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The relationship between debrisoquine oxidation phenotype and the pharmacokinetics and pharmacodynamics of propranolol

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1 The pharmacokinetics and pharmacodynamics of propranolol (80 mg by mouth) were studied in seven extensive and four poor metabolisers of debrisoquine.
2 Evidence for impairment of the 4′-hydroxylation of propranolol was found in poor metabolisers. However, no significant difference was detected in the oral clearance of unchanged drug between the two groups of debrisoquine oxidation phenotypes.
3 Poor metabolisers of debrisoquine did not experience more intense or more prolonged β-adrenoceptor blockade than extensive metabolisers of debrisoquine.

Keywords Propranolol clearance metabolism debrisoquine oxidation phenotype

Introduction

An association between debrisoquine oxidation phenotype and the ability to metabolise the β-adrenoceptor antagonists, metoprolol and bufuralol, has been described. Population studies also suggest that the metabolism of these drugs is under common genetic control (Lennard *et al.*, 1982a, b, 1983a; Dayer *et al.*, 1982, 1983). Furthermore, there is evidence that metoprolol produces more intense and more prolonged β-adrenoceptor blockade in poor metabolisers of debrisoquine than in extensive metabolisers (Lennard *et al.*, 1982b).

An anecdotal report suggested that the metabolism of propranolol (Shah *et al.*, 1982) may also be under this type of genetic control. Von Bahr *et al.* (1982a) have reported evidence for a correlation between the 4′-hydroxylation of propranolol and that of debrisoquine in human liver microsomes. The aim of the present study was to explore the relationship between debrisoquine oxidation phenotype and the pharmacokinetics and pharmacodynamics of propranolol in a panel of healthy volunteers.

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Methods

Eleven healthy non-smoking men aged 31 ± 5 (s.d.) years consented to take part in the study, which was approved by the Royal Hallamshire Hospital Ethics Committee. All subjects had normal hepatic and renal function. Oxidation phenotype was determined by measurement of urinary debrisoquine/4-hydroxydebrisoquine ratios as described elsewhere (Lennard *et al.*, 1982b) and the panel consisted of seven extensive and four poor metabolisers of debrisoquine.

After a 12 h fast a venous blood sample was taken and 80 mg of propranolol hydrochloride was given orally. Further blood samples were taken at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after dosing. Plasma was separated and then stored at −20 °C until analysis.

Before dosing with propranolol the subjects were exercised on a bicycle ergometer for 4 min at a work-load sufficient to achieve a heart rate

greater than 140 beats/min. This test was repeated using the same work-load at 2, 12 and 24 h after dosing. Post-exercise heart rates were measured from an ECG recording using the mean of the first 4 beats after cessation of exercise. β-adrenoceptor-blockade was expressed as the percentage reduction in post-exercise heart rate compared with pre-dosing values.

Plasma concentrations of unchanged propranolol and of 'total' propranolol and 4′-hydroxypropranolol after enzyme hydrolysis of the sample were measured by the h.p.l.c. method of Lo *et al.* (1982). Conjugated metabolites were hydrolysed by incubating plasma samples with a mixture of β-glucuronidase and aryl sulphatase (G-0751, Sigma) at 37 °C for 3 h. This procedure converts quantitatively the glucuronide but not the sulphate conjugate to the aglycone (Walle *et al.*, 1983).

The areas under the plasma concentration-time curve for unchanged propranolol (AUC_o), total (free plus conjugated) 4′-hydroxypropranolol [$(AUC_{m1})_o$] and conjugated propranolol [$(AUC_{m2})_o$] were calculated using the trapezoidal rule with extrapolation to infinity. The oral clearance of propranolol was calculated by dividing the dose by the AUC_o. The ratios $(AUC_{m1})_o/AUC_o$ and $(AUC_{m2})_o/AUC_o$ were used as measures of the partial intrinsic clearance of propranolol down the 4′-hydroxylation and conjugation pathways, respectively (see **Appendix**).

