THE INFLUENCE OF VARIOUS FACTORS ON THE *In vitro* DISTRIBUTION OF HALOPERIDOL IN HUMAN BLOOD

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1 Haloperidol is $89.6 \pm 0.3\%$ bound (mean $\pm$ s.e. mean) in human plasma under *in vitro* conditions and the free drug distributes rapidly between the plasma and the cellular elements of blood. The cell/plasma partition ratio was 1.12 ± 0.06 (mean $\pm$ s.e. mean).

2 Alteration of plasma binding by dilution with buffer showed that uptake of haloperidol by the cellular elements of blood was proportional to free drug concentration.

3 Bishydroxycoumarin (95 or 286 $\mu\text{g/ml}$) reduced plasma binding of haloperidol and the displaced haloperidol was taken up by the cellular blood elements.

4 The experiments indicate that the cellular compartment of blood as well as the plasma compartment may act as a *sink* for haloperidol and drug displacement interactions should therefore be interpreted with a knowledge of both of these compartments.

Introduction

Investigations of interactions between drugs due to displacement from binding sites have, in the main, involved consideration of the plasma protein binding of the interacting drugs, although there are exceptions (Stockley, 1974). In whole blood, however, drugs have access not only to plasma proteins but also to the cellular blood elements and partition between plasma and red blood cells, for example, has been demonstrated for several therapeutic agents (Hinderling, Bres & Garrett, 1974; Hughes, Ilett & Jellett, 1975; Jellett & Shand, 1973; Kurata & Wilkinson, 1974). It is possible that drug molecules bound to the cellular blood elements could be displaced by other agents and thus the cellular elements as well as the plasma proteins could be the source of drug displaced during drug interactions. The present study therefore investigated the *in vitro* distribution of haloperidol in human blood in order to evaluate the significance of the cellular blood compartment in the distribution of this drug.

Methods

Fresh blood was obtained from normal volunteers of either sex and heparin (3u/ml) was added. All the following procedures were then carried out at 37°C. The blood was distributed in 10 ml aliquots and in the case of plasma dilutions, cells and plasma were separated (1200 g for 10 min), a

volume of plasma was removed and replaced with isotonic phosphate buffer (K_2HPO_4 , 1.41; NaH_2PO_4 , 0.26; NaCl, 8.10 g/l; pH 7.4) and the cells were then resuspended. Radiolabelled haloperidol at a concentration of 40 or 95 ng/ml was added in a 0.1 ml volume of the above buffer. In some experiments bishydroxycoumarin was added to the blood 5 min before the addition of haloperidol. The samples were incubated for 15 min unless otherwise stated with gentle mixing and the haematocrit was then measured. Plasma and cells were separated by centrifugation and ^{14}C or ^3H content of the plasma was determined by liquid scintillation counting.

Having measured the concentration of haloperidol in the plasma the cell (C)/plasma (P) drug concentration ratio was determined from the haematocrit (H) and the blood (B)/plasma ratio according to the following equation:

$$\frac{C}{P} = \frac{B/P - (1-H)}{H}$$

Plasma binding was measured by equilibrium dialysis as previously described (Hughes *et al.*, 1975). Preliminary experiments showed that there was no breakdown of radiolabelled haloperidol during dialysis.

All results are expressed as mean $\pm$ s.e. mean and Student's *t*-test or a paired *t*-test was used to evaluate statistical significance.

Drugs used

Amitriptyline (Merck, Sharpe & Dohme, U.S.A.), bishydroxycoumarin (BHC, Sigma), diphenylhydantoin (Parke Davis & Co., Australia), haloperidol (^{14}C , 20 $\mu\text{Ci}/\text{mg}$; ^3H , 133 $\mu\text{Ci}/\text{mg}$; G.D. Searle, U.K.), Heparin Injection B.P., procainamide hydrochloride (E.R. Squibb, Australia), ($\pm$)-propranolol hydrochloride (I.C.I., Australia).

Results

Measurement of chloroform/buffer partition coefficient

Measurements were made at 37°C as previously described (Hughes *et al.*, 1975). The values obtained after mixing for 30 or 60 min were not significantly different ($t = 0.77$, $P > 0.4$) indicating that equilibrium had been obtained. Observations made at these two times have therefore been combined to give a mean value of 10.55 ± 0.24 .

Effect of time of incubation on plasma concentration of haloperidol

Total plasma concentrations of [^3H]-haloperidol found after incubation of whole blood containing [^3H]-haloperidol (95 ng/ml) for 5, 15, 30 and 60 min were 84.6 ± 2.3 , 85.1 ± 1.8 , 85.2 ± 3.0 and 86.0 ± 2.0 , respectively (mean $\pm$ s.e. mean for blood from five subjects). A paired t -test showed that there was no significant difference ($P > 0.1$) between the plasma concentrations measured at any of the time periods indicating that the distribution of haloperidol had reached equilibrium within 5 minutes.

Measurement of normal plasma binding and cell/plasma partition ratio for haloperidol (40 ng/ml blood)

In plasma from eleven normal volunteers the protein binding of haloperidol *in vitro* was remarkably constant. A mean value of $89.6 \pm 0.3\%$ (range 88.1–91.4) was obtained while the cell/plasma partition ratio was 1.12 ± 0.06 (range 1.47–0.78).

