THE DISTRIBUTION OF PROPRANOLOL, PINDOLOL AND ATENOLOL BETWEEN HUMAN ERYTHROCYTES AND PLASMA

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1 This study aimed (1) to measure the whole blood to plasma (WB:P) and red blood cell to plasma (RBC:P) concentration ratios of propranolol in healthy volunteers and two types of patients, and (2) to compare the concentration ratios of the lipophilic drug propranolol with moderately lipophilic pindolol and hydrophilic atenolol.

2 There was no significant difference between the WB:P and RBC:P ratios of propranolol concentration in healthy volunteers and neurological patients compared with hypertensive patients. The mean $\pm$ s.d. WB:P ratios of propranolol concentration in the three groups were 0.74 ± 0.03 , 0.71 ± 0.05 , and 0.76 ± 0.08 respectively. The mean RBC:P ratios were 0.39 ± 0.08 , 0.36 ± 0.11 , and 0.47 ± 0.15 respectively. WB:P and RBC:P concentration ratios of propranolol were linearly correlated with the free fraction of drug in plasma. Propranolol was 90% bound in plasma.

3 The mean WB:P and RBC:P ratios of pindolol in seven volunteers were 0.69 ± 0.08 and 0.37 ± 0.14 respectively. Pindolol was $71.4 \pm 8.6\%$ bound to plasma proteins. The concentration of pindolol in the RBC was linearly correlated with that unbound in plasma.

4 In four healthy volunteers, the mean WB:P concentration ratio of atenolol was 1.07 ± 0.25 and the mean RBC:P ratio was 1.15 ± 0.55 .

5 The similarity of the RBC:free plasma drug concentration ratios for all three drugs suggests that the use of organic solvent partition coefficients for the prediction of *in vivo* distribution may be unreliable.

Introduction

It is important to realise that some drugs have a significantly higher affinity for the erythrocyte than for plasma, and the concentration of these compounds in the blood is substantially higher than in plasma (Beerman *et al.*, 1975; Beerman, Groschinsky-Grind & Rosén, 1976). Plasma concentration has therefore to be used with caution in relating pharmacokinetic data to organ blood flow. Drugs are transported to sites of elimination by blood. Provided that the drugs rapidly equilibrate between erythrocytes and plasma, as for propranolol, blood may be regarded as a single compartment and therefore the concentration in blood rather than in plasma should be used when the function of the organ of elimination is related to systemic clearance. Evans & Shand (1973) have shown that the concentration ratio of propranolol between erythrocytes and plasma is proportional to the degree of protein binding in plasma, suggesting that the whole blood to plasma (WB:P) ratio of propranolol concentrations was a measure of the volume of distribution of the drug. Based on an haematocrit of 0.45, the WB:P ratio of propranolol

concentration in six healthy volunteers reported by Evans & Shand (1973) ranged between 0.7 and 0.85 and was linearly related to the fraction of free drug in plasma. In contrast, Sawchuk, Robayo & Miller (1974) found a significantly higher concentration of propranolol in whole blood of hypertensive patients than in the plasma, the mean ratio being 1.3. In the latter study there was substantial intersubject variation in the WB:P ratio, ranging from 0.5 to 1.9. It was suggested that the binding of drugs to plasma proteins may differ between healthy and hypertensive subjects. In this study red blood cell to plasma (RBC:P) and WB:P concentration ratios of propranolol were measured in healthy volunteers; in a group of patients undergoing neurological investigation who were taking part in a study of the penetration into the central nervous system of β -adrenoceptor antagonists given in therapeutic doses; and in hypertensive patients who were taking propranolol. Binding of drug to plasma proteins was measured in the first two groups and its influence on the above drug ratios investigated. The study was extended to the partition-

ing of two less lipid-soluble β -adrenoceptor antagonists, pindolol and atenolol, between erythrocytes and plasma.

Methods

In the following experiments blood samples were taken into lithium-heparin, and plasma and whole blood stored at -20°C until assayed. Binding of drug to plasma protein was measured by equilibrium dialysis for 5 h at 37°C and pH 7.40 across a Cuprophan membrane (Enka, GmbH, thickness $11\text{ }\mu\text{m}$).