The statistical methods used were the Mann-Whitney U test and the Spearman rank correlation (r_s).

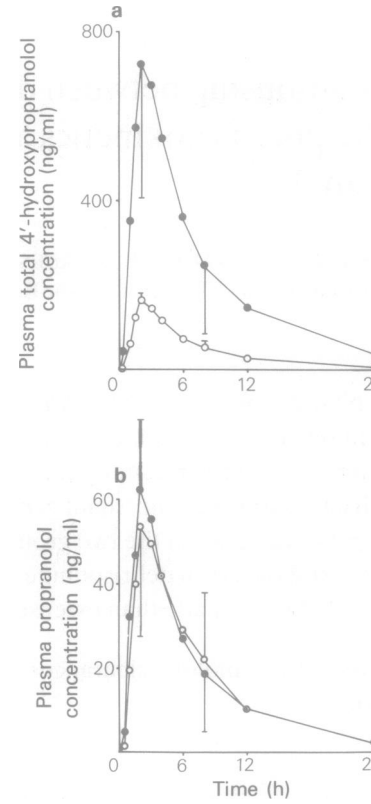

Figure 1 Plasma total 4′-hydroxypropranolol (**a**) and unchanged propranolol (**b**) concentrations *vs* time after administration of a single 80 mg oral dose of propranolol hydrochloride to seven extensive metabolisers (●) and four poor metabolisers (○) of debrisoquine. Vertical bars represent s.d.

Results

At all sampling times, plasma total 4′-hydroxypropranolol concentrations were about five to seven times higher in extensive metabolisers compared to poor metabolisers of debrisoquine (Figure 1a). Mean values (± s.d.) at 2, 12 and 24 h were 719 ± 313, 145 ± 93 and 35 ± 28 ng/ml, respectively, in extensive metabolisers and 152 ± 23, 26 ± 6 and 5 ± 4 ng/ml in poor metabolisers. Values for the ratio $(AUC_{m1})_o/AUC_o$ correlated inversely with the urinary debrisoquine/4-hydroxydebrisoquine ratio ($r_s = -0.93$, $P < 0.001$) (Table 1, Figure 2a).

In contrast, the mean plasma concentrations of unchanged propranolol were similar in the two oxidation phenotypes (Figure 1b). Mean values (± s.d.) at 2, 12 and 24 h were 62 ± 42, 10 ± 9 and 2 ± 1 ng/ml in extensive metabolisers and 54 ± 26, 10 ± 7 and 2 ± 2 ng/ml in poor metabolisers. There was no relationship between the AUC_o values for unchanged propranolol and the urinary debrisoquine/4-hydroxydebrisoquine ratio ($r_s = 0.56$, $P > 0.05$) (Figure 2b). The 95% confidence limits for the difference between the mean AUC_o values for extensive and poor metabolisers of debrisoquine were −436 to +436 ng ml^{-1} h. The elimination half-life of unchanged propranolol was not significantly different in the two phenotypes (Table 1).

The area under the plasma concentration-time curve for conjugated propranolol [$(AUC_{m2})_o$] was significantly higher in poor metabolisers (3050 ± 606 ng ml^{-1} h) than in extensive metabolisers (1985 ± 796 ng ml^{-1} h) ($P = 0.012$). However, values of the ratio $(AUC_{m2})/AUC_o$, a measure of partial metabolic clearance by conjugation, were not significantly different between the two phenotypes and were not related to the urinary debrisoquine/4-hydroxydebrisoquine ratio ($r_s = 0.40$, $P > 0.05$) or to the partial clearance to 4′-hydroxypropranolol ($r_s = 0.16$, $P > 0.05$).

Table 1 Pharmacokinetic measurements and β-adrenoceptor antagonist effect following administration of a single oral 80 mg dose of propranolol hydrochloride to seven extensive (EM) and four poor (PM) metabolisers of debrisoquine.

