Inhibition of CYP2D6 activity by treatment with propranolol and the role of 4-hydroxy propranolol

K. ROWLAND, W. W. YEO, S. W. ELLIS, I. G. CHADWICK, I. HAQ, M. S. LENNARD, P. R. JACKSON, L. E. RAMSAY & G. T. TUCKER
Department of Medicine and Pharmacology, University of Sheffield, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF

- 1 The 4-hydroxylation of propranolol by rat and human liver microsomes is associated with formation of a chemically reactive species which binds irreversibly to cytochrome P450D6 (CYP2D6) destroying its catalytic function. Therefore, the effect of propranolol treatment (80 mg twice daily) on debrisoquine phenotype was examined, to see if it resulted in phenocopying *in vivo*. The role of 4-hydroxypropranolol (4OHP) in the inhibition of CYP2D6 activity was also studied using microsomes from yeast expressing CYP2D6 and from human livers; metoprolol was used as the CYP2D6 substrate.
- 2 Although a significant effect on apparent oxidation phenotype was demonstrated, the absolute change in the urinary debrisoquine/4-hydroxydebrisoquine ratio (D/4HD) was small, such that no extensive metaboliser who received propranolol treatment was reclassified as a poor metaboliser. The *in vitro* studies indicated that 4OHP is a potent inhibitor of metoprolol metabolism ($K_i \sim 1 \mu\text{M}$). This inhibitory effect was enhanced when 4OHP was pre-incubated in the presence of a NADPH generating system and human liver microsomes. The effect was decreased significantly when reduced glutathione was added to the pre-incubation mixture. Metabolism of 4OHP occurred when incubated with human liver microsomes in the presence of a NADPH generating system and irrespective of CYP2D6 phenotype; yeast expressing CYP2D6 did not metabolise 4OHP.
- 3 We conclude that, although treatment with propranolol 80 mg twice daily significantly decreases the catalytic function of CYP2D6, the inhibition is insufficient to result in phenocopying. The reactive intermediate produced by further metabolism of 4OHP is probably scavenged effectively *in vivo* by glutathione and other nucleophiles.

Keywords propranolol phenocopying CYP2D6 irreversible binding

Introduction

Administration of propranolol causes significant inhibition of oxidative drug metabolism in animals and man [1]. Furthermore, there is evidence for some drugs (including propranolol itself) that this inhibition is not mediated solely by competition from unchanged propranolol. Thus, liver microsomes from rats pre-treated *in vivo* with propranolol exhibit impaired hydroxylation of propranolol [2, 3], lignocaine [4, 5], imipramine [3] and debrisoquine [3], despite negligible residues of unchanged propranolol

remaining from pre-treatment. Since the *N*-dealkylation of propranolol, lignocaine and imipramine was not inhibited, these observations indicate a selective effect of a metabolite of propranolol on the activity of CYP2D1, the rat isoform of cytochrome P450 known to be a major catalyst of the hydroxylations [3]. Studies using radiolabelled propranolol provide strong support for the role of an irreversibly bound metabolic intermediate of propranolol in the impairment of monooxygenase activity in both rat and

human liver microsomes [3–7]. Since there is a delay between the appearance of 4-hydroxypropranolol (4OHP) and the time course of irreversible binding when propranolol is incubated with rat liver microsomes, Masubuchi *et al.* [6] have, therefore, suggested that a further metabolite of 4OHP is the reactive species responsible for inhibition of hydroxylase activity.

CYP2D6, the human orthologue of rat CYP2D1, exhibits a major genetic polymorphism, and has been shown to make a major contribution to the 4-hydroxylation of propranolol in man [8, 9]. Therefore if 4OHP is a proximate metabolite for a reactive compound that binds and inactivates CYP2D6, this raises a concern that treatment with propranolol may cause phenocopying *in vivo*, that is the conversion of the extensive metaboliser phenotype (EM) to that of an apparent poor metaboliser (PM). We have investigated this possibility in a clinical study and have carried out *in vitro* experiments, with human liver microsomes and yeast expressing CYP2D6, to clarify the role of 4OHP in the inhibition of CYP2D6.

