Partial metabolic clearances as determinants of the oral bioavailability of propranolol

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1 The objective of this study was to determine the relationships between the total oral clearance of propranolol and the partial clearances through its primary metabolic pathways, i.e. glucuronidation, side-chain oxidation and ring oxidation.

2 Seven young, white males were given single 80 mg oral doses of the drug together with tritium-labelled propranolol. Plasma propranolol was measured by GC/MS and fourteen metabolites were measured in urine by h.p.l.c. with radiometric detection.

3 The total oral clearance of propranolol in these subjects varied about three-fold, from 27.5 to 71.4 ml min⁻¹ kg⁻¹.

4 The clearance through glucuronidation was very similar in all subjects, ranging from 6.8 to 9.9 ml min⁻¹ kg⁻¹.

5 The clearance through side-chain oxidation varied 2.4-fold, from 10.9 to 25.8 ml min⁻¹ kg⁻¹. Increased clearance through this pathway correlated with increased total oral clearance ($r = 0.84$; $P < 0.02$).

6 The clearance through ring oxidation varied as much as 5.6-fold, from 7.5 to 41.8 ml min⁻¹ kg⁻¹. Increased clearance through this pathway correlated highly with increased total oral clearance ($r = 0.94$; $P < 0.002$).

7 These observations indicate that the intersubject variability in the oral clearance of propranolol in young, white males is due mainly to differences in the activity of the ring oxidation pathway, to some extent in the side-chain oxidation pathway, but not to differences in the glucuronidation pathway.

8 The partial metabolic clearance approach adopted in this study may be useful in the investigation of factors influencing the oral bioavailability of propranolol.

Keywords propranolol oral clearance metabolism partial clearance

Introduction

The contribution of presystemic hepatic metabolism to the highly variable oral bioavailability of propranolol is well established (Shand *et al.*, 1970; Gibaldi *et al.*, 1971). The enzymatic

mechanisms involved have been more difficult to assess because of the multiplicity of metabolites formed from this drug. Recently, for the first time, the quantitative metabolic fate of pro-

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propranolol in man was determined (Walle *et al.*, 1985b). Measurements of fourteen metabolites in urine allowed a complete description of the metabolism through the three primary pathways, i.e., glucuronidation, side-chain oxidation and ring oxidation (Figure 1), each of which may reflect a distinct enzyme activity.

In the present study the relationships between the total oral clearance of propranolol and its partial clearances through the primary pathways were investigated in a group of young, white males. The main aim was to determine which primary metabolic pathway makes the most important contribution to interindividual variability in the oral clearance of propranolol.

Figure 1 Primary pathways of propranolol metabolism.

Methods

Subjects

Seven healthy white male volunteers, 22–30 years of age and weighing 61 to 101 kg (Table 1), consented to participate in the study, which was approved by the University Human Research Committee. The subjects were admitted to a General Clinical Research Center and each underwent clinical examinations including medical history, physical examination, selected blood chemistry tests, complete blood counts (including haematocrit), urinalysis, ECG and chest X-ray. None of the subjects were smokers or had taken any other drugs for at least 1 week prior to the study. Each subject was fed an isocaloric diet (15% protein, 50% carbohydrate, 35% fat).

Experimental protocol

An oral dose consisting of one 80 mg tablet of propranolol hydrochloride (Inderal) and 40 μ Ci

of racemic 4- 3 H]-propranolol hydrochloride (Amersham Corp., Arlington Heights, IL), dissolved in 50 ml of distilled water, was administered in the morning after an overnight fast. A light breakfast was eaten 2 h after the dose.

Blood samples (8 ml) were drawn by syringe from an antecubital vein through an indwelling cannula or by direct venepuncture at 0.5, 1, 1.5, 2, 3, 4, 6, 10, 16, 24 and 36 h after the dose. The cannula was kept patent by the addition of 1 ml sodium heparin solution (50 u ml^{-1}) after each blood sampling. The first 2 ml of each blood sample were discarded. Blood samples, placed in heparinized polypropylene tubes, were centrifuged (2000 g, 15 min, 4° C) and plasma was transferred to glass tubes and kept frozen at -70° C until analysed.

Urine was collected at 24 h intervals for 96 h in refrigerated glass bottles containing sodium bisulphite (about 2%, w/v) to prevent oxidation of ring hydroxylated metabolites (Walle *et al.*, 1975). When each collection was complete the volume was measured and an aliquot frozen at -70° C until analysed.

