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INHIBITION OF DESMETHYLIMIPRAMINE 2-HYDROXYLATION BY DRUGS IN HUMAN LIVER MICROSOMES

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Abstract—The 2-hydroxylation of desmethylimipramine (DMI) correlates strongly with the 4-hydroxylation of debrisoquine (D) both in human volunteers and *in vitro* comparing human liver microsomes from different individuals. D competitively inhibits the 2-hydroxylation of DMI *in vitro* suggesting that DMI is hydroxylated by the 'debrisoquine hydroxylase' which is under monogenic control in man. We have characterized the effect of drugs on the hydroxylation of DMI in human liver microsomes by measuring the formation of 2-OH-DMI with HPLC using fluorescence detection. Amitriptyline, nortriptyline and metoprolol inhibited the hydroxylation of DMI competitively indicating interaction with the catalytical site for DMI 2-hydroxylation. Antipyrine and amylobarbitone at concentrations similar to their K_m-values for metabolism did not inhibit DMI-hydroxylation. Thus, for these compounds there was a good correspondence between the drugs' capacity to inhibit DMI 2-hydroxylation competitively *in vitro* and their apparent metabolism by the 'debrisoquine hydroxylase' *in vivo* in man. Thioridazine, chlorpromazine, quinidine and quinine also inhibited DMI-hydroxylation competitively. Thioridazine was an unusually potent inhibitor (apparent inhibition constant $K_i = 0.75$ μM). Quinidine was also an unusually potent inhibitor ($K_i = 0.27$ μM) and much more efficient than its isomer quinine ($K_i = 12$ μM). Theophylline could inhibit DMI hydroxylation but with atypical kinetics. We suggest that this simple DMI *in vitro* test as well as earlier described inhibition tests with debrisoquine, sparteine and bufuralol can be used to screen if drugs interact with the 'debrisoquine hydroxylase' in human liver.

There are marked interindividual differences in steady state plasma levels of desmethylimipramine (DMI) after fixed doses [1] due to genetically determined variability in drug oxidation [2]. Steady-state plasma levels of DMI are dependent on the debrisoquine (D) hydroxylation phenotype determined as the ratio between D and 4-OH-D in urine [3, 4]. This ratio correlates strongly to a DMI hydroxylation index formed by relating the concentrations of DMI and 2-OH-DMI in urine [5]. Three to ten per cent of European Caucasians are slow hydroxylators of D, a property which is a monogenic autosomal recessive trait [6, 7]. Such individuals seem to lack or have an abnormal cytochrome P-450 isozyme [8, 9] and are also slow in metabolizing some other drugs [10].

The rates of 2-hydroxylation (aromatic) of DMI and 4-hydroxylation (benzylic) of D also correlate among human livers *in vitro* [5]. These findings *in vivo* and *in vitro* suggest that the two hydroxylations are catalyzed by the same cytochrome P-450 isozyme or, alternatively, are commonly regulated. The former possibility is supported by the fact that D competitively inhibits the 2-hydroxylation of DMI *in vitro* [5].

The purpose of this investigation was to study the effect of various drugs on 2-hydroxylation of DMI in human liver microsomes. Among the drugs chosen nortriptyline and metoprolol seem to be oxidized by the debrisoquine hydroxylase while antipyrine and amylobarbitone have been shown not to depend on the debrisoquine hydroxylation phenotype for their metabolism. It has been proposed that *in vitro* inhibition studies with debrisoquine [11], sparteine [12] and bufuralol [13] can be used to screen if drugs interact with debrisoquine hydroxylase. The idea was also to evaluate whether such a simple *in vitro* inhibition test with DMI also could be used to screen if various drugs interact with this enzyme.

MATERIAL AND METHODS

Chemicals and reagents. Desmethylimipramine (DMI) hydrochloride and *N*-desmethylclomipramine (internal standard) were obtained from Ciba–Geigy (Basel, Switzerland), 2-OH-DMI from Regis Chemical Company (Chicago, Illinois), pig heart isocitric dehydrogenase from Boehringer and Soehne (Mannheim, West Germany) and NADPH and DL-isocitrate from Sigma Chemical Company (St Louis, Missouri). The drugs came from their respective manufacturers. Analytical grade chemicals and solvents were from Merck (Darmstadt, West Germany).