Methods

Clinical study

Twenty hypertensive patients (11 men), aged 33–73 years, who were taking atenolol 50–100 mg daily and had no contraindication to propranolol treatment consented to a randomised open label parallel group study comparing the effect on debrisoquine oxidation phenotype of propranolol 80 mg twice daily vs atenolol 50–100 mg daily for 2 weeks. Thereafter all patients were changed back to atenolol. The study was approved by the hospital Ethics Committee. The debrisoquine phenotype was determined by a standard method [10] at baseline, at the end of weeks 1 and 2 of treatments, and weekly for a further 2 weeks after returning to atenolol treatment. Other antihypertensive drugs and medications were held constant throughout the study. Results were expressed as the mean change in the log urinary metabolic ratio of debrisoquine to its 4-hydroxy product (D/4HD), and were compared using the Mann-Whitney test.

In vitro experiments

The mechanism of inhibition of CYP2D6 activity by 4OHP was investigated using metoprolol as a model substrate [11] and microsomes from human liver samples and yeast expressing recombinant human CYP2D6 [12]. All of the α -hydroxylation and two-thirds of the *O*-demethylation of metoprolol by human liver microsomes is catalysed by CYP2D6 [11]. Further experiments were performed to determine whether CYP2D6 catalyses the metabolism of 4OHP.

Drugs and chemicals The hydrochloride salt of 4OHP was obtained from ICI Pharmaceuticals (Zeneca) (Macclesfield, UK), SKF525A was from

SmithKline Beecham (Welwyn, Herts, UK) and mexiletine HCl was a gift from Boehringer Ingelheim (Bracknell, Berks, UK). Reduced glutathione and ketoconazole were purchased from the Sigma Chemical Co. (Poole, UK), phenacetin from Hopkin and Williams Ltd (Chadwell Heath, UK) and sulphaphenazole was a gift from Ciba-Geigy (Basle, Switzerland). Metoprolol tartrate (racemate), α -hydroxymetoprolol *p*-hydroxybenzoate and *O*-demethylmetoprolol base were obtained from Hassle (Molndal, Sweden), and guanoxan from Pfizer Central Research (Sandwich, UK). All other chemicals were of analytical reagent grade.

Human liver and yeast microsomes Samples of human liver, coded HL3–HL7, were obtained from five renal transplant donors with the approval of the hospital Ethics Committee and the local Coroner. Clinical details of the donors have been published previously [7, 11]. Evidence based on analyses of debrisoquine 4-hydroxylase and metoprolol oxidase activity indicate that the donor of HL3 was phenotypically a PM and that the remainder of the livers were from EM subjects [11]. Human liver and yeast microsomes were prepared as described previously [11, 13]. Microsomal protein content was measured by the method of Lowry *et al.* [14] using bovine serum albumin as the standard.

Incubation conditions

a) Inhibition of metoprolol metabolism by 4OHP

The control incubation mixture comprised 0.1 ml liver microsomal suspension (equivalent to 0.2 mg microsomal protein), 0.2 ml 1.15% KCl (w/v) containing metoprolol (40 μ M) and 0.2 ml of a NADPH generating system dissolved in 0.2 M potassium phosphate buffer (pH 7.4). The NADPH generating system comprised 2 μ M glucose-6-phosphate (G6P), 0.2 μ M NADP, 0.2 μ M G6P-dehydrogenase and 2 μ M MgCl₂. The reaction was started by the addition of metoprolol and the NADPH-generating system and was carried out at 37° C for 10 min. The reaction was stopped by transferring 0.4 ml of the incubate to plastic vials containing 50 μ l 6% (w/v) perchloric acid (PCA). The control experiment was carried out using microsomes from HL4, 5, 6 and 7. The incubations were then repeated under the following conditions: (a) pre-incubation with 4OHP (0.4 μ M) for 10 min without NADPH generating system; (b) pre-incubation for 10 min with 4OHP (0.4 μ M) and NADPH generating system; (c) pre-incubation for 10 min with 4OHP (0.4 μ M), NADPH generating system and reduced glutathione (1 mM).

