DIRECT MEASUREMENT OF PROPRANOLOL BIOAVAILABILITY DURING ACCUMULATION TO STEADY-STATE

A.J.J. WOOD, K. CARR, R.E. VESTAL†, S. BELCHER, G.R. WILKINSON & D.G. SHAND

Division of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA

- 1 A high performance liquid chromatographic method for the determination of propranolol in human plasma and blood has been developed and used to confirm that cumulation occurred during chronic oral administration, steady-state being achieved within 48 h of beginning 80 mg of the drug every 8 h.
- 2 The method was adapted to measure [H^3]-propranolol and native drug in the same blood sample and was applied to determine simultaneously the disposition of i.v. ([H^3]-propranolol) and orally (non-labelled) administered drug after single oral dose of 80 mg and when steady-state had been established on an 80 mg, 8-hourly regimen.
- 3 Using this approach it was possible to show that a reduced oral clearance at steady-state was associated with a smaller reduction in systemic (i.v.) clearance and no change in liver blood flow. A direct estimate of bioavailability was also possible and was found to be increased at steady-state compared with a single oral dose.
- 4 We conclude that the accumulation of propranolol during the attainment of steady-state is due to a reduction in intrinsic clearance, resulting in reduced presystemic hepatic extraction.

Introduction

The pharmacokinetics of the β -adrenoceptor blocking drug, propranolol, have been extensively investigated. Most of the information has been obtained using the fluorometric assay technique of Shand, Nuckolls & Oates (1970), though some data have been obtained using the gas liquid chromatography (g.l.c.) methods described by Walle (1974) and diSalle, Baker, Baraggi, Watkins, Chidsey, Figerido & Morselli (1973). We have developed another method using high performance liquid chromatography (HPLC) and compared it with the fluorometric technique under two sets of conditions: (1) in research studies to determine the time taken to reach steady-state during chronic oral administration in which the subject's own blank plasma was used and (2) in random clinical samples using blank plasma from normal volunteers.

As this method proved convenient and reliable, we have adapted it to measure both radio-labelled and native propranolol in the same blood sample. This has allowed us to simultaneously measure the pharmacokinetics of propranolol after both intravenous (as [H^3]-propranolol) and oral (as unlabelled drug) administration, a technique that is particularly useful in the investigation of non-linear kinetics (Houghton & Richens, 1974). In addition, with fully absorbed, highly extracted drug like propranolol, the derived data can be used to estimate liver blood flow and in-

trinsic clearance as well as giving a direct method for measuring bioavailability under non-linear conditions (Wilkinson & Shand, 1975).

We have therefore used this approach to further investigate the accumulation of propranolol that is known to occur during the attainment of steady-state with repetitive oral administration (Evans & Shand, 1973).

Methods

HPLC assay method

All blood samples were withdrawn into a heparinized glass tube with a ground glass stopper. For the assay of plasma, 4 ml was alkalinized with 200 μ l 5N NaOH: for whole blood 4 ml was alkalinized with 100 μ l 5N NaOH. To this was added an internal standard which was synthesized by refluxing a mixture of 0.5 g propranolol, 5 ml water, 1 ml 10N NaOH and 30 ml ethyliodide for 24 h. The resulting mixture was extracted with 100 ml heptane, the organic phase separated and evaporated to dryness under reduced pressure at 37°C. The internal standard, whose mass spectrum and fragmentation pattern after formation of trimethylsilyl and acetate ester was consistent with it being *N*-ethylpropranolol, was diluted with 1:1 mixture of water: methanol to give a solution of approximately 2 μ g/ml, of which 100 μ l was added to the sample

†Present address: Dept. of Medicine, VA Hospital, 5th and Fort Street, Boise, Idaho 83702, USA

together with 500 μ l water (or propranolol standard) and 11 ml 1.5% iso-amyl alcohol in heptane. The samples were gently shaken in a 17 ml glass tube with a Teflon-lined screw cap for 20 min and centrifuged for 10 min at approximately 800 g. Approximately 10 ml of the organic phase was transferred to a vial and the organic solvent was evaporated to dryness. The residue was reconstituted in 250 μ l of the mobile phase and 200 μ l was injected onto the liquid chromatogram for quantification.

