Br J clin Pharmac 1995; **39**: 433–439

Imipramine metabolism in relation to the sparteine and mephenytoin oxidation polymorphisms—a population study

H. MADSEN, K. KRAMER NIELSEN & K. BRØSEN
Department of Clinical Pharmacology, Institute of Medical Biology, Odense University, Odense, Denmark

1 Sparteine and mephenytoin phenotyping tests were carried out in 327 healthy Danish subjects. Two weeks later each subject took 25 mg imipramine followed by urine collection for 24 h. The urinary content of imipramine, desipramine, 2-hydroxy-imipramine and 2-hydroxy-desipramine was assayed by h.p.l.c.

2 The medians of the hydroxylation ratios (i.e. 2-hydroxy-metabolite over parent compound) were 6 to 14 times higher in 300 extensive metabolizers of sparteine (EM_S) as compared with 27 poor metabolizers (PM_S), but none of the ratios separated the two phenotypes completely.

3 There were 324 EM of mephenytoin (EM_M) and three PM (PM_M) in the sample. The demethylation ratios between desipramine, 2-hydroxy-desipramine and their corresponding tertiary amines showed statistically significant correlations with the mephenytoin S/R isomer ratio (Spearman's r_s: –0.20 and –0.27, $P < 0.05$).

4 The demethylation ratios were higher in 80 smokers than in 245 non-smokers. This indicates that CYP1A2, which is induced by cigarette smoking, also catalyzes the *N*-demethylation of imipramine.

5 CYP2D6 genotyping was carried out by PCR in 325 of the subjects, and the D6-wt allele was amplified in 298 EM_S, meaning that they were genotyped correctly. One PM_S was D6-wt/D6-B, another PM_S had the genotype D6-wt/ and hence both were misclassified as EM_S. The remaining 25 PM_S were D6-A/D6-B ($n = 5$), D6-B/ ($n = 18$) or D6-D/D6-D (no PCR amplification, $n = 2$). Thus, the specificity for genotyping PM_S was 100% and the sensitivity was 92.4%.

6 There were 198 apparently homozygous EM_S (D6-wt/) and 98 heterozygous EM_S (D6-wt/D6-A or D6-wt/D6-B). The sparteine metabolic ratio was lower and the hydroxylation ratios were higher in the homozygotes compared with the heterozygotes. However, for all of the ratios there was a considerable overlap between the two genotypes.

Keywords imipramine sparteine mephenytoin CYP2D6 CYP2C19 CYP1A2

Introduction

The major pathways of imipramine metabolism are *N*-demethylation to the active metabolite desipramine and aromatic hydroxylation to 2-hydroxy-imipramine and 2-hydroxy-desipramine. Aliphatic hydroxylation to 10-hydroxy-imipramine and 10-hydroxy-desipramine, *N*-oxidation to imipramine-*N*-oxide and side chain dealkylation to iminodibenzyl are considered less important pathways [1]. The hydroxylated metabolites are predominantly excreted as glucuronide conjugates in the urine. After administration of ^{14}C-labelled imipramine to patients about 40% of the radioactivity was recovered in urine over 24 h and about 70% over 72 h [2].

It was suggested nearly 20 years ago, that the *N*-demethylation of imipramine and the 2-hydroxylation of imipramine and desipramine are catalyzed by different P450 isoforms [3, 4]. Indeed, subsequent *in vivo* studies in healthy volunteers and patients showed that CYP2D6, the source of the sparteine/debrisoquine oxidation polymorphism, is the major enzyme catalyzing

Correspondence: Dr Hanne Madsen, Department of Clinical Pharmacology, Institute of Medical Biology, Odense University, Winsløwparken 19, DK-5000 Odense C, Denmark

the 2-hydroxylation but is not involved in the *N*-demethylation of imipramine [5–8]. This was later confirmed *in vitro* [9–12]. CYP2D6 is also a major enzyme catalyzing the 2-hydroxylation of desipramine [5–8, 13–15]. The *N*-demethylation but not the 2-hydroxylation of imipramine covaries with S-mephenytoin hydroxylase (CYP2C19) activity *in vivo* [16, 17] and *in vitro* [18].

CYP2D6 is encoded by the functional allele D6-wt (wild type) in extensive metabolizers (EM_s), and the absence of the enzyme in poor metabolizers (PM_s) [19] is caused by inactivating mutations in the CYP2D6 gene, the most common of which are designated D6-A, D6-B and D6-D [20]. It is possible to test for D6-wt, D6-A, and D6-B by the polymerase chain reaction (PCR) [20]. The D6-D is a complete deletion of the CYP2D6 gene [21] and, therefore, is not amplified by PCR. Thus, EM_s and PM_s can be distinguished by genotyping, phenotyping or both [22].