Table 1 Subject characteristics and pharmacokinetic parameters after an 80 mg oral dose of propranolol

Subject	Age (years)	Weight (kg)	$t_{1/2}$ (h)	AUC_{po} (ng ml^{-1} h)	CL_{po} (ml min^{-1})	CL_{po} (ml min^{-1} kg^{-1})
1	27	88	2.9	550	2420	27.5
2	24	67	4.4	478	2807	41.9
3	24	75	3.4	332	4028	53.7
4	30	61	3.3	313	4270	70.0
5	24	101	2.6	273	4909	48.6
6	24	89	2.9	262	5118	57.5
7	22	74	3.1	253	5284	71.4
Mean	25	79	3.2	352	4119	52.9
s.e. mean	1	5	0.2	44	425	5.8

Analytical methods

Plasma concentrations of propranolol were measured by gas chromatography-mass spectrometry (Walle *et al.*, 1975) using hexadeuterium-labelled propranolol as internal standard (Walle *et al.*, 1978b).

Urinary metabolites were measured by liquid scintillation spectrometry as described by Walle *et al.* (1985b). Briefly, all glucuronic acid conjugates present in urine were hydrolysed enzymatically. The metabolites were then fractionated by differential solvent extraction into basic and acidic extracts and a nonextractable fraction. Individual metabolites in these three fractions were separated by reversed-phase h.p.l.c. using stepwise gradient elution. Each of fourteen metabolites were collected and their radioactivity counted.

These fourteen metabolites account for all of the radioactivity excreted during the 24 h after dosage. Ninety-one percent of total metabolites ultimately appearing in urine are excreted during the first 24 h. The metabolites were grouped according to the three initial pathways, i.e. glucuronidation, side-chain oxidation and ring oxidation (Walle *et al.*, 1985b).

Propranolol glucuronide is the sole metabolite of initial glucuronidation.

Initial side-chain oxidation results in the formation of desisopropylpropranolol glucuronide, hydroxylated desisopropylpropranolol glucuronide and sulphate, propranolol glycol glucuronide, hydroxylated propranolol glycol glucuronide and sulphate, naphthoxylactic acid (main side-chain oxidation metabolite), naphthoxyacetic acid and hydroxylated naphthoxylactic acid glucuronide.

Initial ring oxidation results in the formation of ring hydroxylated propranolol glucuronide and sulphate (main ring oxidation metabolites) as well as two radioactive peaks containing multiple minor metabolites.

The metabolites demonstrating both side-chain and ring oxidation have been assigned to initial side-chain oxidation. It has been shown for these metabolites that side-chain oxidation precedes ring oxidation (Walle *et al.*, 1985b).

Several of the main metabolites, e.g. propranolol glucuronide (Walle *et al.*, 1979c), naphthoxylactic acid (Walle *et al.*, 1979a) and 4-hydroxypropranolol glucuronide (Walle *et al.*, 1980) were measured in plasma by previously published GC/MS techniques.

Pharmacokinetic calculations

The area under the plasma concentration-time curve (AUC) for propranolol was calculated by

the trapezoidal rule and extrapolated to infinity. The oral clearance (CL_{po}) was then calculated as $Dose/AUC$ expressed in both $ml\ min^{-1}$ and $ml\ min^{-1}\ kg^{-1}$. The weight correction was introduced, since the subjects' weights varied considerably (Table 1). The elimination half-life ($t_{1/2}$) of propranolol was obtained from the plasma concentration-time curve by linear regression analysis.

The partial oral clearance through each of the three primary pathways, i.e. glucuronidation, side-chain and ring oxidation (CL_m) was calculated as follows:

$$CL_m = CL_{po} \times f_m$$

where f_m is the fraction of the dose excreted as metabolites in the urine. The fraction of metabolites available for renal elimination was assumed to be unity. This approach has previously been used in studies of the metabolism of antipyrine, a compound also eliminated through three primary metabolic pathways (Bax *et al.*, 1981; Teunissen *et al.*, 1984; Breimer, 1983).

Results

The pharmacokinetic parameters for propranolol after the single 80 mg oral dose in the seven subjects studied are shown in Table 1. Whereas very little interindividual variation was observed in the half-life (range 2.6–4.4 h), there was an approximately three-fold variability in the oral clearance, from 27.5 to 71.4 $ml\ min^{-1}\ kg^{-1}$.

