this kind of analysis to other drugs known to increase both dopamine and DOPAC brain levels.

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Erythrocyte binding of cephalosporins

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A rapid sample preparation and HPLC technique was used to study the erythrocyte binding properties of six cephalosporins at therapeutic concentrations. The negligible red blood cell partition coefficients indicated that only small amounts of the cephalosporins were taken up by the blood cells at all drug concentrations. Thus, plasma concentrations were about twice as high as the respective blood concentrations. Blood/plasma ratios solely depended on the haematocrit and were independent of the extent of protein binding.

All drugs that are distributed to their target sites in the body via the systemic circulation come into contact with blood cells. In contrast to extensive studies of the interaction of numerous drugs with plasma protein, very little information is available about drug-blood cell interaction. The significance of drug binding to plasma proteins is widely recognized and its investigation is a standard procedure for the characterization of new drugs. However, drug binding to blood cells has not been given the same attention, and there is no reason to assume it may be of less significance, since erythrocytes account for approximately half of the blood volume. Furthermore, drugs bound to erythrocytes may provide a novel drug delivery system (Lewis & Alpar 1984). In previous studies (Derendorf & Garrett 1983; Derendorf et al 1984; Garrett & Hunt 1974) it has been shown that drug binding of lipophilic drugs to erythrocytes can be described by a red blood cell partition coefficient:

$$D = \frac{C_{RBC}}{C_{pw}} = \frac{A_{tot} - C_{pw} \cdot V_{pw}}{C_{pw}(V_B - V_{pw})} \quad (1)$$

where D is the red blood cell partition coefficient, C_{RBC} is the concentration of the drug in the erythrocytes, C_{pw} is the concentration in plasma water (protein-free plasma), A_{tot} is the total amount of drug added to a red blood cell suspension, V_B is the volume of the red blood cell suspension and V_{pw} is the volume of plasma water. Drug binding to glass can be compensated for by preparing the calibration curve for C_{pw} in plasma water, i.e. under the same conditions as in the absence of erythrocytes. The volume V_{pw} can be calculated from the total volume V_B and the haematocrit H.

It can be assumed that only free drug in blood is able to diffuse into the blood cells. The results obtained can be confirmed with the separate evaluation of binding when drug is added to whole blood. The relation of drug concentration in blood (C_B) and plasma (C_p) after equilibration between blood cells, plasma proteins and plasma water is reached, can be described by equation 2:

$$\frac{C_B}{C_p} = D \cdot H \cdot (1 - f_b) + 1 - H \quad (2)$$

where f_b is the fraction bound to plasma protein in the concentration studied. This fraction can be determined by equilibrium dialysis, ultrafiltration or ultracentrifugation. A comparison of erythrocyte binding in the presence (eqn 2) and absence (eqn 1) of plasma protein after equilibrium is achieved will confirm the model if agreement is shown (Derendorf & Garrett 1983).

The aim of the present study was to investigate systematically the binding of a series of cephalosporins to erythrocytes.

Materials and methods

Materials. All chemicals were either USP (US Pharmacopeia), NF (National Formulary) or ACS (American Chemical Society) quality and were used without further purification. Cefotaxime sodium, desacetylcefotaxime, ceftazidime, cephalexin, cefazolin and cefoxitin were used as supplied (Hoechst-Roussel, Somerville, NJ; Glaxo, Greenford; Lilly, Indianapolis). Blood was obtained from human donors.

Chromatographic conditions. The column used was a C_{18}-Mikrobondapak equipped with a guard column. The mobile phase consisted of 0·007 M phosphoric acid in water and acetonitrile in a mixture of 85 : 15 for cefotaxime sodium, desacetylcefotaxime, ceftazidime, and cephalexin, and a mixture of 70 : 30 for cefazolin and cefoxitin. The flow rate was 1·3 mL min^{-1}, sensitivity 0·001 AUFS, and the wavelength 254 nm. The chart speed was 0·3 cm min^{-1} and all assays were performed at room temperature.

Sample preparation. Stock solutions were prepared by dissolving 10 mg of drug in 10 mL of isotonic saline. Standards were prepared for a range of 1 to 250 μg mL^{-1} for all compounds investigated.

Plasma or plasma water (1·0 mL) and acetonitrile (3·0 mL) were mixed and vortexed for 5 s. The mixture was centrifuged for 20 min at 3300 rev min^{-1}. One mL of the resulting supernatant was extracted with 1 mL of a mixture of chloroform and 1-butanol (3 : 1) vortexed for 10 s, centrifuged for 5 min at 3300 rev min^{-1} and then 20 μL of the resulting aqueous supernatant were injected into the HPLC.

Red blood cell partition studies. Human blood was centrifuged for 15 min at 2000 rev min^{-1}. The plasma was removed and modified eutonic Hank's solution (Lewis & Alpar 1984) was added to the erythrocytes. The red blood cells were gently suspended and centrifuged for 10 min at 2000 rev min^{-1}; this washing procedure was repeated three times to remove all plasma protein. Red blood cell suspensions in the isotonic buffer were spiked with the drugs being investigated to yield total drug concentrations of 5 to 50 μg mL^{-1}. The haematocrit was determined routinely using a microcentrifuge with capillary tubes. The pH was controlled before and after addition of the drug. The spiked suspensions were allowed to equilibrate for 30 min and then centrifuged for 10 min at 2000 rev min^{-1}. The 30 min equilibration time was shown to be sufficient; no difference in the results was found when the samples were incubated for longer time (up to 24 h). An aliquot of the supernatant solution was analysed, and the red blood cell partition coefficient was calculated.