The concentration of 4OHP used was 0.4 μ M because previous experiments had indicated that this caused approximately 40% inhibition of both pathways of metoprolol metabolism. Since we were trying to establish whether the pre-incubation of 4OHP led to a further increase in the inhibition it was necessary to select a 4OHP concentration that did not completely inhibit the metabolism of metoprolol when co-incubated.

The original control experiment was repeated using

microsomes prepared from yeast expressing CYP2D6 (59–71 pmol P450 mg⁻¹ protein) and two human livers (HL5 and HL7; 195–221 pmol P450 mg⁻¹ protein) with metoprolol (10, 20, 40 µM), a NADPH generating system and with 4OHP (2–10 µM).

b) *Metabolism of 4OHP* 4OHP (4 µM) was incubated with microsomal suspensions (0.1 ml containing 0.4 mg protein) from three human livers (HL3, HL5 and HL7) and yeast expressing CYP2D6, in the presence of a NADPH generating system (0.2 ml) in a total incubation volume of 0.5 ml. The reaction was started by the addition of microsomes and the NADPH generating system and was carried out at 37° C for 15 min. The reaction was stopped by the addition of 50 µl 6% (w/v) PCA.

The experiments with human liver microsomes were repeated in the absence of a NADPH generating system and in the presence of SKF525A (100 µM), carbon monoxide, phenacetin, ketoconazole or sulphaphenazole (10–50 µM in each case).

Analytical methods

a) *Metoprolol metabolites* The metabolites were assayed using the h.p.l.c. method described previously by Otton *et al.* [11].

(b) *4-Hydroxypropranolol* Ascorbic acid (25 mg) was added to the incubation mixture to prevent decomposition of 4OHP and 0.4 ml of the incubate was then transferred to plastic vials containing mexiletine (40 µl of a 0.06 mg ml⁻¹ solution) which was added as an internal standard. The samples were centrifuged at 3000 rev min⁻¹ for 10 min and aliquots of the supernatant were injected directly on to the column.

H.p.l.c. was performed using a Z-module column system containing a cartridge packed with Nova-Pak C18 (Waters Associates), a model 6000A pump (Waters Associates), a model 7125 Rheodyne injector (H.p.l.c. Technology, Macclesfield, UK) and a model 970FS Schoeffel/Kratos fluorescence detector (H.p.l.c. Technology). The mobile phase was water/acetonitrile (80:20, v/v) containing 1% (v/v) triethylamine adjusted to pH 2.7 with orthophosphoric acid, and was delivered at a flow rate of 2.5 ml min⁻¹. The retention times of 4OHP and mexiletine under these conditions were 3.5 min and 4.5 min, respectively. The detector was operated at an excitation wavelength of 205 nm with a 340 nm emission cut-off filter. The limit of determination of 4OHP was 15 pmol ml⁻¹ and the coefficient of variation of the assay was less than 5%.

Data analysis

Inhibition constants (K_i) were determined from Dixon plots by linear regression. Analysis of variance followed by pairwise comparisons was used to test for differences.

Results

The effect of propranolol treatment on CYP2D6 phenotype

One patient in the propranolol group was a poor metaboliser and was, therefore, excluded from the analysis as predefined by the protocol. The mean change from baseline in the log urinary metabolic ratio of debrisoquine to its 4-hydroxy product (D/4HD) over 2 weeks differed significantly for the two treatment groups [median 0.59 propranolol vs -0.03 atenolol; difference 0.6 (95% CI 0.2–1.2; $P = 0.01$; Figure 1)].