Propranolol

Group 1a comprised six healthy subjects (three men, three women) aged 22 to 36 years (mean = 27.3) who took propranolol 80 mg orally twice daily for 14 days. On the study day subjects took the morning dose 3 h before and a light breakfast 1.5 h before samples were collected. Venous blood (28 ml) was taken from each subject. Plasma was separated from 22 ml and frozen in 2 ml portions; 6 ml were frozen whole after the packed cell volume (PCV) had been measured using a micro-haematocrit centrifuge (Hawksley). The binding of drug to plasma protein was measured for each subject.

Group 1b comprised eight patients undergoing neurological investigations, seven men and one woman, aged 18 to 53 years (mean = 36.2). Five subjects took a single dose of propranolol 160 mg orally and three took 80 mg 12 hourly for 3 days before sampling. Venous blood (50 ml) was taken from each subject: 5 ml were used to measure whole blood propranolol concentration; 4 ml to measure the PCV; and plasma concentration and protein binding of propranolol were measured in the remainder.

Group 2 comprised fourteen hypertensive patients (ten men and four women) aged between 44 and 71 years (mean = 60.2). All were taking propranolol 80 to 640 mg daily as part of their treatment and ten patients also took a diuretic. From each patient 25 ml of venous blood were taken between 2.25 and 5.5 h after the last dose of drug (but 15 h later in one case) and between 10.00 and 12.30 h. Plasma was separated from 20 ml of blood and frozen in 2 ml portions; 5 ml of blood were frozen whole after the PCV had been measured using the micro-method described above.

Concentrations of propranolol in all fluids were measured by a modification of the fluorimetric method of Shand, Nuckolls & Oates (1970).

Pindolol

Seven subjects (five men and two women) aged 24 to 65 years (mean = 42.0) who were undergoing neurological investigations participated. Each took either a

single oral dose of pindolol 10 mg (five subjects) or 10 mg twice daily for 3 or 6 days (two subjects). Venous blood (50 ml) was taken from each subject 2.5 to 4.5 h after the last dose of pindolol. Plasma was separated from 40 ml of blood and frozen in 5 ml samples. Blood (6 ml) was frozen whole and 4 ml used to determine the PCV. Protein binding was measured in the plasma of each subject using the method described for propranolol. Pindolol concentration in all samples was measured by a modification of the fluorimetric method of Pacha (1969).

Atenolol

At least 1 h after a light breakfast, six healthy subjects (five men and one woman) aged 21 to 32 years (mean = 26.5) took a single dose of two tablets of atenolol 100 mg (total 200 mg) with 50 ml water. Blood samples (15 ml) were collected from three subjects before the dose (0 h) and 1.5, 3, 6 and 8 h thereafter, and from the other three subjects at 1, 3 and 6 h. Five ml of each sample were frozen whole after the measurement of PCV; plasma was separated from the remaining 10 ml and frozen at -20°C until atenolol concentration was assayed using a modification of the fluorimetric method of Kaye (1974).

Results

Propranolol

The mean $\pm$ s.d. WB:P ratio for Group 1a was 0.74 ± 0.03 and the mean RBC:P ratio was 0.39 ± 0.08 (Table 1). Propranolol was $88.62 \pm 0.93\%$ protein bound in plasma. There was a linear correlation between both WB:P and RBC:P and the fraction of unbound drug ($r = 0.92$ $P < 0.01$ and $r = 0.93$, $P < 0.01$ respectively). This is shown in Figure 1 together with the results from Group 1b. There was a good correlation between the concentration of free propranolol in plasma and the erythrocyte concentration ($r = 0.97$; $P < 0.01$); the slope of this line was 2.98.

The mean WB:P and RBC:P ratios of propranolol concentration in Group 1b were 0.71 ± 0.05 and 0.36 ± 0.11 respectively (Table 2). Propranolol was $91.22 \pm 1.94\%$ bound to protein in plasma. There was a significant linear relationship between the concentration of propranolol in the erythrocyte and that free in plasma ($r = 0.88$; $P < 0.01$); the slope of the line was 2.75.