Phenotype	*Subject*	*Urinary debrisoquine/ 4-Hydroxy-debrisoquine ratio*	*4'-Hydroxypropranolol AUC_o* / *Propranolol AUC_o*	*Propranolol glucuronide AUC_o* / *Propranolol AUC_o*	*AUC propranolol (ng ml^{-1} h)*	*Propranolol elimination half-life (h)*	*% Reduction in exercise heart rate* 2 h	12 h	24 h
EM	1	0.21	27.6	5.82	208	2.69	18	4	0
	2	0.68	12.5	3.69	862	4.63	29	18	7
	3	0.57	7.3	3.95	501	5.34	23	13	7
	4	0.41	36.6	9.06	134	3.35	29	15	9
	5	0.19	41.0	14.58	103	3.27	26	14	1
	6	0.84	12.1	3.58	519	4.56	29	10	8
	7	5.07	2.8	5.02	587	4.16	31	—	1
	Mean	1.16	20.0	6.53	416	4.00	26	12	5
	s.d.	1.74	15.0	4.03	279	0.93	5	5	4
PM	1	41	2.4	6.59	514	5.06	28	13	4
	2	40	4.1	10.70	260	4.40	20	2	0
	3	33	4.8	12.45	188	2.56	28	13	4
	4	208	1.4	5.26	702	5.02	23	12	−10
	Mean	81	3.2	8.75	416	4.26	25	10	0
	s.d.	85	1.6	3.38	236	1.17	4	5	7
P values EM *vs* PM			0.012	NS	NS	NS	NS	NS	

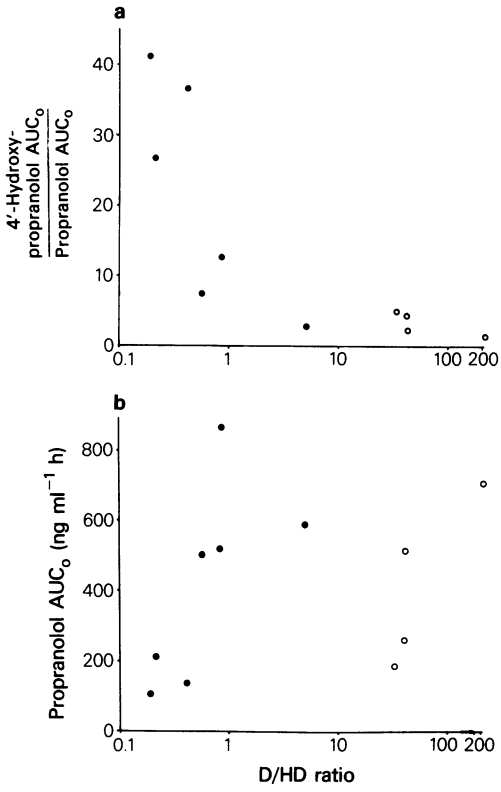

Figure 2 Relationship between the urinary debrisoquine/4-hydroxy-debrisoquine ratio (D/HD) and (**a**) the 4'-hydroxypropranolol AUC_0/propranolol AUC_0 ratio and (**b**) propranolol AUC_0. ● – extensive metabolisers, O – poor metabolisers of debrisoquine.

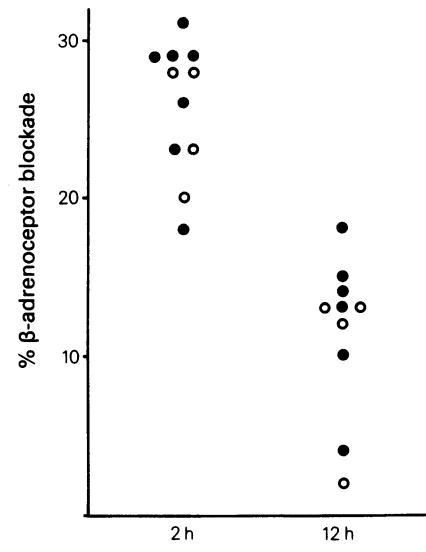

Figure 3 Percentage β-adrenoceptor antagonist effect (% β-adrenoceptor-blockade) at 2 and 12 h after dosing with propranolol. ● – extensive metabolisers, O – poor metabolisers of debrisoquine.