When the time-course of change in D/4HD ratio was examined, a significant difference between groups was already present after 1 week of treatment (difference 0.6; 95% CI 0.3–1.3; $P = 0.01$). One week after discontinuation of propranolol and reintroduction of atenolol there was no significant difference between the groups (difference 0.27, 95% CI -0.3 to 0.7, $P = 0.35$; Figure 1). The variance related to propranolol treatment was about half that observed between patients in the 19 extensive metabolisers. The magnitude of the change (0.6; 95% CI 0.2–1.2, $P = 0.01$) was not sufficient to result in phenocopying—the conversion of the extensive metaboliser phenotype to that of an apparent poor metaboliser. The changes in D/4OHD ratio in the nine EM patients treated with propranolol are shown in Figure 2, with reference to the antimode separating extensive from poor metabolisers found in a previous population study.

The effect of 4OHP on microsomal CYP2D6 activity

Preincubation of 4OHP (0.4 µM) without the NADPH generating system showed that it was a potent inhibitor of metoprolol metabolism by human liver microsomes. Thus, the formation of the α -hydroxy and *O*-demethyl products of metoprolol was decreased to two-thirds of control values (Table 1;

Figure 1 Mean change from baseline in the log urinary metabolic ratio of debrisoquine/4-hydroxydebrisoquine for nine patients treated with propranolol 80 mg twice daily for weeks 1 and 2 followed by a further 2 weeks (3 and 4) of atenolol treatment (hatched bar) vs 10 patients continuing on atenolol 50–100 mg daily for the full 4 weeks (solid bar). * $P < 0.04$, ** $P < 0.01$.

Figure 2 Change in the metabolic ratio of debrisoquine (D/4-OHD) in nine patients treated with propranolol (arrows) superimposed on the frequency distribution of log urinary D/4-OHD ratios for 324 British Caucasians (unpublished data). Antimode (large arrow) delineates the division between extensive (□) and poor metaboliser (■) status.

Table 1 The effect of co-incubation of 4OHP (0.4 μ M) on the α -hydroxylation (α OH) and *O*-demethylation (ODM) of metoprolol (40 μ M) by human liver microsomes

Experiment	% inhibition	
	α -OH	ODM
a	37 $\pm$ 12	33 $\pm$ 7
b	76 $\pm$ 29**	67 $\pm$ 18*
c	55 $\pm$ 16 [†]	49 $\pm$ 11 [‡]

** $P < 0.01$, * $P < 0.05$ vs a; [†] $P < 0.01$, [‡] $P < 0.05$ vs b.

Results are expressed as mean ($\pm$ s.d.) percentage inhibition of the control activity in the absence of 4OHP.

a = pre-incubation with 4OHP for 10 min without NADPH.

b = pre-incubation for 10 min with 4OHP and NADPH.

c = pre-incubation for 10 min with 4OHP, NADPH and reduced glutathione (1 mM).

Each experiment was replicated 4 times using microsomes from four different livers.

experiment (a)). A Dixon plot of the inhibition of α -hydroxylation of metoprolol by 4OHP was unable to distinguish between competitive and non-competitive inhibition (Figure 3). The apparent K_i value for the inhibition of α -hydroxylation was 1 μ M (Figure 3). Preincubation of 4OHP with the NADPH generating system resulted in a further decrease in metoprolol metabolism to one-third of control values (Table 1; experiment (b)). The concentration of 4OHP remaining in the incubation mixture was approximately 0.2 μ M when metoprolol was added. The addition of reduced glutathione to the preincubation mixture with 4OHP and NADPH attenuated the decrease in metoprolol metabolism (Table 1; experiment (c)). 4OHP inhibited the metabolism of metoprolol by CYP2D6 in yeast (Figure 4). The rates of α -hydroxylation of metoprolol (40 μ M) in the absence of 4OHP, using microsomes from HL5, HL7 and yeast expressing CYP2D6 were 29, 15 and 21 pmol product mg^{-1} protein min^{-1} , respectively.

The metabolism of 4OHP

4OHP (4 μ M) was metabolised by microsomes from three human livers (HL3, HL5 and HL7) irrespective

Figure 3 Dixon plot for inhibition of the α -hydroxylation of metoprolol when coincubated with 4OHP in human liver microsomes from HL5 (human liver 5). Each point represents the mean result of duplicate incubations. (I, inhibitor; V, nmol metabolite produced min^{-1} mg^{-1} protein.)

