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The Effects of Haematocrit, Plasma Protein Concentration and Temperature of Drug-containing Blood In-vitro on the Concentrations of the Drug in the Plasma

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Abstract—Factors which influence the plasma drug concentrations in whole blood have been investigated in-vitro using human blood containing radio-labelled phenytoin or chlorpromazine. Phenytoin (3·55 μg mL^{-1}, 0·01 mM) or chlorpromazine (2·74 μg mL^{-1}, 0·01 mM) was mixed with normal or modified blood and the plasma drug level was measured. Plasma phenytoin and chlorpromazine levels decreased with decrease in the protein concentration of plasma, but were not influenced by addition of γ-globulin to the blood specimen. Plasma phenytoin levels increased with an increase in haematocrit from 20 to 45%, whereas the chlorpromazine level remained constant. The partition coefficients of phenytoin and chlorpromazine between blood cells and plasma were almost the same at various haematocrit values. By cooling the blood containing each drug to 4°C, plasma phenytoin and chlorpromazine levels were higher compared with those at 37°C. Similar temperature effects on the drug levels in plasma were obtained when the washed erythrocytes were resuspended in albumin medium, but not when resuspended in saline.

Although only free drug in plasma is pharmacologically active, therapeutic schedules being evaluated are often based on the total drug concentration in serum or plasma. In addition to plasma proteins, erythrocytes are known to bind certain drugs, such as phenytoin (Sherwin et al 1976), cyclosporin A (Lemaire & Tillement 1982), chlorpromazine (Bickel 1975; Casper et al 1980), nicardipine (Urien et al 1985) and amiodarone (Maling et al 1989). It has recently been reported that, with increasing haematocrit values, the plasma concentration of cyclosporin A (Rosano 1985) and busulfan (Ehrsson & Hassan 1984) decreased, whereas cephalosporin levels increased (Derendorf 1987), without any change in total drug concentration in whole blood. Furthermore, the plasma concentration of sodium valproate (Shirkey et al 1985) and of tricyclic antidepressants (Javaid et al 1985) decreased with decreasing plasma protein concentration. Therefore, attention should be paid to these pathophysiological factors in determining the significance of plasma drug levels in the blood.

We now present our in-vitro data on certain factors which may influence plasma drug levels; variations of haematocrit, decrease in plasma total proteins and increase in γ-globulin as the pathophysiological factors to be expected, and the storage temperature of the blood sample before separation of the plasma. Phenytoin (PHT) and chlorpromazine (CPZ) were used as model drugs, because both are widely used clinically and are of moderate hydrophobicity (Tamura et al 1987).

Materials and Methods

Materials

Chemicals. CPZ was obtained from Sigma Chemical Co. (USA) and PHT (diphenylhydantoin) from Nacarai Tesque Inc. (Japan). [^{3}H]CPZ (17·1 Ci $mmol^{-1}$) and [^{14}C]PHT (55·8 mCi $mmol^{-1}$) were obtained from New England Nuclear (USA). These drugs were dissolved in phosphate-buffered saline (PBS, 140·5 mM NaCl, 10 mM phosphate buffer, pH 7·4). Human serum albumin (HSA, essentially globulin-free, prepared from Cohn Fraction V), and human serum γ-globulin (purified from Cohn Fraction II and III, electrophoretic purity of 99%) were purchased from Sigma Chemical Co. (USA). All other reagents were of pure grade.

Blood. Blood from healthy volunteers in our university (age: 19–39, haematocrit value; 37–53%, plasma total protein; 7·4–7·9 g dL^{-1}) was mixed with heparin (50 units mL^{-1}). The haematocrit value was determined routinely using capillary tubes and a microcentrifuge.

Preparation of modified blood. (1) Low haematocrit blood was prepared by dilution of whole blood with corresponding plasma obtained by mild centrifugation (1800 *g*, 10 min). (2) Blood with lower concentration of plasma protein was prepared as follows: plasma was filtered through an ultrafiltration membrane (YMT, Amicon Corp.) to obtain protein-free plasma, which was then mixed with corresponding high haematocrit blood to make 45% haematocrit blood. The protein concentration of each plasma sample was determined by the method of Lowry et al (1951). (3) Blood with increased γ-globulin content was prepared by addition of γ-globulin to the whole blood.

Preparation of erythrocyte suspension. Erythrocytes separated from the blood by centrifugation, were washed three times with 10-fold volumes of PBS and resuspended in PBS or in PBS containing 5% HSA to make 45% haematocrit blood.

Methods

Treatment of blood and erythrocyte suspension with drugs. One volume of each PHT (35·5 μg mL^{-1}, 0·1 mM) or CPZ

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solution (27·4 μg mL^{-1}, 0·1 mM) containing 0·1–0·2 μCi of each isotope-labelled drug was added to 9 volumes of blood or erythrocyte suspension and the mixture was incubated at 37°C for 30 min. For temperature studies, the mixture was then incubated at 4°C for 30 min.