All analyses were performed utilizing an M6000 solvent delivery system, a U6K universal injector and a C₁₈ μ -Bondapak column, 300 mm $\times$ 3.9 mm i.d. (Waters Assoc., Inc., Milford, Mass.). The propranolol and internal standard, *n*-ethylpropranolol, were measured with a fluorometric detector (Aminco Fluoro-Monitor, American Instruments Co., Silver Springs, MD.) employing a 70 μ l Suprasil flow cell. A General Electric Germicidal lamp was used in combination with 295 nm interference filter and a Kodak #34 secondary filter. Two mobile phases were employed and delivered at a rate of 2.0 ml/min at ambient temperature. Solvent phase 1 consisted of methanol: water: acetic acid (700:600:6 v/v/v); solvent phase 2 of water: acetonitrile: acetic acid (500:225:5 v/v/v).

The concentration of propranolol was determined by comparing peak height ratios of propranolol and the internal standard from plasma or blood to peak height ratios of known concentrations of propranolol and the internal standard added to drug free plasma or blood.

Comparison of assay methods

The above HPLC method was compared with the fluorometric technique of Shand *et al.* (1970) in two groups of samples (a) routine clinical samples from patients receiving propranolol for whom no blank plasma was available, necessitating the use of donor plasma and (b) a study in five normal volunteers from whom plasma was obtained prior to the administration of propranolol 80 mg. p.o. every 8 h. Plasma samples were then obtained in the fasting state 2, 4, 6 and 8 h after the first dose, then daily before each morning dose for 3 days. On the last day samples were also obtained 2, 4, 6 and 8 h after dosing. All samples were frozen and assayed within one month.

Simultaneous determination of i.v. and oral kinetics

This study was carried out in six male normal volunteers aged 25–72 years. Informed consent was obtained and the protocol approved by the Vanderbilt University Committee for the Protection of Human Subjects. On the first day of the study, they received 80 mg propranolol orally, fasting, and 40 μ Ci [³H]-propranolol (New England Nuclear 4.5 mCi/mg). Blood samples were obtained at 5, 15, 30, 60, 120,

240, 360 and 480 min after drug administration. Thereafter the subjects received 80 mg propranolol every 8 for 2 days and the i.v. study was repeated on the morning of the third day. In these studies, propranolol concentrations were determined in 4 ml blood using solvent system 1. The [³H]-propranolol was assayed by obtaining the effluent solvent representing the fluorescent peak on the chromatogram, which was then added to 10 ml Unogel® and counted by liquid scintillation spectrometry. The radio-activity was compared with a standard to which [³H]-propranolol has been added.

On the basis of the data the following calculations were performed.

Systemic clearance (*Cl*_s) was computed from the [³H]-propranolol concentrations as:

$$Cl_s = \frac{D_{iv}}{AUC_{iv}}$$

where *D*_{iv} is the dose of [³H]-propranolol given i.v. and *AUC*_{iv} is the total area under the concentration/time curve extrapolated to infinity.

Volume of distribution was computed as

$$V_d \beta = \frac{Cl_s \cdot T_{1/2}}{0.693}$$

where *T*_{1/2} is the half-life measured from the terminal portion of the concentration/time curve from 1–8 h.

The apparent oral clearance, *Cl*_{o(1)} after the first dose was measured as

$$Cl_{o(1)} = \frac{D_o}{AUC_{o(1)}}$$

where *D*_o is the oral dose and *AUC*_o is the area under the concentration/time curve extrapolated to infinity. During steady-state, *Cl*_{o(ss)} was calculated as

$$Cl_{o(ss)} = \frac{D_o}{AUC_{o(ss)}}$$

where *AUC*_{o(ss)} is the area under the curve during the 8-h dosage interval.

It has previously been shown (Wilkinson & Shand, 1975) that if the venous equilibration model of Rowland, Benet & Graham (1973) applies and the drug is fully absorbed and metabolized only by the liver then:

$$Cl_o = Cl_{i(tot)}$$

in which *Cl*_{i(tot)} is the intrinsic clearance of the drug, a measurement of the activity of the drug metabolizing enzymes.