Figure 4 Inhibition of the metabolism of metoprolol (40 μ M) by 4OHP when coincubated with microsomes from yeast expressing CYP2D6. Values are expressed as mean ($\pm$ s.d.) percentage of the control in the absence of the inhibitor. The experiment was replicated three times. Δ ODM; $\square$ α OH.

Figure 5 Metabolism of 4OHP (4 μ M) by human liver (HL) microsomes ($\blacktriangle$ HL3, $\triangle$ HL5, $\circ$ HL7) and microsomes from yeast ($\square$) expressing CYP2D6. Values are expressed as mean ($\pm$ s.d.) percentage of the starting concentration of 4OHP remaining in the incubation mixture. The experiments were replicated three times.

of CYP2D6 phenotype (Figure 5). This reaction required the presence of a NADPH generating system and it was inhibited by SKF525A (100 μ M) and the presence of carbon monoxide (Figure 6). Phenacetin, ketoconazole and sulphaphenazole did not inhibit the

Figure 6 Effects of different incubation conditions on the metabolism of 4OHP (4 μ M) by human liver microsomes. Values are expressed as mean ($\pm$ s.d.) percentage of 4OHP metabolism after a 15 min incubation. The experiments were replicated three times using microsomes from three different livers, HL3, HL5 and HL7. (CTRL, active microsomes and NADPH generating system present; NOMS, without microsomes; XMS, heat inactivated microsomes; CO, carbon monoxide; P, phenacetin; K, ketoconazole; S, sulphaphenazole.)

metabolism of 4OHP (Figure 6). Yeast expressing CYP2D6 did not metabolise 4OHP (Figure 5).

There was an inconsistency with respect to enzyme activity, between the data obtained from the two experiments described above. The experiment described in Table 1 indicated that there was 50% disappearance of 4OHP (0.4 μ M) in 10 min whereas the results in Figure 5 showed that virtually all of the 4OHP (4 μ M) was metabolised in approximately 5 min. The same batch of microsomes was used for both experiments which were carried out several months apart. We presume, therefore, that some of the activity of the enzyme metabolising 4OHP had been lost on storage prior to carrying out the experiment with the lower (0.4 μ M) concentration of 4OHP. However, this would not alter the conclusion of the study.

Discussion

The clinical study examining the effect of propranolol on CYP2D6 phenotype, showed that there was significant inhibition of the metabolism of debrisoquine to 4-OH debrisoquine after 1 week of treatment with propranolol 80 mg twice daily (Figure 1). However, the magnitude of the change (0.6; 95% CI 0.2–1.2, $P = 0.01$) was not sufficient to result in phenocopying—the conversion of the extensive metaboliser phenotype to that of an apparent poor metaboliser. Inspection of Figure 2, which illustrates the observed changes in ratio against the data from a local population study separating extensive from poor metabolisers, reveals that a change in metabolic ratio even larger than the calculated upper 95% confidence

interval of 1.2 would be necessary to convert the majority of extensive metabolisers into apparent poor metabolisers. The change in D/4OHD ratio in extensive metabolisers occurred quickly as a significant difference between atenolol and propranolol groups was already present after 1 week of treatment, and this difference was abolished 1 week after reintroduction of atenolol in the propranolol group (Figure 1). The variance related to propranolol treatment was about half that observed between patients in the 19 extensive metabolisers. This suggests that inter-individual variation in constitutive CYP2D6 activity between patients is likely to overwhelm any clinical consequences attributable to differences in activity caused by treatment with propranolol. However, the effect in some individuals may still be large enough for significant drug interactions to occur when propranolol and other drugs metabolised by CYP2D6 are co-prescribed.