To reconfirm the observed red blood cell partition coefficients, the ratio between the drug concentrations in whole blood and plasma was determined. Whole blood (5 mL) was spiked with different amounts of the drugs to give whole blood concentrations between 1 and 100 μg mL^{-1}. The blood was allowed to equilibrate for 30 min and then centrifuged for 10 min at 3000 rev min^{-1}. An aliquot of the supernatant plasma was analysed. The blood-plasma ratio was calculated (eqn 2).

Results and discussion

Chromatographic separation. With a mobile phase of 0·007 M phosphoric acid in water and acetonitrile (85 : 15), cefotaxime and desacetylcefotaxime are well separated and can be assayed simultaneously (Yost & Derendorf 1985). The retention times for a flow rate of 1·3 mL min^{-1} are 8·7 min (cefotaxime), 3·5 min (desacetylcefotaxime), 4·2 min (ceftazidime) and 7·6 min (cephalexin). The retention times for cefazolin and cefoxitin using a different mobile phase (0·007 M phosphoric acid in water–acetonitrile, 70 : 30) were 2·8 and 5·2 min, respectively.

Sample preparation. Sample preparation utilized a deproteinization and sample concentration technique that was developed for the simultaneous determination of cefotaxime and its metabolite desacetylcefotaxime (Yost & Derendorf 1985). After deproteinization with acetonitrile and extraction with a mixture of chloroform–1-butanol (3 : 1), a phase separation was obtained leaving the cephalosporins in the aqueous part and extracting most of the interfering endogenous material. The aqueous phase was injected directly into the HPLC. As part of the plasma water was dissolved in the acetonitrile–1-butanol–chloroform layer, the concentration of the cephalosporin in the aqueous phase was significantly higher than in the original plasma or plasma water sample. Therefore, the usual diluting effect of the deproteinization could be avoided. This sample preparation technique resulted in linear calibration curves for all the cephalosporins being investigated in a concentration range up to 100 μg mL^{-1}.

Erythrocyte partition coefficients. The red blood cell partition coefficients (D) were determined (eqn 1) for 10 different drug concentrations in the therapeutic range. In all cases the RBC-partition coefficient was

Table 1. Erythrocyte partition coefficients and blood-plasma ratios for the investigated cephalosporins

Compound	D[a]	$\frac{C_B}{C_p}$[b]
Cefotaxime	0·11 (0·03)	0·56 (0·03)
Desacetylcefotaxime	0·06 (0·08)	0·59 (0·06)
Ceftazidime	0·09 (0·09)	0·59 (0·07)
Cephalexin	<0·02	0·49 (0·06)
Cefazolin	0·08 (0·04)	0·52 (0·10)
Cefoxitin	0·06 (0·04)	0·53 (0·03)

[a] Mean (s.d.) of the red blood cell partition coefficients over a concentration range of 5–50 μg mL^{-1} (n = 10).

[b] Mean (s.d.) of the blood-plasma ratios over a concentration range of 1–100 μg mL^{-1} (n = 12). Haematocrit H = 0·45.

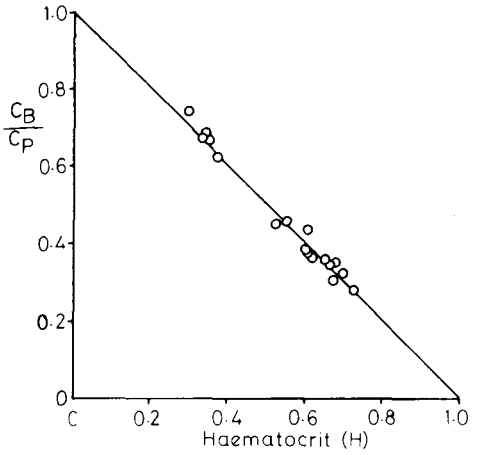

FIG. 1. Ratio of the concentration of cefotaxime in blood over the concentration in plasma (blood–plasma ratio C_B/C_p) as a function of the haematocrit H. Circles indicate experimentally determined blood-plasma ratios for different haematocrits; line represents equation 2.

low. The relatively high variability in the coefficient can be explained by the fact that for negligible D values, equation 1 represents the small difference of two large numbers. No obvious concentration-dependency could be observed. The measured pH in the erythrocyte suspensions ranged from 7·4 to 7·6. The average RBC-partition coefficients are listed in Table 1. Cephalexin binding was so low (<0·02), that no accurate determination was possible.

Blood-plasma ratio. In a second series of experiments blood was spiked with known amounts of the cephalosporins and the resulting plasma concentrations were measured over a concentration range from 1 to 100 μg mL^{-1}. The blood-plasma ratios are given in Table 1. In all cases plasma concentrations were higher than blood concentrations confirming that cephalosporin was excluded from the erythrocytes. For the case of total exclusion (D = 0), the blood-plasma ratio (eqn 2) is solely a function of the haematocrit H and is independent of the degree of plasma protein binding ($C_B/C_p = 1 - H$). The experimentally determined blood–plasma ratios for cefotaxime in blood samples with various haematocrits (Fig. 1) are consistent with equation 2. This was also true for the other cephalosporins.

Drug distribution in a blood sample. In cases of high erythrocyte binding it may be advantageous in pharmacokinetic studies to analyse blood rather than plasma for optimum analytical sensitivity. However, blood analysis would be disadvantageous when non-partitioning erythrocytes 'dilute' the blood concentration from that of the plasma. Thus as in the case of the cephalosporins plasma concentrations exceed blood concentrations. It is obvious and has been shown (Garrett & Lambert 1973) that using blood, plasma or plasma water as the reference body fluid to study the pharmacokinetics will result in different pharmacokinetic parameters.

The present studies show that the cephalosporins investigated do not diffuse into the erythrocytes to any significant degree. Further studies are needed to determine the mechanisms of drug uptake by red blood cells in detail.

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