Human liver microsomes. Adult human liver specimens came from our liver bank [14]. The liver specimens were obtained from kidney transplant donors with total cerebral infarction shortly after circulatory arrest and the samples were frozen in small cubes in liquid nitrogen within half an hour after the patients' death. This procedure was approved by the Swedish Board of Health and Welfare. Microsomes were prepared as previously described [14] and protein

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assayed [15]. The microsomes were stored at −80°, at a protein concentration of 10 mg/ml.

The rate of hydroxylation of DMI in the livers was determined in a system containing microsomes (0.5 mg protein/ml), 50 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM $NADP^+$, 5 mM D,L-isocitrate, and 0.5 mg pig heart isocitric dehydrogenase per 1 ml incubation mixture. Incubations were performed at 37° in air for 15 min with different inhibitor drug concentrations at three fixed concentrations of DMI (10, 25, 50 μM). Before the final design of each experiment we screened if a low and a high concentration of the compounds inhibited the hydroxylation of DMI (10 μM). The reaction was started by addition of microsomes and stopped with 300 μl 25% ice-cold ammonia. 2-OH-DMI was quantitated using a modification [5] of the method by Sutfin and Jusko [16]. Standard curves were prepared by adding 2-OH-DMI to microsomes and cofactors. To ensure that no metabolism occurred ammonia was added before the microsomes. Comparison of the formation rate of 2-OH-DMI at various inhibitor concentrations was accomplished by using the method of Dixon [17].

RESULTS

The 2-hydroxylation of DMI was linear for at least 20 min and to 1 mg/ml of microsomal protein. The apparent K_m was about 20 μM. Figure 1 and Table 1 show the effect of various drugs on the 2-hydroxylation of DMI in human liver microsomes *in vitro*. Amitriptyline, nortriptyline and metoprolol inhibited the hydroxylation competitively while anti-