The mean WB:P drug concentration ratio for the hypertensive patients in Group 2 was 0.76 ± 0.08 and the mean RBC:P ratio was 0.47 ± 0.15 (Table 3). There was no significant difference between the results in Group 1 and Group 2. Ten of the fourteen patients in Group 2 were taking diuretics in addition to propranolol but this did not appear to influence the results.

Table 1 Individual and mean $\pm$ s.d. WB:P and RBC:P ratios of propranolol concentration, packed cell volume (PCV) and percentage of drug protein bound in plasma in six healthy subjects (Group 1a) after taking propranolol 80 mg orally twice daily for 2 weeks.

Age (years)	Sex	WB:P	PCV	RBC:P	% protein bound
36	M	0.72	0.47	0.42	88.9
25	F	0.79	0.43	0.51	85.2
25	F	0.71	0.43	0.27	91.0
30	M	0.72	0.43	0.34	90.6
26	F	0.75	0.41	0.36	89.4
22	M	0.77	0.42	0.42	86.6
Mean 27.3		0.74	0.43	0.39	88.6
$\pm$ s.d. 5.0		0.03	0.02	0.08	0.9

Figure 1 Whole blood: plasma (WB:P) and red blood cell:plasma (RBC:P) concentration ratios of (a) propranolol and (b) pindolol v free drug fraction in plasma.

Table 2 Individual and mean $\pm$ s.d. WB:P and RBC:P ratios of propranolol concentration, packed cell volume (PCV) and percentage of drug protein bound in plasma in eight patients (Group 1b) 3 h after taking a single or multiple oral doses

Age (years)	Sex	Dose	WB:P	PCV	RBC:P	% protein bound
43	M	160 mg	0.79	0.48	0.55	89.4
22	M	160 mg	0.74	0.37	0.31	90.7
40	M	160 mg	0.71	0.41	0.30	94.4
18	M	160 mg	0.71	0.38	0.23	90.0
30	F	160 mg	0.74	0.45	0.43	88.5
53	M	80 mg 12 hourly	0.64	0.47	0.23	92.6
31	M	80 mg 12 hourly	0.66	0.55	0.39	92.4
53	M	80 mg 12 hourly	0.70	0.52	0.43	91.8
Mean 36.2			0.71	0.45	0.36	91.2
$\pm$ s.d. 13.2			0.05	0.06	0.11	1.9

Table 3 Individual and mean $\pm$ s.d. WB:P and RBC:P ratios or propranolol concentration, packed cell volume (PCV) and additional medication in 14 patients with hypertension taking various oral doses of propranolol (Group 2)

Age (years)	Sex	Dose	WB:P	PCV	RBC:P
61	M	80 mg 8 hourly	0.61	0.50	0.22
57	M	60 mg 8 hourly	0.69	0.44	0.30
44	M	40 mg 8 hourly	0.74	0.40	0.37
68	M	80 mg 12 hourly	0.66	0.56	0.40
62	M	80 mg 6 hourly	0.75	0.47	0.47
54	M	80 mg 12 hourly	0.87	0.46	0.72
55	F	20 mg 12 hourly	0.81	0.38	0.49
51	M	160 mg 12 hourly	0.89	0.42	0.73
66	M	80 mg 12 hourly	0.75	0.44	0.43
66	M	40 mg 12 hourly	0.78	0.45	0.42
59*	F	160 mg 8 hourly	0.86	0.38	0.64
68	M	160 mg 6 hourly	0.79	0.48	0.57
71	F	40 mg 8 hourly	0.73	0.44	0.39
61	F	80 mg morning	0.74	0.45	0.42
Mean 60.2			0.76	0.45	0.47
$\pm$ s.d. 7.5			0.08	0.05	0.15

* = Blood sample was taken 15 h after last dose of propranolol; in all other subjects samples were taken between 2 and 6 h after last dose.