The relationship between genetic polymorphism in drug oxidation and imipramine metabolism has not been studied in a large population of randomly selected subjects. Thus, imipramine, sparteine, mephenytoin and CYP2D6 genotype testing was carried out in 327 healthy Danish volunteers. The main purpose for carrying out the study was to investigate the possibility of using imipramine as a probe drug for phenotyping. Another aim of the study was to document the sensitivity and specificity of CYP2D6 genotyping in a large population of subjects who had not previously been phenotyped.

Methods

Subjects

The subjects of the study were 327 healthy Danes recruited among students and staff at Odense University. There were 206 men and 121 women. The median age was 23.7 years (range 20–45 years). None of the participants had daily intake of alcohol or drugs (including oral contraceptives). The volunteers participated on the basis of written and verbal information, and the protocol was approved by the Danish National Board of Health and the Ethics committee of Vejle and Funen.

Phenotyping

Oral intake of a combination of 100 mg sparteine sulphate (Depasan, Giulini Pharma GmbH, Hannover Germany) and 100 mg racemic mephenytoin (Mesantoin, Sandoz Pharmaceutical Corp., East Hannover, New Jersey, USA) was followed by urine collection for 12 h. The urine volume was recorded and an aliquot of 10 ml was kept frozen at −20° C until assay, which took place less than 1 month after the test. Sparteine, 2,3-didehydrosparteine and 5,6-didehydrosparteine were assayed by gas chromatography [23]. A metabolic ratio (MR) was calculated as sparteine/(2,3- plus 5,6-didehydrosparteine). EM_s were defined according to an MR less than 20 and the PM_s according to an MR above 20 [24]. R- and S-mephenytoin were assayed by gas chromatography [25]. In Danes [26] an S/R ratio above 0.9 defines poor metabolizers of mephenytoin (PM_M) and an S/R below 0.9 defines extensive metabolizers (EM_M).

The imipramine test was performed about 2 weeks after the sparteine/mephenytoin test. After voiding, each subject ingested 25 mg imipramine hydrochloride (25 mg Tofranil tablets, Ciba Geigy Corp., Basel, Switzerland) followed by urine collection for 24 h. The urinary pH was measured, the urinevolume was recorded, and an aliquot of 20 ml was kept frozen at −20° C until assay. Imipramine, desipramine, 2-hydroxy-imipramine and 2-hydroxy-desipramine after β-glucuronidase treatment were determined by h.p.l.c. [27]. The detection level was 10 nmol l^{-1} for all four compounds. After deconjugation the detection level was 100 nmol l^{-1} for the hydroxylated metabolites.

Genotyping

A blood sample was drawn by venepuncture into an EDTA vacutainer (Venoject), and the blood was transferred immediately to a 50 ml capped polypropylene tube (Falcon) and kept at −20° C until extraction of DNA for PCR analysis. DNA for PCR amplification was extracted from 0.5 ml blood as described previously [28]. The DNA was tested for D6-wt, D6-A and D6-B by a two-step PCR amplification procedure [20]. This method does not distinguish the D6-wt/D6-D from D6-wt/D6-wt. Lack of amplification product in PM_s is considered as diagnostic for the genotype D6-D/D6-D.

Statistics

The pharmacokinetic indices for imipramine in EM_s, and PM_s, in heterozygous and homozygous EM_s, and smokers and non-smokers were compared by the median differences and 95% confidence intervals. Correlations between the pharmacokinetic indices and the mephenytoin S/R ratio were assessed by the Spearman rank correlation test (r_s). All statistical tests were carried out using the MEDSTAT program version 2.12.

Results

Twenty-seven (8.3% (5.5–11.8)) of the 327 individuals in the population studied were phenotyped as PM_s. The median MR in the 27 PM_s was 71 (range: 23–126) and the median MR in 300 EM_s was 0.39 (range: 0.09–12) (Figure 1). The S/R ratio was 0.93, 0.97 and 1.01 in three PM_M and ranged from <0.1 to 0.5 in 324 EM_M. As expected there was no correlation between MR and S/R (r_s = 0.015, P = 0.80, n = 327).