The composition of the metabolites in urine and the partial clearances through the primary pathways, i.e. glucuronidation, side-chain oxidation and ring oxidation, in these subjects are shown in Table 2. There was very little variation in the clearance through glucuronidation (range 6.8 to 9.9 $ml\ min^{-1}\ kg^{-1}$), only a moderate 2.4-fold variation in the clearance through side-chain oxidation (range 10.9–25.8 $ml\ min^{-1}\ kg^{-1}$) but a large, 5.6-fold, variation in the clearance through ring oxidation (range 7.5–41.8 $ml\ min^{-1}\ kg^{-1}$). Comparing the sum of the clearances by these pathways with the total oral clearance of propranolol (cf. Table 1), it is evident that the recovery of the metabolites excreted in urine during the 24 h study period was complete (92–105%).

Relationships between the partial clearances and oral drug clearance, expressed in $ml\ min^{-1}\ kg^{-1}$, in the individual subjects are shown in Figure 2. Clearance through ring oxidation was correlated highly with oral clearance of propranolol ($r = 0.94$; $P < 0.002$). There was a significant but smaller correlation between clearance through side-chain oxidation and total drug

Table 2 Urinary excretion (%) and partial clearances (CL_m) according to the primary metabolic pathways after a single 80 mg oral dose of propranolol

Subject	Glucuronidation		Side-chain oxidation		Ring oxidation	
	%*	CL_m ($ml\ min^{-1}$)	%*	CL_m ($ml\ min^{-1}$)	%*	CL_m ($ml\ min^{-1}$)
1	24.7	598	39.7	959	27.2	650
2	17.5	489	42.3	1186	45.9	1286
3	18.4	743	44.2	1778	38.7	1560
4	12.9	549	36.9	1574	44.0	1879
5	16.0	788	50.4	2475	33.6	1646
6	16.5	846	39.8	2038	46.9	2403
7	10.1	533	32.1	1695	58.6	3093

*% of total metabolites excreted during 24 h after the dose.

clearance ($r = 0.84$; $P < 0.02$), whereas the clearance through glucuronidation was independent of the oral clearance of propranolol. The same relationships were obtained when the clearances were expressed in $ml\ min^{-1}$, however, with slightly lower correlation coefficients.

The biological fate of the total radioactivity in plasma, more than 95% of which consists of metabolites, was also examined. The plasma $t_{1/2}$ (0–24 h) was 3.8 ± 0.2 h (mean $\pm$ s.e. mean), which was not significantly different from that of propranolol (Table 1). The renal clearance of the total radioactivity was $87 \pm 6\ ml\ min^{-1}$ (range 67 to $114\ ml\ min^{-1}$) and this was not significantly different from creatinine clearance, $120 \pm 11\ ml\ min^{-1}$ (range 81 to $162\ ml\ min^{-1}$).

The half-lives of propranolol glucuronide, naphthoxylactic acid and 4-hydroxypropranolol glucuronide were also similar to that of propranolol and high correlations were observed between their plasma AUCs in the individual subjects and the amounts excreted in urine as previously noted (Walle *et al.*, 1979a, b, c, 1980).

The rate of appearance of metabolites in plasma, the time to reach peak concentrations and their elimination half-lives were the same when measured by radioactive and nonradioactive techniques, indicating that there is no difference in the kinetics of propranolol metabolism whether the drug is given in solution (radioactively labelled drug) or as a tablet.

Discussion

This study has resolved the oral clearance of propranolol into its partial metabolic clearances by glucuronidation, side-chain oxidation and ring oxidation. It is likely that each of these three metabolic pathways reflects a distinct enzyme or enzyme system activity, which together govern the overall metabolism and bioavailability of propranolol.

The application of the partial metabolic clearance approach is of particular value when the biological fate of a drug is known completely. This appears to be so for propranolol. Thus, the drug is completely absorbed following oral doses (Paterson *et al.*, 1970) and metabolism takes place exclusively in the liver (Shand & Rangno, 1972; Kornhauser *et al.*, 1978; Lo *et al.*, 1982). Virtually the entire dose appears as metabolites in urine (Paterson *et al.*, 1970; Walle *et al.*, 1985b). The metabolites have the same half-lives as propranolol, as observed in this study as well as in previous reports (Walle *et al.*, 1979a, c, 1980), indicating that the rate of elimination of the metabolites into urine is at least as rapid as the

Figure 2 Relationships between total oral clearance of propranolol and its partial clearances by glucuronidation, side-chain oxidation and ring oxidation.

rate of their formation in the liver; the renal clearance of total metabolites is slightly less than the glomerular filtration rate. Thus, urinary excretion of metabolites appears to reflect their net formation in the liver. Although enterohepatic recirculation of propranolol glucuronide has been suggested (Walle *et al.*, 1979b), it is probably minor and will therefore not give rise to any major alterations of the three primary pathways as reflected in the urinary metabolite excretion. It is therefore suggested that the partial metabolic clearances for propranolol calculated from urinary excretion data reflect the activities of the hepatic enzymes responsible for glucuronidation, side-chain oxidation and ring oxidation.