Pindolol

The results are shown in Table 4. The mean WB:P concentration ratio of pindolol was 0.69 ± 0.08 and the mean RBC:P ratio was 0.37 ± 0.14 . Pindolol was $71.4 \pm 8.6\%$ protein bound in plasma. A plot of each ratio against free drug fraction in plasma is shown in Figure 1. There was a linear relationship between erythrocyte concentration and the concentration of unbound pindolol in plasma ($r = 0.99$; $P < 0.001$); the slope of the regression line was 1.016.

Atenolol

Table 5 shows the results for four of the six subjects. (Whole blood concentrations of atenolol were undetectable in two subjects after their blood appeared to gel on addition of sodium hydroxide and ethyl acetate.) The mean WB:P atenolol concentration

Table 5 Individual and mean $\pm$ s.d. WB:P and RBC:P ratios of atenolol concentration and packed cell volume (PCV) in four subjects at various times after taking atenolol 200 mg orally

<i>Age (years)</i>	<i>Sex</i>	<i>Time (h) after dose</i>	<i>WB:P</i>	<i>PCV</i>	<i>RBC:P</i>
25	F	1.5	1.37	0.49	1.76
		3	1.16	1.50	1.32
		6	1.30	0.47	1.64
		8	1.45	1.47	1.96
32	M	1.5	0.94	0.45	0.87
		3	0.94	0.44	0.87
		6	0.95	0.44	0.89
		8	0.96	0.42	0.96
21	M	3	0.83	0.47	0.63
		6	0.90	0.47	0.79
21	M	3	1.39	0.44	1.90
		6	0.67	0.44	0.24
Mean			1.07	0.46	1.15
± s.d.			0.25	0.02	0.55

ratio was 1.07 ± 0.25 and the mean RBC:P ratio was 1.15 ± 0.55 . In two subjects there was marked variability in the concentration ratios obtained. There is no obvious explanation for this except that perhaps the method of measuring atenolol in whole blood is not precise for all subjects.

Table 4 Individual and mean $\pm$ s.d. WB:P and RBC:P ratios of pindolol concentration, packed cell volume (PCV) and percentage of drug protein bound in plasma in seven subjects 3 h after taking either a single or multiple oral doses

<i>Age (years)</i>	<i>Sex</i>	<i>Dose</i>	<i>WB:P</i>	<i>PCV</i>	<i>RBC:P</i>	<i>% protein bound</i>
38	F	10 mg	0.67	0.45	0.26	73.5
52	M	10 mg	0.66	0.49	0.31	79.8
30	M	10 mg	0.59	0.56	0.26	81.5
65	M	10 mg	0.66	0.52	0.34	64.7
24	F	10 mg	0.84	0.48	0.66	61.7
42	M	10 mg	0.72	0.50	0.45	61.7
43	M	12 hourly	0.68	0.45	0.29	76.8
		10 mg				
Mean	42.0	12 hourly	0.69	0.49	0.37	71.4
± s.d.	13.6		0.08	0.04	0.14	8.6

