytoin [13]	100	200	73	78	97
IIC10	$P450_{TB}$	Tolbutamide [14, 25]	100	500	86	81	88
IID6	$P450_{DB}$	Quinidine [15]	0.05	500	53	49	66
IIE1	j	Acetone† [16]	1500	5000	74	87	80
IIIA3	$P450_{NF}$	Nifedipine [17]	10	200	73	90	95

* Incubation of 1.5 mg microsomal protein/mL with metronidazole 100 μM and inhibitor for 90 min; values are the mean of duplicate experiments.
† Rat data.

in the data analysis of the inhibition of metronidazole hydroxylation by microsomes from livers HL1 and HL3, the K_i ± SD values were found to be 16 ± 2 and 14 ± 4 μM, respectively, and the Dixon and Cornish-Bowden plots (not shown) were compatible with competitive inhibition. The possibility that the effect of phenacetin was mediated through paracetamol generated by O-deethylation could be

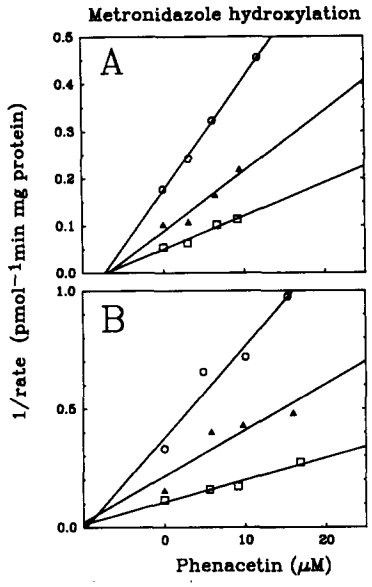

Fig. 2. Dixon plots illustrating the inhibition of metronidazole hydroxylation by phenacetin in microsomes from two human livers, HL1 (A), HL3 (B). The log average phenacetin concentration over the incubation period was defined as the inhibitor concentration. Substrate concentrations were 50 μM (○), 100 μM (▲), and 200 μM (□).

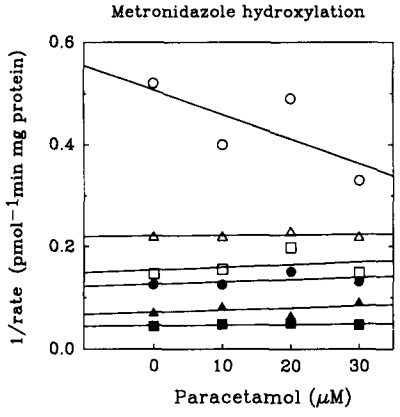

Fig. 3. Dixon plots illustrating the inhibition of metronidazole hydroxylation by paracetamol in microsomes from two human livers, HL1 (filled symbols), HL3 (open symbols). Substrate concentrations were 50 μM (○●), 100 μM (△ ▲), and 200 μM (□ ■).

discounted since paracetamol *per se* had no effect on metronidazole hydroxylation (Fig. 3).

The effect of metronidazole on the O-deethylation of phenacetin was investigated on two occasions using microsomes from liver HL1. The results indicated no inhibition but rather slight activation of the deethylation rate (Fig. 4).

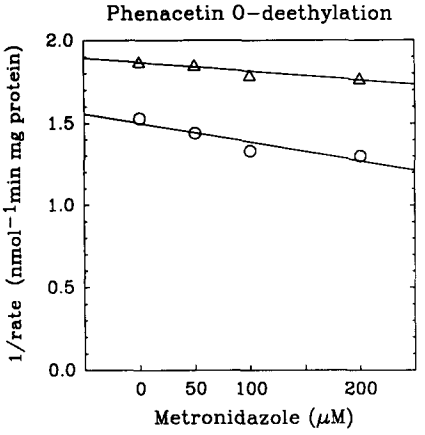

Fig. 4. The inhibition of phenacetin O-deethylation by metronidazole estimated on two occasions in microsomes from a human liver (HL1).

DISCUSSION

Our findings show that metronidazole is hydroxylated in human liver microsomes by a NADPH dependent process, most likely catalysed by the cytochrome P450 system. The biphasic kinetics of this reaction indicate that at least two enzymatic sites are involved, although allosteric changes of a single site cannot be excluded. The characterization of the particular cytochrome(s) P450 involved in the high affinity component of metronidazole hydroxylation by means of inhibition with substrates of some of the specific P450 forms may have been confounded by the biphasic kinetics. Thus, substantial inhibition of the low affinity site or minor inhibition of the high affinity site may have been missed. However, with this reservation the minimal effects of mephenytoin, tolbutamide, quinidine, acetone and nifedipine in concentrations in excess of their reported K_m values and/or K_i values with respect to prototype substrates appear to exclude the significant involvement of the corresponding cytochromes P450 IIC8-10, IID6, IIE1 and IIIA, respectively (Table 4 [8, 13–17, 25]). Moreover, metronidazole, propranolol and phenacetin do not inhibit the hydroxylation of tolbutamide by human liver microsomes whereas cimetidine and nifedipine do inhibit this reaction [8, 25]. In microsomes from the liver of a putative poor metabolizer of debrisoquine, metronidazole was hydroxylated at a rate comparable to that seen in the other livers. This further supports the view that cytochrome P450IID6 is not involved in the reaction. It should be noted that, although acetone is an inducer, substrate and inhibitor of rat cytochrome P450IIE1, its interaction with the human form has not been investigated to our knowledge. Nevertheless, the human and rat form of cytochrome P450IIE1 are very similar regarding amino acid sequence and substrate selectivity and no differences with respect to acetone would be expected [27].