β-adrenoceptor blockade did not correlate with the urinary debrisoquine/4-hydroxydebrisoquine ratio at either 2 h ($r_s = 0.06$, $P > 0.05$) or 12 h ($r_s = -0.28$, $P > 0.05$) (Figure 3). At 24 h, β-adrenoceptor blockade was less than 10% in all subjects (Table 1).

Discussion

The inverse relationship between the ratio $(AUC_{ml})_0/AUC_0$ and the urinary debrisoquine/4-hydroxydebrisoquine ratio provides *in vivo* evidence that the 4'-hydroxylation of propranolol and that of debrisoquine are closely linked, supporting the *in vitro* findings of Von Bahr *et al* (1982a). Our results, therefore, are consistent with impaired 4'hydroxylation of propranolol in poor metabolisers of debrisoquine. This conclusion rests on a number of assumptions (**Appendix**), however. In particular, the renal clearance of the metabolite in the two debrisoquine phenotypes is assumed to be the same.

4'-Hydroxypropranolol and its conjugate(s) are minor urinary metabolites of propranolol, accounting for 6–27% of an 80 mg oral dose in man (Schneck *et al.*, 1980; Silber *et al.*, 1983; Walle *et al.*, 1983). Therefore, impairment of this pathway alone would not be sufficient to exert a sizeable influence on the kinetics of the parent drug. Thus, the lack of relationship between the oral clearance of unchanged propranolol and debrisoquine oxidation phenotype in this study suggests that the control of other pathways of propranolol metabolism is not linked to that of the 4-hydroxylation of debrisoquine. Confidence limits for these data are wide and proof of no difference in the oral clearance of propranolol in the two phenotypes would require a study with larger numbers of subjects. On the other hand, large differences in metoprolol AUC between extensive and poor metabolisers of debrisoquine were observed in a study of similar design and size (Lennard *et al.*, 1982b).

Other known routes of propranolol metabolism are *N*-dealkylation followed by side-chain deamination and glucuronidation of parent drug (Smith & Tucker, 1980). As with the 4-hydroxylation of debrisoquine *N*-dealkylation reactions are catalysed by the mixed-function oxidases. However, there are no precedents for *N*-dealkylation reactions being under polymorphic control of the debrisoquine-type (Lennard *et al.*,

1983b). Using the ratio $(AUC_{m2})_o/AUC_o$ as an index of the partial clearance of propranolol to its conjugated products we found no connection between these routes of propranolol metabolism and debrisoquine oxidation phenotype or partial clearance to 4'-hydroxypropranolol.

The lack of relationship between debrisoquine oxidation phenotype and β-adrenoceptor blockade was consistent with similar plasma concentrations of unchanged drug in both phenotypes. This assumes, however, that only unchanged drug contributes to the pharmacological effect. It has been shown in dogs that 4'-hydroxypropranolol and propranolol are equipotent in their β-adrenoceptor blocking properties (Fitzgerald & O'Donnell, 1971). 4'-Hydroxypropranolol is formed in significant amounts only after oral dosing in man (Paterson *et al.*, 1970) and indirect evidence based on a disparity between the extent of β-adrenoceptor blockade achieved after oral and intravenous propranolol suggested that 4'-hydroxypropranolol contributes significantly to β-adrenoceptor blockade after a single oral dose of drug (Cleavland & Shand, 1972; Coltart & Shand, 1970). If this is the case and if it is assumed that differences in the plasma concentration of total metabolite (unconjugated plus conjugated) reflect differences in those of unconjugated metabolite alone, less pharmacological activity would be anticipated in poor compared with extensive 4'-hydroxylators of propranolol given similar concentrations of parent drug. This was not observed in the present study, suggesting therefore that 4'-hydroxypropranolol may not have contributed significantly to the β-adrenoceptor-blocking effects of the parent drug. Thirty per cent of the metabolic products of propranolol have not been characterised in humans (Walle *et al.*, 1983) and others have suggested that some of these metabolites may make a substantial contribution to the β-adrenoceptor-blocking action of propranolol (Bai & Abramson, 1983). The present study indicates that the extent of formation of these metabolites may not be related to the polymorphic oxidation of debrisoquine.