To explain why phenocopying did not occur *in vivo* we studied the effects of 4OHP on CYP2D6 activity and the metabolism of 4OHP *in vitro*. In keeping with the potent inhibition of propranolol-7-hydroxylase by 4OHP observed in rat liver microsomes [6], we found a low K_i value (1 μ M) for the effect of 4OHP on the α -hydroxylation of metoprolol (Figure 3). The fact that inhibition of metoprolol metabolism was enhanced when half of the 4OHP was metabolised during pre-incubation and before addition of metoprolol (Table 1), confirms a major contribution to inhibition from a further metabolite of 4OHP. Attenuation of this effect by pre-incubation with glutathione suggests the formation of an adduct with the reactive 4OHP metabolite, sequestering it away from CYP2D6. The extent to which this might occur *in vivo* affords a possible explanation for the relatively small effect of propranolol treatment on the debrisoquine/4OH debrisoquine ratio.

The findings that 4OHP was metabolised by human liver microsomes irrespective of CYP2D6 phenotype and was not metabolised by microsomes from yeast expressing CYP2D6 indicate that the reactive product of 4OHP is not generated by CYP2D6 itself. Thus the mechanism of non-competitive inhibition of CYP2D6 substrates does not involve specific suicidal inactivation of the enzyme. Since 4OHP was capable of inhibiting the metabolism of metoprolol by CYP2D6 expressed in yeast, yet is not metabolised by this enzyme, at least part of the inhibition of CYP2D6 seen after treatment with propranolol may be due to parent drug, intact 4OHP or both, in addition to that mediated through irreversible binding of a reactive species. Conversely, whether the reactive product of 4OHP metabolism contributes to inhibition of cytochromes P450 other than CYP2D6 is unknown. The *N*-dealkylations of propranolol, lignocaine and imipramine appear to be relatively insensitive [2–5], but antipyrine and theophylline, which are not substrates of CYP2D6, are inhibited *in vivo* by propranolol treatment.

The results of experiments to identify the enzyme responsible for the metabolism of 4OHP seem to eliminate CYPs 1A2, 2C9 and 3A4, since corresponding probe inhibitors (phenacetin, sulphaphenazole and

ketoconazole) had no effect. The potent inhibitory effect of the general cytochrome P450 inhibitor SKF525A and the reduction of metoprolol metabolism caused by the presence of carbon monoxide suggests that the metabolism of 4OHP is P450-dependent. Two isomeric methoxyhydroxy metabolites have been found in human plasma and urine during chronic propranolol therapy [16]. Walle *et al.* [16] have suggested that the most likely precursor of these metabolites would be the catechol intermediate 3,4-dihydroxypropranolol [16]. It is possible that this catechol might undergo further oxidation to a reactive quinone [17] which binds irreversibly to microsomal protein [18]. Quinones are capable of forming adducts with glutathione and this could account for the increased catalytic activity of CYP2D6 observed when reduced glutathione was added to the incubation mixture (Table 1c).

In conclusion, we have shown that propranolol 80 mg twice daily decreases the catalytic function of CYP2D6 significantly, but the inhibition is insufficient to cause phenocopying. The latter might have been anticipated from evidence suggesting that the metabolism of propranolol *in vitro* results in the formation of an irreversibly bound intermediate that inactivates CYP2D6. However, it seems likely that the appearance of such a reactive intermediate might be decreased *in vivo* by parallel conjugation of 4OHP and scavenging by glutathione and other nucleophiles. This possibility emphasises the importance of interpreting *in vitro* data on drug metabolism in relation to both activating and inactivating enzymes [19].

This work was supported in part by a research studentship (KR) from SmithKline Beechams Pharmaceuticals.