The median urinary recovery of imipramine and its three metabolites was 25% of the dose in EM_s and 6.3% in PM_s (Table 1). The higher recovery in EM_s was due to a much faster excretion of 2-hydroxy-

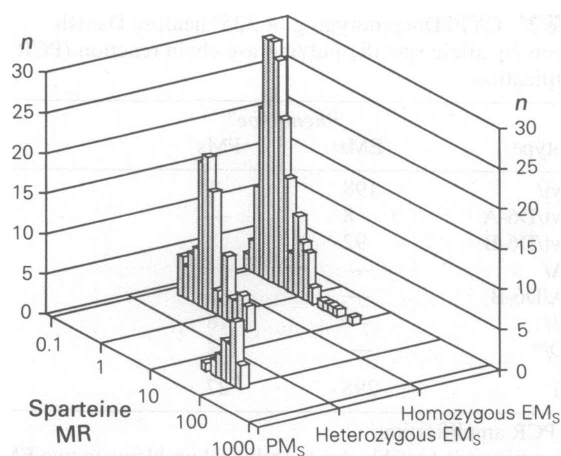

Figure 1 The sparteine metabolic ratio (MR) in 27 poor metabolizers (PM_s), 100 heterozygous extensive metabolizers (EM_s) (D6-wt/D6-A and D6-wt/D6-B) and 198 apparently homozygous EM_s (D6-wt/).

imipramine and 2-hydroxy-desipramine in this phenotype.

The putative indices for hydroxylation (the 2-hydroxy metabolites over parent compound ratios) were much higher in EM_S than in PM_S, but none of the ratios separated the two phenotypes completely (Table 2 and Figure 2). The corresponding demethylation ratios (Table 2) all showed a weak negative correlation with the S/R ratio. Two subjects did not disclose their smoking habits, 245 were non-smokers and 80 were smokers. Both of the demethylation ratios were higher in smokers than non-smokers. The median differences [95% confidence interval] between non-smokers and smokers were −0.3 (−0.7; 0.1) and −0.2 (−0.3; −0.03) for desipramine *vs* imipramine and 2-hydroxy-desipramine *vs* 2-hydroxy-imipramine, respectively.

None of the hydroxylation ratios listed in Table 2 was statistically significantly different between non-smokers and smokers (data not shown).

Table 1 Urinary recoveries (% of dose) of imipramine and its metabolites in relation to the sparteine (CYP2D6) and the mephenytoin (CYP2C19) oxidation polymorphisms

	CYP2D6			*CYP2C19*
	EM_s *(n = 300)* *Median (range)*	PM_s *(n = 27)* *Median (range)*	*Median difference (95% confidence interval)*	*Spearman's* r_s *with mephenytoin S/R*[a] *(n = 327)*
IP	0.15 (0–1.4)	0.18 (0.04–1.5)	−0.03 (−0.07; 0.02)	0.12*
DMI	0.30 (0.03–1.9)	0.80 (0.0–1.9)	−0.50 (−0.70; −0.4)	−0.06 (NS)
2-OH-IP	11 (1.6–30)	2.5 (0.2–4.6)	8.8 (7.3; 10)	0.14*
2-OH-DMI	12 (3.3–24)	2.5 (1.0–6.5)	8.9 (7.8; 10)	−0.19*
Total[b]	25 (7.6–40)	6.3 (1.8–11)	18 (16; 20)	0.0 (NS)

[a]Spearmans r_s: *P: < 0.05, NS: $P > 0.05$.
[b]The sum of IP, DMI, 2-OH-IP and 2-OH-DMI.
IP: imipramine; DMI: desipramine; 2-OH-IP: 2-hydroxy-imipramine and 2-OH-DMI: 2-hydroxy-desipramine.
EM_s: extensive metabolizers of sparteine, PM_s: poor metabolizers of sparteine.

Table 2 The metabolic ratios of imipramine in relation to the sparteine (CYP2D6) and mephenytoin (CYP2C19) oxidation polymorphisms

	CYP2D6			*CYP2C19*
	EM_s *(n = 300)* *Median (range)*	PM_s *(n = 27)* *Median (range)*	*Median difference (95% confidence interval)*	*Spearman's* r_s *with mephenytoin S/R*[a]
Hydroxylation ratios				
2-OH-IP / IP	80 (6–1274)	13 (0–39)	65 (47; 89)	−0.04 (NS)
2-OH-DMI / DMI	39 (4.4–478)	2.8 (1.1–12)	35 (27; 45)	−0.05 (NS)
2-OH-IP + 2-OH-DMI / IP + DMI	51 (4.9–777)	4.8 (1.1–12)	46 (35; 59)	−0.02 (NS)
Demethylation ratios				
DMI[b)] / IP	2.2 (0.2–35)	4.8 (1.0–14)	−2.1 (−3.1; −1.2)	−0.20*
2-OH-DMI / 2-OH-IP	1.1 (0.2–4.9)	1.0 (0.3–14)	0.0 (−0.3; 0.2)	−0.27*

[a]Spearmans r_s: *P: < 0.05, NS: $P > 0.05$.
[b]Imipramine was undetectable in three subjects.
IP: imipramine; DMI: desipramine; 2-OH-IP: 2-hydroxy-imipramine and 2-OH-DMI: 2-hydroxy-desipramine.
EM_s and PM_s: extensive metabolizers and poor metabolizers of sparteine.