In rat hepatocytes, 3-methylcholanthrene pretreatment increased the rate and affinity of metronidazole hydroxylation and its inhibition by α-naphthoflavone [5]. This suggests that cytochrome P450IA1 and/or IA2 have a high affinity for metronidazole hydroxylation. Phenacetin, caffeine, α-naphthoflavone, and, probably, theophylline are

all substrates and thus inhibitors of cytochrome P450IA2 [11, 12, 23]. Nevertheless, the hydroxylation of metronidazole was inhibited only by phenacetin with an estimated K_i value comparable to the reported K_m values. The competitive nature of this inhibition may have been obscured by the large differences in turnover rate between the two compounds and the biphasic kinetics of metronidazole hydroxylation. However, metronidazole did not inhibit the O-deethylation of phenacetin. If two compounds are substrates of a single cytochrome P450, they should show reciprocal competitive inhibition [6–9]. Thus, the effect of phenacetin on metronidazole hydroxylation cannot be taken as evidence of a common enzyme catalyzing both oxidations. By the same token, phenacetin inhibits the hydroxylation of debrisoquine suggesting coregulation of their metabolism, yet other studies have demonstrated that different enzymes are involved and that the inhibition is not reciprocal [6, 28]. The mechanism of the inhibition of metronidazole hydroxylation by phenacetin remains to be determined. We have shown that it is not related to the paracetamol generated by O-deethylation.

A number of other *in vivo* and *in vitro* findings supports the independence of metronidazole hydroxylation and the metabolism of phenacetin, caffeine and theophylline in man. Thus, smoking is associated with increased rates of caffeine and theophylline metabolism *in vivo* and the O-deethylation of phenacetin *in vivo* and *in vitro*, whereas the clearance of metronidazole is unrelated to tobacco consumption [1, 29–32]. Cimetidine decreases the elimination rate of theophylline and caffeine but not the clearance of metronidazole by hydroxylation [4, 33, 34]. In human liver microsomes nifedipine and cimetidine potently inhibit theophylline demethylation [21], whereas in the present study metronidazole hydroxylation was minimally affected by these compounds. Metronidazole coadministration does not affect the clearance of theophylline [35]. Finally, the clearance of antipyrine by 4-hydroxylation correlates closely with the clearance of theophylline but not with the rate of metronidazole hydroxylation [2, 4, 36, 37]. Accordingly, the collective evidence appears to exclude the possibility that metronidazole is hydroxylated to a significant extent by cytochrome P450IA2 in man.

In healthy volunteers treatment with phenobarbitone or antipyrine increases the clearance of metronidazole by hydroxylation more than it increases the total and partial metabolic clearances of antipyrine [2, 4]. Thus, it is possible that in man metronidazole is hydroxylated mainly by phenobarbitone-inducible cytochromes P450. The final identification may require the isolation of purified cytochromes P450 and/or the use of specific inhibitory antibodies, neither of which were available for the present study.

The minimal effects of antipyrine and cimetidine on the hydroxylation of metronidazole in the human liver microsomes are in agreement with *in vivo* findings in man [4]. Moreover, although the present results cannot exclude that antipyrine is a competitive inhibitor of metronidazole hydroxylation, they are compatible with the notion that different cytochromes P450 are involved in the metabolism of the two drugs [1–5]. The inhibitory effect of propranolol on metronidazole hydroxylation may be related to its irreversible binding to cytochrome P450 [18].

The kinetics of metronidazole hydroxylation by rat and mouse hepatocytes could be described by single site reactions with median K_m values of 0.6 and 1.1 mM, respectively [5, 38]. However, the range of substrate concentrations used in these studies may not have been sufficient to detect multiphasic kinetics. Moreover, in hepatocytes from phenobarbitone pretreated rats the K_m value was doubled whereas 3-methylcholanthrene pretreatment tended to decrease it [5]. Thus, metronidazole may be hydroxylated at several enzymatic sites in rodents as well as in human liver preparations. Indeed, it has been suggested that multiphasic kinetics of oxidative drug metabolism are the rule, rather than the exception, as a consequence of the involvement of multiple forms of cytochromes P450 [6].

The high affinity site of hydroxylation contributed more than 75% of the intrinsic clearance (V_{max}/K_m) of metronidazole by human liver microsomes and showed a 2-fold interindividual variation in K_m and a 15-fold variation in V_{max}. This suggests the 10-fold interindividual variation in both the *in vitro* intrinsic clearance and the *in vivo* clearance of metronidazole in man is mainly related to variation in the amount of the involved enzyme [1, 2]. In man the dose adjusted area under the concentration–time curve of metronidazole is 9% larger after a dose of 2000 mg than after a dose of 500 mg, which yields peak plasma drug concentrations of approximately 200 and 50 μM, respectively [39]. This is consistent with the present *in vitro* K_m values. The acetic acid metabolite of metronidazole could not be quantified in the present study but represents a minor elimination pathway, accounting for approximately 15% of a dose in humans [2].

In conclusion metronidazole appears to be hydroxylated by at least two cytochrome P450 forms in human liver microsomes. The identity of the high affinity site was not established but a number of cytochromes P450 characterized by specific substrates and/or inhibitors were unlikely candidates. Accordingly, the metabolism of metronidazole *in vivo* may reflect the activities of cytochromes P450 different from those studied using previously described probe drugs. Thus, metronidazole may be a useful ingredient of "cocktails" used to study the genetic and environmental regulation of human drug metabolism [1–3].

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