Although it would appear that both ring oxidation and side-chain oxidation are mediated by cytochrome P-450 (Schneck & Pritchard, 1981; Fujita *et al.*, 1982; Larrey *et al.*, 1984), it is not clear whether the same or different isoenzymes are involved in these two pathways. A contribution from amine oxidase (Ziegler, 1980) to side-chain oxidation is a possibility. Thus, more comprehensive studies aimed at characterizing these oxidative pathways are necessary.

A seemingly homogeneous group of subjects, i.e. young, white males, was studied. To minimize the effect of environmental factors on the disposition of propranolol in this group, smokers were excluded, no other concurrent medication was allowed and all subjects received an identical diet while in the clinical research unit. The results demonstrated, as expected, a rather small, three-fold, interindividual variation in the oral clearance in contrast to the very large, 50-fold, variability in the general population (Walle *et al.*, 1985a). The data suggest that in subjects with low total oral clearance of propranolol there are equal contributions from glucuronidation, side-chain

oxidation and ring oxidation. With increasing oral clearance there is a progressively increasing contribution from side-chain oxidation and in particular from ring oxidation. In the subject with the highest oral clearance ring oxidation accounted for 60% of his clearance; side-chain oxidation, 30%; and glucuronidation, only 10%. The interindividual variability in the oral clearance of propranolol was, thus, mainly due to interindividual differences in ring oxidation (which varied as much as six-fold between the subjects), and to a lesser extent to differences in side-chain oxidation. It was not influenced by differences in the glucuronidation pathway. Although it has been suggested previously that ring oxidation may be the 'first choice' metabolic pathway for propranolol *in vivo* in man (Walle *et al.*, 1980), the present study provides more direct evidence that variability in the activity of ring oxidation is the primary determinant of interindividual variability in propranolol disposition.

It is, however, possible that this finding may be valid only in young, white males, as studied here. Many factors are known or suggested to alter the oral clearance of propranolol, e.g. dose (Evans & Shand, 1973; Walle *et al.*, 1978a), duration of treatment (Wood *et al.*, 1978), sex, age and race (Walle *et al.*, 1985a), diet (Fagan *et al.*, 1983) and environmental factors (Routledge & Shand, 1979; Walle *et al.*, 1985a), which could theoretically involve alterations in any of the three primary pathways. The finding that ring oxidation of propranolol is lower in poor oxidizers of debrisoquine without noticeable changes in the total oral clearance (Lennard *et al.*, 1984; Raghuram *et al.*, 1984) would indeed suggest that a pathway other than ring oxidation, e.g. side-chain oxidation, is a more important determinant of the oral clearance of propranolol in

such individuals. A study determining the total clearance by each primary metabolic pathway, as examined here, should be able to clarify this question.

Owing to the complexity of the metabolism of propranolol, the use of radioactively labelled drug was necessary in the present study. The partial clearance determinations were then based on h.p.l.c. measurements of fourteen metabolites (Walle *et al.*, 1985b). Whether a simpler approach based on only a few of the most prominent metabolites and without radioactive drug can be employed, without loss of accuracy, must await studies in more subjects.

In conclusion, studies in the past have shown that the intrinsic hepatic clearance, i.e. the activity of the drug metabolizing enzymes in the liver, is the most important determinant of the disposition of oral doses of propranolol (Kornhauser *et al.*, 1978). In the present study we have resolved the intrinsic hepatic clearance into the partial clear-

ances by each of three probably different enzyme activities, i.e. glucuronidation, side-chain oxidation and ring oxidation, and have shown that it is possible to monitor these activities directly *in vivo*. It should thus be possible to follow studies of these enzyme activities and their regulation *in vitro* with parallel studies *in vivo*. This approach is expected to yield new insights into the biological factors underlying the large interindividual variability in steady-state plasma propranolol concentrations, including the influence of sex, race and age (Walle *et al.*, 1985a), information that may be of value for other drugs as well.

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