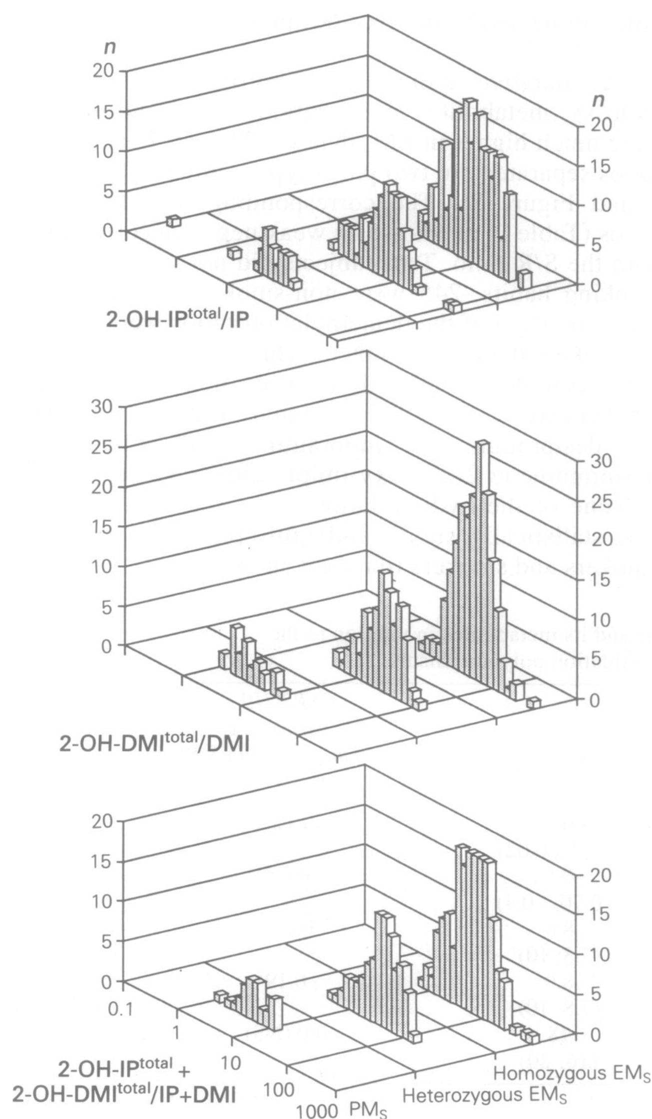

Figure 2 Hydroxylation ratios in 27 poor metabolizers (PM_S), 100 heterozygous extensive metabolizers (EM_S) (D6-wt/D6-A, D6-wt/D6-B) and 198 apparently homozygous EM_S (D6-wt/). IP: imipramine; DMI: desipramine; 2-OH-IP: 2-hydroxy-imipramine and 2-OH-DMI: 2-hydroxy-desipramine.

The median urine pH after the imipramine test was 6.12 (range: 5.00–7.59). The correlation between the pH and the recoveries of imipramine and desipramine were: $r_s = -0.45$ ($P < 0.00001$) and $r_s = -0.26$ ($P < 0.00001$), respectively.

Genotyping was not possible for technical reasons in two of the EM_S. The D6-wt was amplified in all of the 298 EM_S and hence they were all correctly genotyped (Table 3). One hundred and ninety-eight EM_S were D6-wt/, and hence apparently homozygous dominants. One hundred of the EM_S were either D6-wt/D6-A or D6-wt/D6-B and these subjects are certainly heterozygotes (Table 3). Twenty-three of the PM_S were either D6-B/ or D6-A/D6-B, and lack of DNA amplification in two PM_S was considered diagnostic for D6-D/D6-D (Table 3). Two of the PM_S were apparently heterozygous for D6-wt and hence misclassified as EM_S. Thus, genotyping identified 25 PM_S corresponding to 7.7% (5.0–11.2%) of the population. Accordingly, the specificity of the test was 100% and the sensitivity was 92.4% (75.7–99.1%) in the present sample of PM_S.