References

- 1 Bax NDS, Lennard MS, Al-Asady S, *et al.* Inhibition of drug metabolism by β -adrenoceptor antagonists. *Drugs* 1983; **25**: 121–126.
- 2 Schneck DH, Pritchard JF. The inhibitory effect of propranolol pretreatment on its own metabolism in the rat. *J Pharmac exp Ther* 1981; **218**: 575–581.
- 3 Masubuchi Y, Fujita S, Chiba M, Kagimoto N, Umeda S, Suzuki T. Impairment of debrisoquine 4-hydroxylase and related monooxygenase activities in the rat following treatment with propranolol. *Biochem Pharmac* 1991; **41**: 861–865.
- 4 Al-Asady SAH, Black GL, Lennard MS, Tucker GT, Woods HF. Inhibition of lignocaine metabolism by beta-adrenoceptor antagonists in rat and human liver microsomes. *Xenobiotica* 1989; **19**: 929–944.
- 5 Suzuki T, Ishida R, Matsui S, Masubuchi Y, Narimatsu S. Kinetic analysis of mutual inhibition of lidocaine and propranolol in rat liver microsomes. *Biochem Pharmac* 1993; **45**: 1528–1530.
- 6 Masubuchi Y, Suzuki K, Fujita S, Suzuki T. A possible mechanism for the impairment of hepatic microsomal monooxygenase activities after multiple administration of propranolol in rats. *Biochem Pharmac* 1992; **43**: 757–762.
- 7 Shaw L, Lennard MS, Tucker GT, *et al.* Irreversible binding and metabolism of propranolol by human liver microsomes—relationship to polymorphic oxidation. *Biochem Pharmac* 1987; **36**: 2283–2288.
- 8 Otton SV, Gillam EMJ, Lennard MS, Tucker GT, Woods HF. Propranolol oxidation by human liver microsomes—the use of cumene hydroperoxide to probe isozyme specificity and regio- and stereoselectivity. *Br J clin Pharmac* 1990; **30**: 751–760.
- 9 Lennard MS, Jackson PR, Freestone S, *et al.* The relationship between debrisoquine oxidation phenotype and the pharmacokinetics and pharmacodynamics of propranolol. *Br J clin Pharmac* 1984; **17**: 679–686.
- 10 Lennard MS, Silas JH, Smith AJ, Tucker GT. Determination of debrisoquine and its 4-hydroxy metabolite in biological fluids by gas chromatography with flame-ionization and nitrogen-selective detection. *J Chromatography* 1977; **133**: 161–166.
- 11 Otton SV, Crewe HK, Lennard MS, Tucker GT, Woods HF. Use of quinidine inhibition to define the role of the sparteine/debrisoquine cytochrome P-450 in metoprolol oxidation by human liver microsomes. *J Pharmac exp Ther* 1988; **247**: 242–247.
- 12 Ellis SW, Ching MS, Watson PS, *et al.* Catalytic activity of human debrisoquine 4-hydroxylase cytochrome p-450 (CYP2D6) expressed in yeast. *Biochem Pharmac* 1992; **44**: 617–620.
- 13 Ching MS, Lennard MS, Tucker GT, *et al.* The expression of human cytochrome P4501A1 in the yeast *Saccharomyces cerevisiae*. *Biochem Pharmac* 1991; **42**: 753–758.
- 14 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J biol Chem* 1951; **193**: 265–275.
- 15 Bax NDS, Lennard MS, Tucker GT. Inhibition of antipyrine metabolism by beta-adrenoceptor antagonists. *Br J clin Pharmac* 1981; **12**: 779–784.
- 16 Walle T, Conradi C, Walle UK, Gaffney TE. O-methylated catechol-like metabolites of propranolol in man. *Drug Metab Dispos* 1978; **4**: 481–487.
- 17 Hiramatsu M, Kumagai Y, Unger SE, Cho AK. Metabolism of methylenedioxymethamphetamine: formation of dihydroxymethamphetamine and a quinone identified as its glutathione adduct. *J Pharmac exp Ther* 1990; **254**: 521–527.
- 18 Dybing E, Nelson SD, Mitchell JR, Sasame HA, Gillette JR. Oxidation of alpha-methyldopa and other catechols by cytochrome P450 generated superoxide anion: possible mechanism of methyldopa hepatitis. *Mol Pharmac* 1976; **12**: 911–920.
- 19 Park BK. Metabolic basis of adverse drug reactions. *J Roy Coll Phys* 1986; **20**: 195–200.

(Received 16 December 1993,
accepted 11 March 1994)