Effect of dilution of plasma on cell/'plasma' partition coefficient

Measurements of cell/'plasma' partition ratio and % plasma protein binding in dilutions of plasma from one individual showed that this ratio was linearly related to the concentration of free drug in the 'plasma' (Figure 1).

Some of the data points from which this figure is derived are shown in Table 1. It can be seen that as dilution of the plasma increases, protein binding decreases and the total plasma concentration of haloperidol falls while the free (pharmacologically active) plasma concentration rises to a small extent (1.44-fold over the range 90.1 to 70.8% binding).

Using the mean cell/plasma partition ratio calculated from the eleven normal volunteers and the measured percent plasma binding, the concentration of free haloperidol in the plasma can be calculated and compared with the values actually found. As can be seen from Table 1, agreement between measured and calculated values is excellent.

Also shown in Table 1 are the plasma concentrations of free haloperidol calculated on the erroneous assumption that the cellular constituents of blood are not a significant compart-

Table 1 The effect of plasma dilution or addition of bishydroxycoumarin (BHC) on the plasma concentration and protein binding of haloperidol. The concentration of [^{14}C]-haloperidol in the blood was 40 ng/ml.

Haematocrit (%)	Binding conditions	% plasma binding	Measured plasma concentration of haloperidol (ng/ml)		Calculated free plasma concentration of haloperidol (ng/ml)	
			Total	Free	Allowing for cellular compartment	Ignoring cellular compartment
42.3	Control	90.1	39.0	3.9	3.6	6.7
43.0	Dilution	82.0	27.0	4.9	4.8	12.0
43.5	Dilution	70.8	19.4	5.6	5.7	19.6
44.3	BHC	87.1	31.1	4.0	4.1	8.6
	(95 $\mu\text{g}/\text{ml}$)					
44.3	BHC	83.0	26.8	4.6	4.7	11.5
	(286 $\mu\text{g}/\text{ml}$)

Figure 1 The relationship between % free haloperidol in plasma (■, control; ▲, diluted) and cell/plasma partition ratio. The line fitted by least squares regression analysis is described by $y = 0.119x - 0.220$ ($r = 0.99$). Also shown on this graph are the values obtained when plasma protein binding of haloperidol was modified by the addition of various concentrations of bishydroxycoumarin (●, 95 µg/ml; ○, 286 µg/ml).

ment in the distribution of haloperidol. Total plasma concentration in this case obviously remains constant and the free drug concentration rises 2.93-fold when binding is reduced from 90.1 to 70.8%. This is more than twice the observed change in the free concentration of haloperidol in the plasma.

Effect of other drugs on cell/plasma partition of haloperidol

Propranolol (200 mg/ml), amitriptyline (400 ng/ml), procainamide (10 µg/ml), quinidine (6 µg/ml) and diphenylhydantoin (30 µg/ml) did not modify the plasma binding of haloperidol. Bishydroxycoumarin (BHC; 95 or 266 µg/ml blood) reduced plasma binding of haloperidol (Table 1) and this reduction was accompanied by change in total and free plasma haloperidol concentration similar to those seen after plasma dilution.

Discussion

The lack of a significant change in the plasma concentration of haloperidol when a given concen-

tration was incubated with whole blood for varying time periods indicates that the distribution of haloperidol in whole blood reaches an equilibrium state very quickly. Such a fast equilibrium would be expected in view of the moderate chloroform/buffer partition coefficient of haloperidol (Schanker, Nafpliotis & Johnson, 1961).

In normal human blood, haloperidol is approximately 90% bound to the plasma proteins and has a cell/plasma partition coefficient of 1.12. The potential significance of the cellular blood compartment for haloperidol is evident from the fact that the cell/plasma water ratio (10.2) is actually higher than the ratio for bound haloperidol to free haloperidol (8.1) in the plasma.

Since there is a linear relationship between the cell/plasma partition coefficient and % free haloperidol in the plasma, it would appear that the amount of haloperidol in the cellular elements is directly related to the plasma-free drug concentration. As the free drug has a very favourable partition towards cellular elements, they can therefore form a reservoir for free drug displaced from plasma binding sites. This effect is seen in the experiments involving dilution of plasma. Binding to plasma protein is reduced and the total plasma concentration falls, while the free drug concentration in plasma rises to a smaller extent than would be expected from consideration of the plasma in isolation.

The relevance of this function of the cellular elements to displacement interactions between drugs is critically dependent on the characteristics of the 'binding' sites in the cellular elements. In the case of haloperidol, displacement from plasma protein binding sites by BHC does in fact result in a fall in total plasma drug concentration indicating that the cellular elements are acting as a sink. The concentrations of free drug in the plasma rise to a lesser extent than would be predicted from consideration of plasma in isolation. Thus the 'binding' sites in the cellular elements must be less affected by BHC than are those on the plasma proteins.

This situation does not necessarily apply to all drugs. It is possible that with some agents, cellular element 'binding' sites may be more affected than those on plasma proteins. In this case the reverse situation will apply and the cellular elements could act as a source of drug rather than as a sink.

Consideration of displacement interactions in the blood must therefore always be made with a knowledge of both cellular and plasma binding sites and the cell/plasma partition coefficient.

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