Table 3 CYP2D6 genotyping of 325[a] healthy Danish subjects by allele specific polymerase chain reaction (PCR) amplification

Genotype	*Phenotype*[b] EMs	PMs
D6-wt/	198	1
D6-wt/D6-A	8	—
D6-wt/D6-B	92	1
D6-A/	—	—
D6-A/D6-B	—	5
D6-B/	—	18
D6-D/*	—	2
Total	298	27

*No PCR amplification.
[a]Genotyping not possible due to technical problems in two EM.
[b]EM_S and PM_S: extensive and poor metabolizers of sparteine.

The median of the MR in homozygous EM_S was 0.32 (range: 0.09–12) and the corresponding value in heterozygous EM_S was 0.57 (range: 0.17–3.8) (Figure 1). The median difference was –0.23 (–0.30; –0.17). All of the three hydroxylation ratios were statistically significantly higher in homozygous EM_S as compared with heterozygotes (Table 4), but there was a considerable overlap between the two genotypes (Figure 2).

Discussion

Numerous earlier studies [5–15] have shown that CYP2D6 is a major enzyme catalyzing the 2-hydroxylation of imipramine and desipramine. In the present study assessment of the 2-hydroxylation of imipramine and desipramine was performed by calculation of ratios between the hydroxylated metabolites and their parent compounds (Table 2 and Figure 2). It must be borne in mind, that the so-called 'hydroxylation ratios' are also determined by the *N*-demethylation of imipramine and 2-hydroxy-imipramine and by the renal excretion of all four compounds. Despite these confounding factors, we found that the medians of the hydroxylation ratios were 6 to 14 times higher in the EM_S than in the PM_S (Table 2 and Figure 2). This provides strong evidence that the empirically derived indices mark 2-hydroxylation in a population. As expected, the sparteine MR was bimodally distributed (Figure 1) but in agreement with similar studies using desipramine as model drug [29, 30], the hydroxylation ratios of imipramine (Table 2 and Figure 2) showed a less clearcut separation of EM_S and PM_S. There are several possible explanations for this. First, as discussed already, *N*-demethylation contributes to this variability. Second, imipramine and desipramine are weakly basic drugs and, as shown previously [31], we confirm that their urinary excretion rates are pH dependent. Third, and probably most important imipramine and desipramine are oxided to the 2-

Table 4 The metabolic ratios of imipramine in 198 homozygous[a] (D6-wt/) and 100 heterozygous[b] (D6-A/D6-wt and D6-B/D6-wt) extensive metabolizers (EM_s) of sparteine

	Homozygous EM_s Median (range)	*Heterozygous EM_s Median (range)*	*Median difference (95% confidence interval)*
Hydroxylation ratios			
2-OH-IP[†] / IP	94 (6–842)	65 (16–1274)	27 (10–45)
2-OH-DMI / DMI	46 (5.3–448)	30 (4.4–105)	14 (8.3–20)
2-OH-IP + 2-OH-DMI / IP + DMI	62 (7.9–777)	40 (4.9–158)	20 (11–29)
Demethylation ratios			
DMI / IP	2.0 (0.2–21)	2.5 (0.6–35)	–0.4 (11–29)
2-OH-DMI / 2-OH-IP	1.0 (0.2–4.9)	1.2 (0.3–3.0)	–0.2 (–0.4; –0.1)

[a]Probably about 20% of the so-called homozygotes are in fact heterozygotes (see text).
[†]Imipramine was undetectable in two homozygotes and one heterozygote.
[b]Two subjects could not be genotyped because of technical problems.
IP: imipramine; DMI: desipramine; 2-OH-IP: 2-hydroxy-imipramine and 2-OH-DMI: 2-hydroxy-desipramine.
EM_s and PM_s: extensive metabolizers and poor metabolizers of sparteine.

hydroxy metabolites by P450s in addition to CYP2D6 [9]. All three confounding factors are able to mask a bimodal distribution of the hydroxylation ratios [32].