Determination of drug concentration in plasma and of drug distribution between blood cells and plasma. After centrifugation of the prepared samples (1800 *g*, 10 min, 20°C unless otherwise stated) the radioactivity in plasma was measured with a liquid scintillation counter (LSC-1000, Aloka Co., Japan). Because the total radioactivity in whole blood or in the erythrocyte suspension could be quenched by haemoglobin, quench corrections were made by counting the same specimens before and after combustion using an automatic sample combustion system (ASC-113, Aloka Co., Japan). The partition coefficient (D) of the drug between blood cells and plasma was calculated from the concentration in whole blood and plasma and the haematocrit (Ehrsson & Hassan 1984).

Results

The time course of distribution between plasma and blood cells of the added drug was examined. The equilibrium of PHT and CPZ was reached within 10 min incubation and the distribution ratio of either drug did not change up to 60 min of incubation. Thus, the incubation time of 30 min was used in subsequent experiments.

Effect of haematocrit on the drug levels in plasma

A regression analysis between haematocrit and the drug levels in different plasma samples is shown in Fig. 1; significant positive correlation was observed with PHT, but none was seen with CPZ. The partition coefficients of PHT and CPZ between blood cells and plasma were 0·31 ± 0·09 and 1·17 ± 0·12, respectively, and the ratios showed no significant correlation with haematocrit values (data not shown).

Effect of decrease in plasma protein and increase in γ-globulin on the drug levels in plasma

Both PHT and CPZ levels in plasma decreased with decreasing plasma protein concentration, whereas no appreciable change in plasma drug levels was observed upon γ-globulin increase (Table 1).

Effect of temperature during sample storage on the drug levels in plasma

After the treatment of normal blood (haematocrit 45%) with drugs at 37°C for 30 min, the mixture was then kept at 4°C for 30 min. PHT and CPZ levels in plasma at 4°C were 6·01 and 3·26 μg mL^{-1}, which are 14 and 16% higher than the average of 5·28 and 2·80 μg mL^{-1} for the same samples at 37°C, respectively. This temperature effect was reversible; the increased drug levels in plasma returned to almost the same levels as at 37°C by raising the sample temperature again from 4°C to 37°C (Table 2).

To pursue the mechanism of variation of plasma drug levels accompanying a change in temperature of blood storage, erythrocyte suspension prepared with 5% albumin medium or saline medium was used. After incubation of the cell suspension with drug at 37°C, the mixture was put into an ice bath (4°C) and then incubated again at 37°C. In each step, the drug levels in the supernatant after centrifugation were determined. As shown in Table 3, when the cells were resuspended in saline medium, no appreciable change in the drug levels in the supernatant between 37°C and 4°C was observed. However, when the cells were suspended in albumin medium, the PHT and CPZ levels in the supernatant were increased by about 15 and 11%, respectively, at 4°C compared with 37°C.

Discussion

Numerous blood components which can bind certain drugs are expected to lead to competition for the drug; competition between blood cells and plasma proteins determines the drug concentration in plasma. In this study, we have demonstrated some factors influencing drug concentration in plasma.

The effect of haematocrit value

The plasma concentration of drugs with very low affinity (Derendorf 1987) or very high affinity (Rosano 1985) for blood cells is affected by the haematocrit value. Both PHT (Sherwin et al 1976; Tamura et al 1987) and CPZ (Bickel

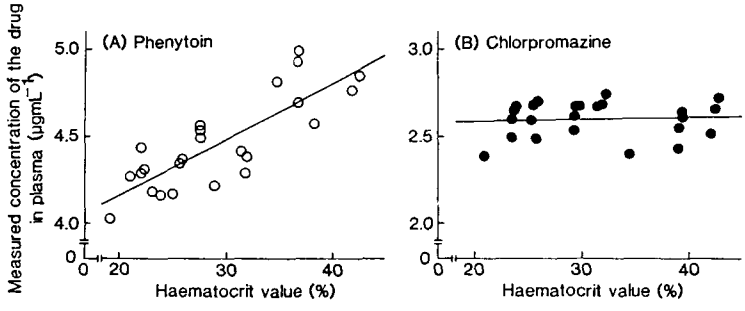

FIG. 1. Effect of haematocrit values of drug-containing blood on the measured concentration of the drug in the plasma. Blood specimens with various haematocrit values were incubated with phenytoin (A) or chlorpromazine (B) at 37°C for 30 min, and then drug concentration in the plasma was measured. The regression lines are: (phenytoin) $Y = 0{\cdot}03X \pm 3{\cdot}54$, $r = 0{\cdot}81$, $P < 0{\cdot}01$, $n = 24$; (chlorpromazine) $Y = 0{\cdot}001X \pm 2{\cdot}56$, $r = 0{\cdot}08$, $n = 24$. Blood contained 3·55 μg mL^{-1} phenytoin or 2·74 μg mL^{-1} chlorpromazine.

Table 1. Effect of total plasma protein decrease and γ-globulin increase in drug-containing blood on the measured concentration of the drug in the plasma. Blood contained 3·55 μg mL^{-1} PHT or 2·74 μg mL^{-1} CPZ.