A further complication in the interpretation of our data is the fact that propranolol undergoes stereoselective disposition, the S(−) enantiomer being more active than the R(+) form (Von Bahr *et al.*, 1982b; Walle *et al.*, 1984). Whether there are significant differences in plasma concentrations of the isomers of parent propranolol and its metabolites between extensive and poor metabolisers of debrisoquine remains to be investigated.

In an anecdotal report it has been proposed that propranolol should be added to the list of β-adrenoceptor blockers which may produce unacceptable side-effects in poor metabolisers of debrisoquine (Shah *et al.*, 1982). Our findings provide no evidence that phenotyping with debrisoquine can identify those individuals who may be most vulnerable to side-effects, if those side-effects are related to plasma propranolol concentrations.

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Appendix

Propranolol undergoes extensive first-pass metabolism after oral administration (Shand *et al.*, 1970). Therefore, when calculating the extent of metabolite production that formed on the 'first pass' and that formed following recirculation through the liver must be considered:

Amount of metabolite i formed = contribution from first-pass + contribution from recirculation

Am_i = E_H × fraction of total metabolised drug accounted for as metabolite i + F_H × fraction of total metabolised drug accounted for as metabolite i.

where E_H and F_H are the hepatic extraction ratio and systemic availability of the drug, respectively.

Assuming that linear kinetics apply, that there is no drug metabolism in the gut and that the liver is 'well-stirred' and the sole site of drug metabolism (Pang & Rowland, 1977), the fraction of total metabolised drug converted to metabolite i is given by

$$\frac{CL_{mi.int}}{CL_{int}}$$

where $CL_{mi.int}$ is the partial intrinsic clearance of drug to metabolite i and CL_{int} is the total intrinsic metabolic clearance of the drug.

Therefore:

$$Am_i = D.E_H.\frac{CL_{mi,int}}{CL_{int}} + D.F_H.(1\text{-}fe).\frac{CL_{mi,int}}{CL_{int}} \quad \ldots(1)$$

where fe = fraction of drug dose, *D*, excreted unchanged in the urine.

If $fe << 1$:

$$Am_i = (E_H + F_H).D.\frac{CL_{mi,int}}{CL_{int}} \quad \ldots(2)$$

But:

$$E_H + F_H = 1$$

And assuming the 'well-stirred' model of hepatic drug clearance and that $fe << 1$:

$$CL_{int} = CL_o$$

where CL_o is the oral clearance of drug.

Replacing D/CL_o by AUC_o, where the latter is the area under the plasma drug concentration-time curve after oral administration:

$$Am_i = AUC_o.CL_{mi,int} \quad \ldots(3)$$

If the metabolite undergoes no further metabolism and is excreted unchanged in the urine, its renal clearance is given by

$$CL_R(mi) \quad \frac{Am_i}{(AUC_{mi})_o} \quad \ldots(4)$$

where $(AUC_{mi})_o$ is the area under the plasma metabolite concentration-time curve after oral

administration of drug.

Substituting Am_i in equation (3) into equation (4) and rearranging gives:

$$\frac{(AUC_{mi})_o}{AUC_o} = \frac{CL_{mi.int}}{CL_R(mi)} \quad \ldots (5)$$

Therefore, assuming that $CL_R(mi)$ is constant, the ratio $(AUC_{mi})_o/AUC_o$ reflects the partial intrinsic clearance of drug to metabolite i.