CYP2D6 genotyping does not require ingestion of a test drug followed by collection of urine, and the test result is not influenced by concomitant drug intake. Of 325 subjects 25 (7.7% (5.0–11.2)) were genotyped as PM_s (Table 3). This is in broad agreement with the frequency of 5% (3.4–6.6%) reported among 720 genotyped but not phenotyped British subjects [33]. In agreement with previous studies based on panels of phenotyped subjects [20, 22, 28, 29], we report a specificity of 100% and a sensitivity of about 90%. Accordingly, and shown directly for the first time, more than 99% of randomly selected subjects are genotyped correctly (Table 3). The misclassification in some PM_s is due to rare, inactivating mutations other than D6-A and D6-B. Based on the allele frequencies calculated in a large Danish panel study [28], it is estimated that about 10% of the 198 D6-wt/ EM_s (Tables 3 and 4) are in fact heterozygous EM_s carriers of one of the rare inactivating mutations. Approximately 10% are heterozygous EM_s with the D6-wt/D6-D genotype. This means that probably as many as 20% of the 198 D6-wt/ individuals are actually heterozygous EM_s. In spite of this, the apparently homozygous dominants had statistically significantly lower MR and higher hydroxylation ratios than the heterozygotes (Table 4, Figures 1 and 2). Thus, the 'gene dose' effect reported in previous panel studies [22, 28, 29] was confirmed. As reported previously the MR and the hydroxylation ratios (Figures 1 and 2, Table 4) displayed marked interindividual variability within each of the two EM genotypes, and hence there was a considerable overlap in the indices between heterozygotes and homozygotes for D6-wt. The variability is probably caused by a heterogeneity in the expression of the so-called wild-type allele [22, 34–36].

About 3.3% of white subjects are PM_M [37]. The source of this polymorphism in mephenytoin oxidation is CYP2C19 [38, 39]. The most common mutation in Caucasian PM_M, m1, is a single base (G → A) change in exon 5 of the CYP2C19 gene, and this results in the formation of a truncated, non-functional enzyme. We have previously shown [16] that the *N*-demethylation clearance of imipramine in PM_M is only about 50% of the value in EM_M and in agreement with subsequent population and *in vitro* studies [11, 16], this strongly suggests, that *N*-demethylation in part is catalyzed by CYP2C19. We show here that both of the putative demethylation ratios (Table 2) were statistically significantly correlated with the mephenytoin S/R ratio. Although the correlations were poor as judged by r_s-values of –0.20 and –0.27 (Table 2), this nevertheless suggests that the two ratios do mark *N*-demethylation. The poor correlation is due to several factors. First, the S/R ratio is a relatively poor discriminator of CYP2C19 activity among the EM_M. Second, the so-called demethylation ratios are also influenced by 2-hydroxylation and renal excretion of the compounds. Third, *N*-demethylation is also catalyzed by CYP1A2 and CYP3A4 [11]. CYP1A2 is a constitutively expressed enzyme which is induced in smokers [41] and potently inhibited by fluvoxamine [10, 42]. It was shown 20 years ago that smokers had lower plasma concentrations of imipramine than non-smokers [43]. Induction of the *N*-demethylation of imipramine was confirmed by our data showing higher demethylation ratios in smokers as compared with non-smokers.

In conclusion the results of the present study show that the regioselective oxidation of imipramine may,

with certain limitations, serve to assess both CYP2D6 and CYP2C19 activity in a population. They also provide additional evidence for a role of CYP1A2 in the *N*-demethylation of imipramine. The complex elimination of imipramine involves both polymorphic and non-polymorphic oxidation as well as pH-dependent renal excretion. This makes imipramine an interesting model drug for pharmacokinetic studies but a less suitable drug for phenotyping purposes.

This study was supported by The Danish Medical Research Council (12-0282-1), Grosserer C. P. Frederiksen's grant for Medical Students and Young Doctors and the Danish Foundation for the Advancement of Medical Science. The technical assistance of Mrs Annelise Casa and Mrs Taybeh Aliari, as well as the secretarial assistance of Mr Henrik Horneberg, is acknowledged.