Discussion

Jellet & Shand (1973) showed that propranolol in blood partitions between erythrocytes and free drug in plasma by passive diffusion. The distribution of propranolol between phosphate buffered saline and the RBC was independent of drug concentration between 50 and 1000 ng ml⁻¹, and propranolol was associated with both membrane and cell contents. Normally the interior of the erythrocyte is at pH 7.21 to 7.26 (Gram, 1924), and propranolol itself has been shown to lower the intracellular pH (Pendleton *et al.*, 1972), therefore one would expect a slight accumulation of weak bases, such as the β -adrenoceptor antagonists, within the erythrocyte. However, the RBC:phosphate buffer concentration ratio was reported to be 5.5 (Jellet & Shand, 1973; confirmed in our laboratory) and the mean RBC:free plasma concentration ratio in the present experiments was 4.0 suggesting that for lipid-soluble drugs such as propranolol, partitioning into the membrane is of greater importance. The WB:P propranolol concentration ratios in healthy subjects of 0.7 to 0.85 reported by Evans & Shand (1973) are similar to those in Group 1 of the present study (0.71 to 0.79 for Group 1a, and 0.64 to 0.79 for Group 1b). Similarly, the RBC:P ratios of Evan & Shand (1973) which varied between 0.3 and 0.7 agreed well with the results of the present investigation (0.27 to 0.51 in Group 1a and 0.23 to 0.55 in Group 1b). The hypertensive patients studied by Sawchuk *et al.* (1974) were found to have a significantly higher concentration of propranolol in whole blood than in plasma, the mean ratio being 1.3. In the latter study, there was substantial intersubject variation in the WB:P ratio; values ranged between 0.5 and 1.9. It is widely believed that the pharmacological response to a drug is a function of its free concentration in plasma. Sawchuk *et al.* (1974) suggested that the wide range of WB:P ratios of propranolol concentrations in their subjects might reflect differences in protein binding which might in turn given rise to a variable pharmacological response in hypertensive patients even where total plasma concentrations of propranolol are similar. The results obtained for the hypertensive patients in Group 2 of the present work (WB:P was 0.61 to 0.89; RBC:P was 0.22 to 0.73) were not significantly different from those in the normal subjects comprising Group 1. Protein binding of drug was not measured in Group 2 because there was no difference in the results from these patients and the controls. There is no obvious explanation why the results of Sawchuk *et al.* (1974) differ from those in this study and that of Evans & Shand (1973).

The erythrocyte concentration of pindolol was linearly related to the concentration of free drug in plasma. As with propranolol, both WB:P and RBC:P

pindolol ratios were proportional to the free fraction of drug. The RBC:free pindolol concentration ratio was 1.27 which was less than that of propranolol (4.0) and probably reflects the lower lipid-solubility of pindolol. Since the concentration ratios of pindolol and propranolol between whole blood and plasma are proportional to the free drug in plasma, it is likely that measurement of the WB:P ratio in addition to the concentration in plasma might be helpful when considering their pharmacological or therapeutic effects.

A RBC:P ratio of 1.2 has been reported for hydrophilic atenolol (Graham *et al.*, 1973) which was the same as that of 1.15 in the present study. Atenolol is 3 to 6% bound to plasma protein (Barber *et al.*, 1978; Henry & Mitchell, 1981). Assuming a constant 5% binding to plasma protein, the RBC:free plasma drug ratio was 1.10, only slightly lower than that of pindolol: possibly atenolol is associated more with the intracellular than the membrane component of the erythrocyte. The RBC concentration of atenolol appeared to be proportional to free plasma drug in 3 of the 4 subjects studied, however, there was considerable between-subject variation and limited data which prevented statistical analysis.

Korten & Miller (1979) investigated the erythrocyte ghost: phosphate buffer partition coefficients of phenobarbitone, pentobarbitone and thiopentone and found these supported the pH-partition hypothesis and correlated much more closely with physiological permeability constants than did partition coefficients using organic solvents. They suggested that partitioning of drugs into erythrocyte ghosts may more usefully predict the distribution of drugs in *in vivo* than does organic solvent partitioning and showed how the latter tended to over-estimate the differences between drugs in relation to their *in vivo* partitioning. The RBC:free plasma concentration ratios of 4.0 for propranolol and 1.3 for pindolol in the present work tend to support this view when compared with their partition coefficients between n-octanolol and phosphate buffer at pH 7.0 and 8.0, of 5.39 and 52.5 respectively for propranolol, and 0.12 and 1.5 respectively for pindolol (Hellenbrecht *et al.*, 1973). This conclusion is probably relatively independent of the biomembrane chosen as a model. Roth & Seeman (1972) have shown that partitioning of chlorpromazine, morphine, phenol and pentobarbitone into erythrocyte ghosts, synaptosomes, or muscle reticulum shows little dependence on the membrane. The use of RBC:P concentration ratio of a drug to help predict its *in vivo* distribution is likely to have the additional advantage of automatically accounting for the degree of protein binding in plasma.

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