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Binding of chlorpromazine, phenytoin and aspirin to the erythrocytes and lipoproteins in whole human blood

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The quantitative binding of ^{14}C-labelled chlorpromazine, phenytoin or aspirin (at 10 μM) to blood cells and plasma lipoproteins in whole human blood or to the washed erythrocytes in an isotonic protein-free medium has been studied. The fractions of chlorpromazine, phenytoin and aspirin bound to the blood cells in whole blood amounted to about 40, 14 and 2% of the total amount added, and those to the lipoproteins amounted to 7, 2 and 1%, respectively. Their binding to the washed erythrocytes in protein-free medium was 95, 76 and 40%, respectively. Their octanol: water partition coefficients were 214, 170 and <0·1, respectively. These results suggest that the amphiphilic drugs with relatively high hydrophobicity may be bound to the blood cells, mainly to erythrocytes, to considerable extents when administered clinically, and also that their binding to plasma lipoproteins may not be negligible.

Many amphiphilic drugs, particularly those having high hydrophobicity, generally have a high affinity to cell membranes (Fujii et al 1979). When these drugs enter the blood stream they associate not only with the plasma proteins but also with the cellular components. They may also be bound to plasma lipoproteins as well as proteins such as albumin and α_1-acid glycoproteins which are already known to bind drugs, since cyclosporin A (Lemaire & Tillement 1982) and some amphiphilic lipids (Fujii et al 1987) are bound to plasma lipoprotein in a proportion comparable with that bound to erythrocytes. For example, when the number of erythrocytes or plasma lipoprotein concentration in the blood changes, as is often observed in certain diseases, the concentration of unbound (free) drug in blood, which is considered to be the pharmacologically active form, may change significantly. Therefore, binding of drugs to these blood components may have some implications in drug pharmacokinetics.

Most experiments on drug binding to blood components have made use of the method of equilibrium dialysis to separate each component and drug solution, enabling the distribution of the drug among various blood components to be calculated from each partition coefficient obtained (cf. Ehrnebo et al 1974; Urien et al 1985). However, there is a possibility that competition for drug binding among other components may occur in blood when numerous components are present. Therefore, more direct measurement of drug distribution in whole blood is needed.

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One difficulty is that the affinity of a drug to these components is often not strong enough to resist washes with saline. We have found that drug molecules such as chlorpromazine, cepharanthine and other amphiphiles are removed from the erythrocyte membrane by saline washes (unpublished results). Furthermore, our preliminary experiments showed that upon electrophoresis of drug-bound plasma lipoproteins, many amphiphilic drugs were dissociated partially from the protein molecules.

In the present study, we have tried to measure directly the binding of some typical amphiphilic drugs to blood cells and plasma lipoproteins in whole human blood in-vitro, by fractionating successively the whole blood pretreated with ^{14}C-labelled drug into the drug-bound components by centrifugation. The drugs we used were two strongly hydrophobic drugs, chlorpromazine and phenytoin, and the weak hydrophobic drug, aspirin.

Materials and methods

Drugs. Chlorpromazine was obtained from Sigma Chemical Co. (USA), and aspirin and phenytoin were obtained from Nakarai Chemicals Ltd (Japan). [^{14}C]Chlorpromazine (108 mCi $mmol^{-1}$) was obtained from Amersham (UK). [^{14}C]Phenytoin (47·05 mCi $mmol^{-1}$) and [^{14}C]aspirin (34·3 mCi $mmol^{-1}$) were obtained from New England Nuclear (USA). These drugs were dissolved in isotonic phosphate-buffered saline (PBS, 10 mM phosphate, 140·5 mM NaCl, pH 7·4). Other chemicals were of reagent grade.

Preparation of blood. Whole blood was taken from normal volunteers in our laboratory. Ethylenediaminetetraacetic acid disodium salt (EDTA) was used as anticoagulant.

Preparation of washed erythrocytes. Whole blood was centrifuged at 2000*g* for 5 min, and plasma and buffy coat were removed. Erythrocytes were washed three times with 10 volumes of PBS and were suspended in PBS to make 50% haematocrit value.

Treatment of whole blood and washed erythrocytes with drugs. One volume of each 0·1 mM drug solution containing 1–2 μCi of each ^{14}C-labelled drug was added

to 9 volumes of whole blood or washed erythrocyte suspension and the mixture was incubated at 37 °C for 20 min.