Apparent liver blood flow $\hat{Q}$ is given by (Wilkinson & Shand, 1975)

$$\hat{Q} = \frac{D_o \cdot D_{iv}}{AUC_{iv} D_o - AUC_{o} D_{iv}}$$

where AUC_o is the $AUC_{o(ss)}$ or $AUC_{o(l)}$ as appropriate.

Bioavailability (F) was calculated as

$$F_{(l)} = \frac{AUC_{o(l)}}{AUC_{iv(l)}}$$

or

$$F_{(ss)} = \frac{AUC_{o(ss)}}{AUC_{iv(ss)}}$$

In theory, again assuming complete absorption and hepatic elimination, bioavailability is determined by flow and intrinsic clearance as

$$F = (1-E) = \frac{Q}{Q + Cl_{i(tot)}}$$

where E is the hepatic extraction ratio.

Results

Comparison of assay procedures

Typical HPLC chromatograms of blank and propranolol-containing blood samples using solvent phase (1) are shown in Figure 1. The assay was capable of detecting 5 ng/ml and standard curves for both plasma and blood were linear up to concentrations of 200 ng/ml. Reproducibility was good, as is shown in Table 1. Similar linear and reproducible data were seen for [H^3]-propranolol. Propranolol was stable when stored for 30 days either at 4°C or -15°C, both in samples to which the drug had been added and in plasma from subjects receiving the drug (Table 2).

Table 1 Linearity and reproductibility of HPLC assay

Propranolol added (ng/ml)	Mean value (ng/ml)	s.e. mean	Coefficient of variation (%)
25	24.63	0.44	1.81
50	49.13	1.59	3.24
75	72.43	3.50	4.84
100	100.29	4.31	4.30
125	131.70	4.96	3.76
200	192.87	5.34	2.77

Figure 1 A typical high performance liquid chromatogram of (a) blank blood and (b) blood to which propranolol 28 ng/ml (Peak I) and the internal standard, N-ethylpropranolol, (Peak II) had been added.

The results from the study of normal subjects shows that propranolol accumulates to reach a steady-state by morning of the third day and that 80% of steady-state levels were achieved by the second day (Figure 2). A total of twenty-two samples from this study were assayed for propranolol by the fluorometric method of Shand *et al.* (1970), utilizing the subject's own plasma as a blank. Good correspondence was noted (Figure 3). Conversely, when a blank was not available in routine clinical samples a much poorer correlation was seen, presumably due to a high blank giving an underestimate of the value with fluorometry compared to HPLC (Figure 4).

Interference by other commonly used drugs in the assay was tested, and none of the drugs listed in Table

Table 2 Stability of propranolol (mean $\pm$ s.e. mean) after 30 days storage at 4°C and -15°C

Sample	n	Day 1	Mean blood propranolol (ng/ml)	
			Day 30	
			4°C	-15°C
Propranolol added (100 ng/ml)	5	99.98 $\pm$ 1.96	104.76 $\pm$ 0.90	101.22 $\pm$ 1.97
Blood after 80 mg p.o.	5	87.88 $\pm$ 7.73	89.88 $\pm$ 9.28	86.84 $\pm$ 9.04

Figure 2 Accumulation of propranolol to steady-state in five subjects (mean $\pm$ s.e. mean).

3 caused a peak at the retention time of propranolol or internal standard. However, it was found that quinidine did interfere when solvent phase (1) was used but not with solvent (2). The two solvents were also compared in duplicate samples from patients receiving propranolol and good correspondence was noted (Figure 5).

Simultaneous i.v. and oral kinetics during accumulation

A significant (almost two-fold) accumulation of propranolol occurred between the first dose and

Figure 3 Comparison of HPLC and fluorometric methods of propranolol assay in study samples ($n = 22$) for which blank plasma from the subjects was available. The best fit of the data is given by $y = 1.238 + 1.004x$ ($r = 0.93$) and was not significantly different from the line of identity.