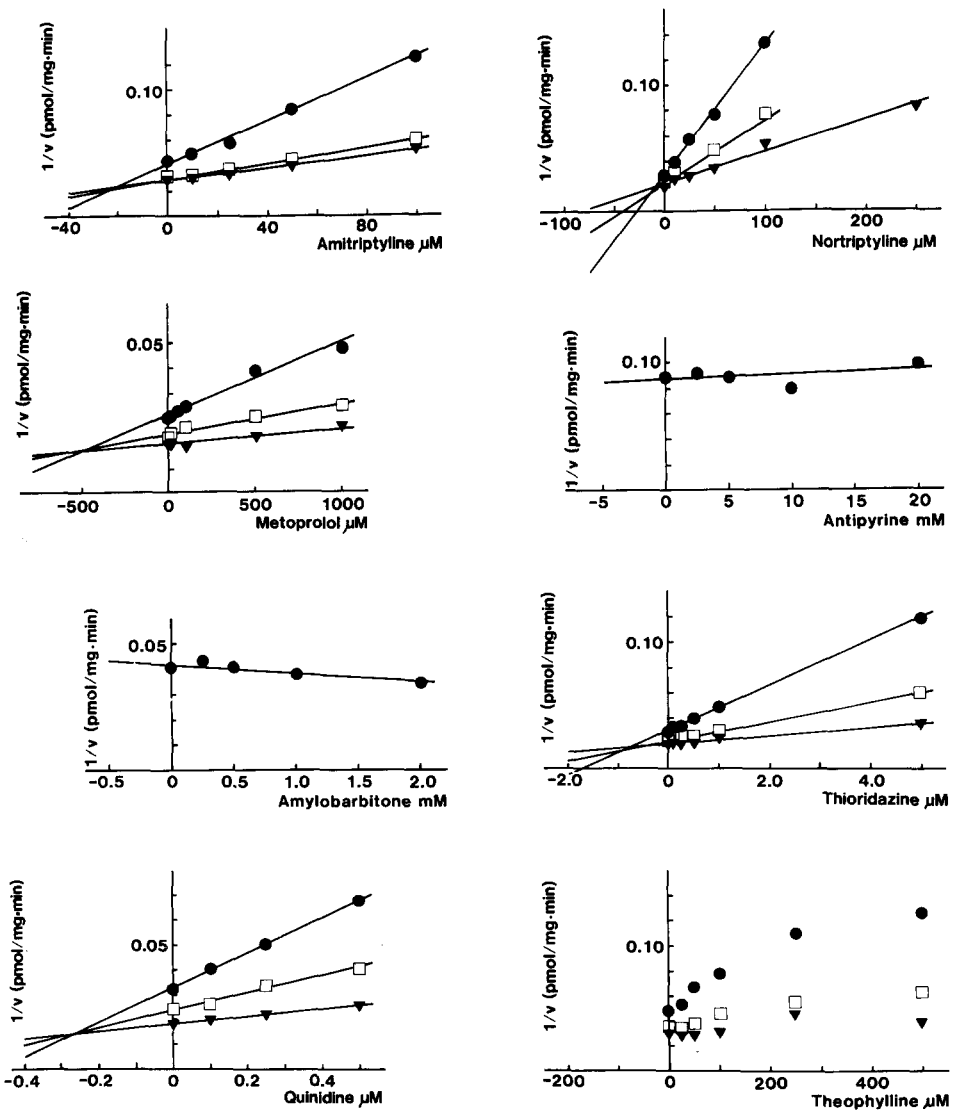

Fig. 1. Effects of different concentrations of drugs (*x*-axis) on the formation rate (v = pmoles/mg × min) of 2-hydroxydesmethylimipramine from different concentrations of desmethylimipramine (10 μM ●—●, 25 μM □—□, 50 μM ▼—▼). Incubations were performed with at least duplicates for 15 min with 0.5 mg of microsomal protein. Lines are obtained by linear regression analysis.

Table 1. Effect of various drugs on 2-hydroxylation of desmethylimipramine in human liver microsomes

Drug	Concentration (μM)	Per cent of control*	K_i† (μM)
Amitriptyline	10	97	20
	25	88	
	100	50	
Nortriptyline	10	77	9
	25	46	
	100	29	
Metoprolol	10	93	570
	100	83	
	500	72	
	2500	49	
Thioridazine	0.25	88	0.75
	1	68	
	5	36	
Chlorpromazine	5	86	6
	25	58	
	50	42	
Quinidine	0.25	70	0.27
	5	42	
	10	35	
Quinine	2.5	97	12
	5	89	
	25	67	
Theophylline	25	100	-
	100	76	
	500	55	

* Microsomes were incubated in duplicates or triplicates for 15 min with 25 μM of desmethylimipramine.

† K_i = apparent inhibition constant. Illustrative value from a representative experiment.

pyrine and amylobarbitone did not. Thioridazine, chlorpromazine, quinidine and quinine inhibited the DMI-hydroxylation competitively. Thioridazine was very potent (apparent inhibition constant $K_i = 0.75$ μM). Also quinidine was an unusually potent inhibitor ($K_i = 0.27$ μM), and much more efficient than its isomer quinine ($K_i = 12$ μM). Theophylline inhibited DMI hydroxylation but with atypical kinetics.

DISCUSSION

Recent studies of the 4-hydroxylation of debrisoquine (D) and 2-hydroxylation of desmethylimipramine (DMI) *in vivo* and in human liver microsomes strongly indicate that the two drugs are hydroxylated by the same cytochrome P-450 isozyme ('debrisoquine hydroxylase') [5]. This study was designed to explore if various drugs could inhibit DMI hydroxylation *in vitro*. Since it has been suggested that such inhibition tests with debrisoquine [11], sparteine [12] and bufuralol [13] could be used as screening tests to identify drugs that interact with the debrisoquine hydroxylase we included drugs for which it is known whether their metabolism depends on the debrisoquine phenotype or not.

Amitriptyline (AT) inhibited DMI-hydroxylation competitively indicating interaction with the same enzymatic site as DMI. This agrees with the findings that the hydroxylation and demethylation of AT *in vivo* in man is related to D-hydroxylation, [18, 19] at least in non-smokers (this lab. unpubl.). Also nortriptyline (NT) was a competitive inhibitor, which is in agreement with the fact that its 10-hydroxylation *in vivo* and *in vitro* in man depend on the debrisoquine hydroxylation phenotype [9, 20]. Its apparent inhibition constant (9 μM) was fairly close to the K_m value for 10-hydroxylation of nortriptyline (about 30 μM; [21]). The inhibition constants for AT and NT are close to those reported by Otton *et al.* [22] with respect to sparteine oxidation.