References

1 Gram LF. Metabolism of tricyclic antidepressants. *Dan med Bull* 1974; **21**: 218–231.
2 Christiansen J, Gram LF, Kofoed B, Rafaelsen OJ. Imipramine metabolism in man. A study of urinary metabolites after administration of radioactive imipramine. *Psychopharmacologia (Berlin)* 1967; **11**: 255–264.
3 Gram LF, Søndergaard I, Christiansen J, Petersen GO, Bech P, Reisby N, Ibsen I, Ortmann J, Nagy A, Dencker SJ, Jacobsen O, Krautwald O. Steady-state kinetics of imipramine in patients. *Psychopharmacology* 1977; **54**: 255–261.
4 Gram LF, Bjerre M, Kragh-Sørensen P, Kvinesdal B, Molin J, Petersen OL, Reisby N. Imipramine metabolites in blood of patients during therapy and after overdose. *Clin Pharmac Ther* 1983; **33**: 335–342.
5 Brøsen K, Klysner R, Gram LF, Otton SV, Bech P, Bertilsson L. Steady-state concentrations of imipramine and its metabolites in relation to the sparteine/debrisoquine polymorphism. *Eur J clin Pharmac* 1986; **30**: 679–684.
6 Brøsen K, Otton SV, Gram LF. Imipramine demethylation and hydroxylation: Impact of the sparteine oxidation phenotype. *Clin Pharmac Ther* 1986; **40**: 543–549.
7 Brøsen K, Gram LF. First-pass metabolism of imipramine and desipramine: Impact of the sparteine oxidation phenotype. *Clin Pharmac Ther* 1988; **43**: 400–406.
8 Brøsen K, Gram LF. Quinidine inhibits the 2-hydroxylation of imipramine and desipramine but not the demethylation of imipramine. *Eur J clin Pharmac* 1989; **37**: 155–160.
9 Brøsen K, Zeugin T, Meyer UA. Role of P450IID6, the target of the sparteine/debrisoquin oxidation polymorphism, in the metabolism of imipramine. *Clin Pharmac Ther* 1991; **49**: 609–617.
10 Skjelbo E, Brøsen K. Inhibitors of imipramine metabolism by human liver microsomes. *Br J clin Pharmac* 1992; **34**: 256–261.
11 Lemoine A, Gautier JC, Azoulay D, Kiffel L, Guengerich FP, Beaune P, Maurel P, Leroux JP. The major pathway of imipramine metabolism is catalyzed by cytochromes P4501A2 and P4503A4 in human liver. *Mol Pharmac* 1993; **43**: 827–832.
12 Coutt R, Su P, Baher GB, Daneshtalab M. Metabolism of imipramine *in vitro* by isozyme CYP2D6 expressed in a human cell line, and observations on metabolite stability. *J Chromatogr* 1993; **615**: 265–272.
13 Bertilsson L, Åberg-Wistedt A. The debrisoquine hydroxylation test predicts steady-state plasma levels of desipramine. *Br J clin Pharmac* 1983; **15**: 388–390.
14 Spina E, Birgersson C, von Bahr C, Erichsson Ö, Mellström B, Steiner E, Sjöqvist F. Phenotypic consistency in hydroxylation of desmethylimipramine and debrisoquine in healthy subjects and in human liver microsomes. *Clin Pharmac Ther* 1984; **36**: 677–682.
15 Spina E, Steiner E, Erichsson Ö, Sjöqvist F. Hydroxylation of desmethylimipramine: Dependence on the debrisoquine hydroxylation phenotype. *Clin Pharmac Ther* 1987; **41**: 314–319.
16 Skjelbo E, Brøsen K, Hallas J, Gram LF. The mephenytoin oxidation polymorphism is partially responsible for the *N*-demethylation of imipramine. *Clin Pharmac Ther* 1991; **49**: 18–23.
17 Skjelbo E, Gram LF, Brøsen K. The *N*-demethylation of imipramine correlates with the oxidation of S-mephenytoin (S/R-ratio)—A population study. *Br J clin Pharmac* 1993; **35**: 331–334.
18 Chiba C, Saitoh A, Koyama E, Tani M, Hayashi M, Ishizaki T. The role of S-mephenytoin 4'hydroxylase imipramine metabolism by human liver microsomes: a two-enzyme kinetic analysis of *N*-demethylation and 2-hydroxylation. *Br J clin Pharmac* 1994; **34**: 237–242.
19 Zanger UM, Vilbois F, Hardwick JP, Meyer UA. Absence of hepatic cytochrome P450bufI causes genetically deficient debrisoquine oxidation in man. *Biochemistry* 1988; **27**: 5447–5454.
20 Heim M, Meyer UA. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet* 1990; **336**: 529–532.
21 Gaedigk A, Blum M, Gaedigk R, Eichelbaum M, Meyer UA. Deletion of the entire cytochrome P450 CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the sparteine/debrisoquine polymorphism. *Am J Hum Genet* 1991; **48**: 943–950.
22 Broly F, Gaedigk A, Heim M, Eichelbaum M, Morike K, Meyer UA. Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of CYP2D6 in a European population. *DNA Cell Biol* 1991; **10**: 545–558.
23 Vinks A, Inaba T, Otton SV, Kalow W. Sparteine metabolism in Canadian Caucasians. *Clin Pharmac Ther* 1982; **31**: 23–29.
24 Brøsen K, Otton SV, Gram LF. Sparteine oxidation polymorphism in Denmark. *Acta Pharmac Toxicol* 1985; **57**: 357–360.
25 Sanz EJ, Villen T, Alm C, Bertilsson L. S-mephenytoin hydroxylation phenotypes in a Swedish population determined after coadministration with debrisoquin. *Clin Pharmac Ther* 1989; **45**: 495–499.
26 Drøhse A, Bathum L, Brøsen K, Gram LF. Mephenytoin and sparteine oxidation: Genetic polymorphisms in Denmark. *Br J clin Pharmac* 1989; **27**: 620–625.
27 Nielsen KK, Brøsen K. High-performance liquid chromatography of imipramine and six metabolites in human plasma and urine. *J Chromatogr* 1993; **612**: 87–94.