Figure 4 Comparison between HPLC and fluorometric assays of propranolol in random clinical samples ($n = 17$). The best fit was given by $y = 21.470 + 0.584x$ ($r = 0.79$) and was significantly different from the line of identity.

steady-state that would not be predicted from half-life averaging 4.33 h and a dosage interval of 8 h. As a consequence, apparent oral clearance was reduced significantly (Table 4). These changes in oral clearance were accompanied by a lesser fall in systemic (i.v.) clearance, without change in liver blood flow. Bioavailability was significantly increased at steady-state compared to the first drug dose, due to a reduced hepatic extraction ratio. The volume of distribution was unchanged (378 ± 52 l after the first dose compared to 334 ± 51 l at steady-state), and $T_{1/2}$ tended to be longer at steady-state (5.4 ± 0.76 h) compared to after the first dose (4.33 ± 0.37 h), but not significantly so.

Table 3 Drugs shown not to interfere with HPLC assay of propranolol

Guanethidine	Warfarin
α -methyl dopa	Insulin
Hydrallazine	Heparin
Prazosin	Codeine
Minoxidil	Perphenazine
Diazepam	Probenecid
Flurazepam	Allopurinol
Clonidine	Digoxin
Chlorpromazine	Digitoxin
Amitriptyline	Paracetamol
Hydrochlorothiazide	Phenergan
Triamterene	Isoniazid
Frusemide	Vancomycin
Chlorthalidone	Gentamycin
Spironolactone	Isosorbide dinitrate

Figure 5 Comparison of solvent 1 (methanol: water; acetic acid) and solvent 2 (water: acetonitrile, acetic acid) used in the HPLC assay. (Scales are ng/ml). The best fit was given by $y = 20.494 + 0.883X$ ($r = 0.95$) and was not different from the line of identity.

Discussion

The presently described HPLC method provides a simple and accurate measure of plasma or blood propranolol concentrations which is superior to the fluorometric method for routine clinical samples, though the latter method gives satisfactory results provided the patient's own blank plasma is available. The interference caused by quinidine was overcome by using a mobile solvent phase of water; acetonitrile; acetic acid.

Using this new method we have confirmed that propranolol accumulated to a greater extent than

predicted from its half-life and dosage interval (Evans & Shand, 1973), and have in addition found that steady-state is established within 48 h of instituting an 8-h regimen in normal subjects. Using a technique of simultaneously measuring oral and i.v. kinetics we have also gained some insight of the kinetic changes that occur during this time period. Because of the increase in drug concentrations, there was a significant reduction in the apparent oral clearance of the drug. According to theoretical arguments previously put forward (Wilkinson & Shand, 1975) the apparent oral clearance should equal intrinsic drug clearance (a measure of the activity of the drug metabolizing enzymes), provided the drug is completely absorbed and metabolized in the liver. Propranolol appears to satisfy both of these conditions, as the drug is fully absorbed and is not significantly eliminated by the kidneys (Paterson, Conolly, Dollery, Hayes & Cooper, 1970). Although some pulmonary extraction has been found in the dog (George, Orme, Buranapong, Macalene, Breckenridge & Dollery, 1976) it was not noted in the monkey (Nies, Evans & Shand, 1973) nor in man (Pessayre, Lebre, Descatoire, Peignieux & Benhamou, 1977). Thus we must attribute the accumulation of propranolol to a reduction in intrinsic clearance. Whether this is due to saturation of a high affinity uptake and storage process or metabolism or both remains unclear.

The changes in apparent oral (or intrinsic) clearance were accompanied by the changes in systemic clearance that would be predicted for a compound with a relatively high hepatic extraction. Because the systemic clearance of this drug is partly flow-dependent, changes in intrinsic clearance should produce lesser alterations in systemic clearance, and this was indeed the case. Apparent liver blood flow was unaltered. The validity of this method for blood flow estimation again depends on the drug being fully absorbed and extracted only by the liver. The fact that the values found are similar to those previously reported supports its validity. The variation between

Table 4 Pharmacokinetic parameters of the first (D_1) and seventh (D_7) dose of 80 mg propranolol given 8-hourly