Determination of drug bound to blood cells and plasma lipoproteins. Blood cells in the whole blood pretreated with drug were separated from plasma by centrifugation at 2000*g* for 5 min at 5 °C. The amounts of the drug bound to the blood cells were calculated from the difference of the radioactivities in the mixture and in the supernatant (Fujii & Tamura 1984).

The amount of drug bound to plasma lipoproteins was obtained from the plasma after removal of blood cells as described. Lipoproteins (very low density lipoprotein, VLDL, low density lipoprotein, LDL, and high density lipoprotein, HDL) in the plasma were separated by ultracentrifugation (Hatch & Lees 1968) as follows. After crystalline KBr was added to the plasma to make the density = 1·21, 4 mL of such plasma was ultracentrifuged at 150 000*g* for 40 h at 4 °C, using a rotor of swing type (80P-7, Hitachi Co. Ltd, Japan). Then, each 0·2 mL fraction from the top was sampled and amounts of the radioactivity, cholesterol, and protein were determined, by using a cholesterol test kit (Wako Pure Chem. Ind. Ltd, Japan) for cholesterol content, and by the method of Lowry et al (1951) for protein content.

Determination of octanol: water partition coefficient. One mL of 0·01 mM drug solution containing trace amounts of ^{14}C-labelled drug was added to 1 mL of octanol and then mixed vigorously at room temperature (20 °C) for 30 min. After centrifugation of the mixture at 2000*g* for 20 min, the radioactivity of the drug in the octanol and buffer layer was measured.

Determination of radioactivity. The radioactivity in each sample was measured with a liquid scintillation counter (Mark III-6880, Searle Analytic Co., USA). When the radioactivity in whole blood or in the erythrocyte suspension was measured, less than 0·05 mL of sample volume was taken, to avoid quenching by haemoglobin.

Results

Octanol: water partition coefficient of each drug. The octanol : water partition coefficients of chlorpromazine, phenytoin and aspirin at pH 7·4 (Table 1) show the former two to have the high coefficients, indicating their relatively high hydrophobicity, whereas aspirin has a low coefficient, representing its low hydrophobicity.

Binding of drug to blood cells in whole blood. When each drug was added to whole blood, equilibration of the drug between plasma and blood cells occurred rapidly; steady state was established during the first 10 min incubation with every drug (data not shown). After each drug was incubated for 20 min with blood, the percentage of drug bound to blood cells was determined (Table 1). About 40, 14 and 2% of the added chlorpromazine, phenytoin and aspirin, respectively, were detected in the blood cell fraction.

Table 1. Octanol : water partition coefficients of the drugs and their binding to the blood cells in whole blood, to the lipoproteins in plasma, and to washed erythrocytes.

		Binding		
Drug	o : w	Cells in whole blood (%)	Lipoproteins in plasma (%)	Washed RBCs (%)
Chlorpromazine	214 ± 32	40·4 ± 5·2	11·8 ± 1·5	95·4 ± 0·5
Phenytoin	170 ± 45	14·3 ± 1·1	2·2 ± 0·5	76·0 ± 1·0
Aspirin	<0·1	2·0 ± 0·8	0·6 ± 0 1	39·5 ± 0·1

The data represent the mean ± s.d. obtained from the five experiments.

Binding of drug to plasma lipoproteins. Fig. 1 shows the profiles of distribution of the [^{14}C]chlorpromazine, cholesterol and protein in the plasma after ultracentrifugation. The radioactivity, cholesterol and protein content in each 0·2 mL fraction from the top of the centrifuging tube after ultracentrifugation are plotted. It is evident from the Fig. that all the lipoproteins (VLDL, LDL and HDL) were recovered in the fraction 1–3 (total of 0·6 mL from the top), because cholesterol was detected only in this fraction. The radioactivity of each drug bound to the lipoproteins was calculated and the results are described as % distribution in the plasma