28 Brøsen K, Nielsen PN, Brusgaard K, Gram LF, Skjødt K. CYP2D6 genotype determination in the Danish population. *Eur J clin Pharmac* 1994; **47**: 221–225.

29 Dahl ML, Johansson I, Palmertz MP, Ingelman-Sundberg M, Sjöqvist F. Analysis of the CYP2D6 gene in relation to debrisoquin and desipramine hydroxylation. *Clin Pharmac Ther* 1992; **51**: 12–17.

30 Dahl ML, Iselius L, Alm C, Svensson JO, Lee D, Johansson I. Ingelman-Sundberg M, Sjöqvist F. Polymorphic 2-hydroxylation of desipramine. *Eur J clin Pharmac* 1993; **44**: 445–450.

31 Gram LF, Kofod B, Christensen J, Rafaelsen OJ. Imipramine metabolism: pH-dependent distribution and urinary excretion. *Clin Pharmac Ther* 1971; **12**: 239–244.

32 Jackson PR, Tucker GT, Lennard MS, Woods HF. Polymorphic drug oxidation: pharmacokinetic basis and comparison of experimental indices. *Br J clin Pharmac* 1986; **22**: 541–550.

33 Smith CAD, Gough AC, Leigh PN, Summers BA, Harding AE, Maranganore DM, Sturman SG, Schapira AHV, Williams AC, Spurr NK, Wolf CR. Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinsons disease. *Lancet* 1992; **339**: 1375–1377.

34 Tyndale R, Aoyama T, Broly F, Matsunaga T, Inaba T, Kalow W, Gelboin HV, Meyer UA, Gonzalez FJ. Identification of a new variant CYP2D6 allele lacking the codon encoding Lys-281: possible association with the poor metabolizer phenotype. *Pharmacogenetics* 1991; **1**: 26–32.

35 Broly F, Meyer UA. Debrisoquine oxidation polymorphism, phenotypic consequences of a 3-base-pair deletion in exon 5 of the CYP2D6 gene. *Pharmacogenetics* 1993; **3**: 123–130.

36 Johansson I, Lundqvist E, Bertilsson, L, Dahl ML, Sjöqvist F, Ingelman-Sundberg M. Inherited amplifications of the active gene in the cytochrome 2D locus as a cause of ultrarapid metabolism of debrisoquine. *Proc Natl Acad Sci USA* 1993; **90**: 11825–11829.

37 Alvan F, Bechtel P, Iselius L, Gundert-Remy U. Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur J clin Pharmac* 1990; **39**: 533–537.

38 Wrighton SA, Stevens JC, Becker GW, VandenBranden M. Isolation and characterization of human liver cytochrome P4502C19: Correlation between 2C19 and S-mephenytoin 4'hydroxylation. *Arch Biochem Biophys* 1993; **306**: 240–245.

39 Goldstein JA, Faletto MB, Romkes-Sparks M, Sullivan T, Kitareewan S, Raucy JL, Lasker JM, Chanayem BI. Evidence that CYP2C19 is the major (S)-mephenytoin 4'hydroxylase in humans. *Biochemistry* 1994; **33**: 1743–1752.

40 de Morais SMF, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J biol Chem* 1994; **269**: 15419–15422.

41 Sesardic D, Boobis AR, Edwards RJ, Davies DS. A form of cytochrome P450 in man, orthologous to form d in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *Br J clin Pharmac* 1988; **26**: 363–372.

42 Brøsen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. *Biochem Pharmac* 1993; **45**: 1211–1214.

43 Perel JM, Shostak M, Gann E, Kantor SJ, Glassman AH. Pharmacodynamics of imipramine and clinical outcome in depressed patients. In *Pharmacokinetics of psychoactive drugs. Blood levels and clinical response*, eds Gottshalk LA, Merlis S. Spectrum, New York 1975, pp. 229–241.

(Received 13 September 1994, accepted 13 December 1994)