Subject	Oral clearance (l/min)		Systemic clearance (l/min)		Liver blood flow (l/min)		Bioavailability (%)	
	D_1	D_7	D_1	D_7	D_1	D_7	D_1	D_7
1	9.78	3.57	1.01	0.86	1.13	1.12	10.4	24.0
2	3.66	2.63	1.11	0.75	1.60	1.06	30.4	28.6
3	5.68	1.69	1.08	0.77	1.33	1.42	19.0	45.6
4	3.77	2.74	1.12	1.04	1.60	1.68	29.8	38.0
5	3.45	1.45	0.62	0.67	0.83	1.26	25.4	46.4
6	6.26	3.61	1.06	0.72	1.27	0.90	16.9	20.0
Mean	5.27	2.62*	1.00	0.80*	1.29	1.24	21.98	33.77*
±s.e. mean	1.07	0.37	0.08	0.05	0.12	0.11	3.23	4.58

* Significantly different from D_1 , $P < 0.05$.

the two studies was, however, quite large in some of the subjects. The reason for this is unclear, but they were allowed 'usual' activity and did eat lunch; factors both known to alter liver blood flow. Finally, the protocol allows for a direct estimate of bioavailability, which was increased with the attainment of steady-state compared to that of the first dose, consistent with a reduction in hepatic extraction during presystemic (first pass) elimination, due to the fall in intrinsic clearance. It should be noted that bioavailability was slightly decreased in one of the subjects (2), even though both systemic and intrinsic clearances were

reduced. This occurred because bioavailability is dependent partly on blood flow (see theoretical section) which also fell, and the decreased availability due to this flow change was enough to offset the increased availability due to the reduction in intrinsic clearance.

This work has been supported by the U.S. Public Health Grants GM 15431, RO 0095 and HL 20770.

We should like to thank Dr M. Smigel for performing the mass spectrometry.

A.J.J. Wood is a Merck International Fellow. Reprint requests should be addressed to D.G.S.

References

- DI SALLE, E., BAKER, K.M., BARAGGI, S.I., WATKINS, W.D., CHIDSEY, C.A., FIGERIDO, A. & MORSELLI, P.L. (1973). A sensitive gas chromatographic method for the determination of the propranolol in human plasma. *J. Chromatogr.*, **84**, 347-353.
- EVANS, G.H., SHAND, D.G. (1973). Disposition of propranolol V. Drug accumulation and steady-state concentrations during chronic oral administration in man. *Clin. Pharmac. Ther.*, **14**, 487-493.
- GEORGE, C.F., ORME, M.L., BURANAPONG, P., MACALENE, D., BRECKENRIDGE, A.M. & DOLLERY, C.T. (1976). Contribution of the liver to overall elimination of propranolol. *J. Pharmacokin. Biopharm.*, **4**, 17-28.
- HOUGHTON, G.W. & RICHENS, A. (1974). Rate of elimination of tracer doses of phenytoin at different steady-state serumphenytoin concentration in epileptic patients. *Br. J. clin. Pharmac.*, **1**, 155-161.
- NIES, A.S., EVANS, G.H. & SHAND, D.G. (1973). The hemodynamic effects of beta-adrenergic blockade on the flow-dependent hepatic clearance of propranolol. *J. Pharmac. exp. Ther.*, **184**, 716-720.
- PATERSON, J.W., CONOLLY, M.E., DOLLERY, C.T., HAYES, A. & COOPER, R.G. (1970). The pharmacodynamics and metabolism of propranolol in man. *Pharmac. Clin.*, **2**, 127-133.
- PESSAYRE, D., LEBREC, D., DESCATOIRE, V., PEIGNIUX, M. & BENHAMOU, J.P. (1978). Mechanism of low drug clearance in patients with cirrhosis. *Gastroenterology* (in press).
- ROWLAND, M., BENET, L.Z. & GRAHAM, G. (1973). Clearance concepts in pharmacokinetics. *J. Pharmacokin. Biopharm.*, **1**, 123-136.
- SHAND, D.G., NUCKOLLS, C.E.M. & OATES, J.A. (1970). Plasma propranolol levels in adults with observations in 4 children. *Clin. Pharmac. Ther.*, **11**, 112-120.
- WALLE, T. (1974). GLC determination of propranolol, other beta-blocking drugs and metabolites in biological fluids and tissues. *J. pharm. Sci.*, **63**, 1885-1891.
- WILKINSON, G.R. & SHAND, D.G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmac. Ther.*, **18**, 377-390.

(Received September 28, 1977)