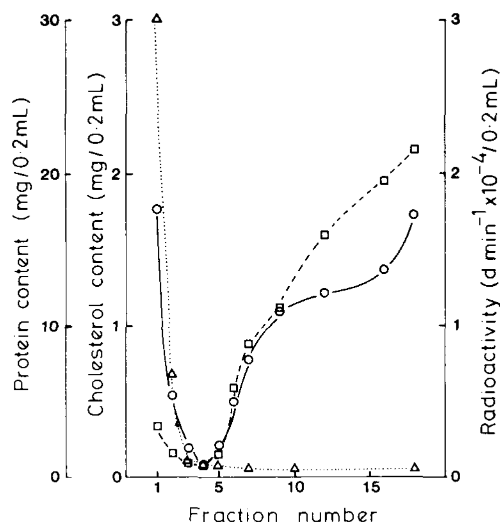

FIG. 1. Ultracentrifugation pattern of proteins and cholesterol in the plasma, and distribution of radioactivity of chlorpromazine in the plasma. Plasma was made density = 1·21 and then it was ultracentrifuged at 150 000*g* for 40 h at 4 °C. Each 0·2 mL from the top of the centrifuge tube was sampled. Solid line indicates radioactivity. Broken and dotted line show protein and cholesterol content, respectively.

(Table 1). About 12 and 2% of chlorpromazine and phenytoin, respectively, are distributed in the lipoprotein fraction, while 0·6% of aspirin existed in the same fraction.

Binding of drug to washed erythrocytes. After incubation of each drug with washed erythrocytes, the percentage binding of the drug to them was examined (Table 1). The amounts of chlorpromazine, phenytoin and aspirin incorporated into the washed cells were about 95, 76 and 40%, respectively.

Discussion

Our results indicate that some amphiphilic drugs added can associate with the blood cells in whole human blood and with lipoproteins in the plasma to considerable extents. From data in Table 1, their supposed distribution in the whole blood was calculated as shown in Table 2.

Table 2. Calculated distribution of the drugs in the blood cells, plasma lipoproteins and others.

	Drug distribution in whole blood (%)		
Drug	Blood cells	Plasma lipoproteins	Others
Chlorpromazine	40	7	53
Phenytoin	14	2	84
Aspirin	2	1	97

From the octanol: water partition coefficients of these drugs obtained in this work (Table 1), it was confirmed that chlorpromazine and phenytoin have relatively high hydrophobicity, whereas aspirin is far less hydrophobic. About 40 and 14% of these hydrophobic amphiphiles added to the blood were found in the blood cell fraction, whereas only 2% of aspirin was bound to the same fraction (Table 2). Such a percentage distribution of phenytoin in blood cells agrees well with the value obtained by equilibrium dialysis (Kurata & Wilkinson 1974). In the absence of the extracellular plasma, as much as 95 and 76% of these drugs were bound to the washed erythrocytes (Table 1). These results are consistent with the previous findings as to the competition of drug binding between the blood cells and plasma proteins (Bickel 1975; Shirkey et al 1985).

The above results mean that although the distribution of these drugs in blood may be determined by their affinity to the blood cells and plasma proteins and by the relative amounts of these components in blood, the hydrophobic drugs sometimes tend to favour the blood cells. The example demonstrated here is the case with chlorpromazine which is distributed in a ratio of about 4 : 6 between the blood cells and plasma in whole blood. The above facts also suggest that these drugs are bound mainly to the erythrocytes among blood cells, as judged by their strong affinity to this cell type in protein-free medium (Table 1) and also because of their abundance in blood.

Binding of hydrophobic drugs to plasma lipoproteins is also sometimes not negligible, as demonstrated with cyclosporin A (Lemaire & Tillement 1982). Indeed, about 12% of chlorpromazine added to the plasma was bound to the lipoprotein fraction obtained by ultracentrifugation of the plasma at d = 1·21 (Table 1). The value means that about 7% of the drug added to the whole blood must be bound to the plasma lipoproteins (Table 2).

It has already been reported that where there is a reduced level of plasma proteins as in some renal diseases (Sherwin et al 1976) or during pregnancy (Wallin & Herngren 1985), the decreased binding of phenytoin or phenobarbitone to plasma proteins is counteracted by their increased binding to the erythrocytes. There is also a report correlating erythrocyte levels of some phenothiazine derivatives with their therapeutic effectiveness (Casper et al 1980). Thus, it is reasonable to suggest that the blood cells, particularly the erythrocytes, and also the plasma lipoproteins in some cases, must be taken into consideration, in addition to albumin and some other plasma proteins, in studying the distribution of certain hydrophobic amphiphiles in the blood components as the possible targets of their binding.

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