The adrenergic beta-adrenoceptor antagonist metoprolol also inhibited DMI 2-hydroxylation competitively. Like nortriptyline the metabolism of this drug is dependent on the debrisoquine hydroxylation phenotype [23, 24]. Similarly, Otton *et al.* [12] have shown that metoprolol competitively inhibits sparteine oxidation *in vitro*. Since sparteine most likely is metabolized by the same enzyme as D [25] this also indicates that metoprolol interacts with the 'debrisoquine hydroxylase'.

We also incubated DMI in the presence of antipyrine. Even at a concentration of 20 mM, which exceeds its K_m-values for metabolism (about 6–7 mM; cf. [11]) this drug did not inhibit DMI hydroxylation, agreeing with the *in vivo* observations in man that the metabolism of antipyrine is independent of the debrisoquine hydroxylation phenotype [26]. Moreover, Boobis *et al.* [11] have shown that antipyrine does not inhibit D hydroxylation in human liver microsomes to an important degree. Also amylobarbitone failed to inhibit DMI hydroxylation at concentrations close to its K_m value of 0.5–2.0 mM [27] agreeing with *in vivo* findings that its metabolic disposition is largely independent of the

debrisoquine phenotype [28] and *in vitro* inhibition studies with debrisoquine [11].

Thioridazine has recently been found to increase the plasma concentrations of DMI in man probably due to inhibition of its metabolism [29]. In agreement with this thioridazine strongly inhibited DMI hydroxylation in human liver microsomes. Chlorpromazine also inhibited DMI hydroxylation competitively. Nortriptyline, which is structurally related to DMI and metabolized by the debrisoquine hydroxylase [9, 20], can increase plasma concentrations of chlorpromazine [30]. These data indicate that chlorpromazine interacts with the enzyme. Findings that chlorpromazine also inhibits sparteine oxidation in human liver *in vitro* with about the same inhibition constant that we find strengthens this view [22].

Otton *et al.* [12] have found that both quinidine and quinine could inhibit sparteine oxidation in human liver *in vitro* and that quinidine was much more potent than quinine. Therefore we also tested the effects of these compounds on the DMI hydroxylation. Quinidine (Q) was found to be an unusually efficient competitive inhibitor of DMI-hydroxylation, and it was much more potent than its isomer quinine. This similarity in stereoselective inhibition indicates indirectly that S and DMI interact with the same cytochrome P-450. The competitive inhibition shows that Q combines with the catalytic enzyme site(s) that 2-hydroxylates DMI. It would be desirable to find out if quinidine inhibits the oxidation of other drugs that are metabolized by the debrisoquine hydroxylase. Although Q inhibits DMI and S oxidation *in vitro* the debrisoquine hydroxylase may not be a major determinant of Q-metabolism *in vivo*. The possibility of such a drug interaction should nevertheless be explored in man.

Dahlqvist *et al.* [31] have shown that the metabolism of theophylline to its three major metabolites in man do not quantitatively depend on the debrisoquine phenotype. *In vitro* theophylline was able to inhibit DMI hydroxylation but the inhibition kinetics were not purely competitive. The mechanism for this apparent *in vivo–in vitro* discrepancy is not clear, but one explanation might be that metabolites of theophylline formed during the incubation had inhibitor effects on DMI hydroxylation. Another possibility is that theophylline interacts with the debrisoquine hydroxylase, but that the drug's metabolism is not quantitatively dependent on this enzyme.

In conclusion, we have found a good correspondence between drugs' capacity to inhibit DMI 2-hydroxylation competitively in human liver microsomes and their apparent metabolism by the debrisoquine hydroxylase *in vivo* in man. Thus this 'DMI test' as well as similar tests with debrisoquine [11], sparteine [12] and bufuralol [13] seem to be useful to screen whether drugs interact with the 'debrisoquine hydroxylase' in man. A competitive inhibition proves that the drug combines with the catalytic enzyme site(s) hydroxylating DMI, and most likely D. It does not prove that the drug is metabolized by this site, although this is likely. A strong correlation between two catalytic activities among liver microsomes from different subjects, as in the case of DMI and D [5] increases the probability that the two drugs are, indeed, metabolized by the same enzyme site. Studies with purified enzymes and inhibiting antibodies should increase our possibilities to predict which compounds and what reactions are metabolized by the debrisoquine hydroxylase. Efficient inhibitors of DMI hydroxylation should be candidates for *in vivo* interaction studies with drugs being metabolized by this enzyme. Early information on this point would be useful during the clinical evaluation of new drugs, particularly in the attempts to identify individuals likely to show unusual drug response.

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