Concentration of protein (g dL^{-1})	Measured concn. of each drug in the plasma (μg mL^{-1})*	
	Phenytoin	Chlorpromazine
Total plasma protein		
7·9 (normal)	5·11 ± 0·20	2·78 ± 0·12
6·4	4·89 ± 0·21	2·34 ± 0·17
4·2	4·24 ± 0·12**	1·73 ± 0·19**
Addition of γ-globulin to normal blood		
None (normal)	4·69 ± 0·14	2·47 ± 0·01
0·2	4·61 ± 0·14	2·47 ± 0·05
0·4	4·50 ± 0·14	2·38 ± 0·06

* Mean ± s.d. (n = 5). ** $P < 0·001$.

Table 2. Effect of storage temperature for drug-containing blood on the measured concentration of the drug in the plasma. Blood contained 3·55 μg mL^{-1} PHT or 2·74 μg mL^{-1} CPZ.

Storage temp. of specimen (Incubation*) (°C)			Measured concn. of each drug in the plasma (μg mL^{-1})**	
1st	2nd	3rd	Phenytoin	Chlorpromazine
37	—	—	5·28 ± 0·13	2·80 ± 0·20
37	4	—	6·01 ± 0·35***	3·26 ± 0·15***
37	4	37	5·38 ± 0·09	2·90 ± 0·15

* Incubation time of 30 min in each step was used. ** Mean ± s.d. (n = 7). *** $P < 0·001$.

The effect of concentration of plasma proteins

Lowering the albumin concentration in plasma yields an increase in the unbound (free) fraction of certain drugs (Javaid et al 1985; Shirkey et al 1985; Melten et al 1986). Our results show that the decrease in the PHT and CPZ in plasma with decrease in the total protein concentration may be attributed to an increase in the free fraction of the drug in plasma, which may result in increased drug binding to blood cells. Since PHT and CPZ levels in plasma were not influenced by an addition of γ-globulin to the blood, the binding affinities of these drugs to γ-globulin appear to be very low, as shown already by an equilibrium dialysis method (Bickel 1975). The possible influence of α_1-acid glycoprotein on the concentration of basic drug in plasma remains to be demonstrated.

The effect of temperature

As can be seen from out data, the plasma PHT and CPZ levels increased by storing the blood containing each drug at 4°C rather than at 37°C, and this temperature effect was reversible. When the washed erythrocytes were suspended in an albumin medium, a similar temperature effect on drug concentration in the supernatant after centrifugation was observed, while this effect was not observed in the absence of the albumin in the medium. Therefore, the temperature effect on the drug levels in plasma may be due to the change in free drug concentration resulting from the temperature-dependent change in the binding affinity of the drug to plasma proteins, most probably to albumin. For several drugs, e.g. theophylline (Shaw et al 1982), phenytoin (Lunde et al 1970) and cyclosporin A (Agarwal et al 1987), a lower proportion of the free fraction at lower temperature was reported.

Table 3. Effect of storage temperature for erythrocyte suspension in the presence or absence of serum albumin (HSA) on the measured concentration of the drug in the supernatant after centrifugation. Erythrocyte suspensions contained 3·55 μg mL^{-1} PHT or 2·74 μg mL^{-1} CPZ.

Storage temp. of cell suspension (Incubation) (°C)			Measured concn.** of each drug in the supernatant after centrifugation (μg mL^{-1})			
			PBS		HSA-containing PBS (5 g dL^{-1})	
1st	2nd	3rd	Phenytoin	Chlorpromazine	Phenytoin	Chlorpromazine
37	—	—	1·43 ± 0·05	0·22 ± 0·01	2·97 ± 0·13	0·73 ± 0·02
37	4	—	1·35 ± 0·07	0·20 ± 0·01	3·43 ± 0·19***	0·81 ± 0·02***
37	4	37	1·43 ± 0·09	0·21 ± 0·01	3·05 ± 0·10	0·73 ± 0·02

* Incubation time of 30 min in each step was used. ** Mean ± s.d. (n = 5). *** $P < 0·001$.

1975; Casper et al 1980; Tamura et al 1987) have moderate affinity for blood cells, the affinity of CPZ being higher than that of PHT. The increase in plasma PHT levels with increasing haematocrit, as observed in the present study, may be due to a drug-concentrating effect by decreasing the plasma volume, nullifying a slight increase in the drug binding to blood cells. On the other hand, maintenance of the constant CPZ level in plasma, even in blood specimens with differing haematocrits may be the result of an increase in the amount of drug bound by an increase in blood cells accompanying the rise in haematocrit value, which counteracted the drug-concentrating effect mentioned above.

In conclusion, our data show that a change in constitution of blood components and/or a difference in the storage temperature of the blood specimen yields an alteration of plasma drug levels. As therapeutic schedules for patients are based on the drug concentration in plasma, it is suggested that the clinical laboratory needs to consider pathophysiological changes in the blood from patients.

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