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DRUG BINDING TO BLOOD CELLS

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INTRODUCTION

The circulating blood volume consists of about 45 percent of blood cells. This fraction may, however, be increased to about 60 percent in newborns, and decreased to as low as 20 percent in anemia, for instance, due to uremia. The major cell body in the blood is the erythrocyte, also called the red blood cell. This cell comprises approximately 95 percent of the total cellular fraction in the blood. Leucocytes and thrombocytes are other cells of interest and comprise the additional 5 percent. In most binding experiments, there is no separation of the different cells of the whole blood, since the erythrocytes are in such excess. Therefore, in the following text, blood cells and red blood cells are used as equivalent expressions.

The major protein of the erythrocyte, hemoglobin, is in excess of albumin in the whole blood; there is about seven to eight times as much hemoglobin as albumin. The diameter of the red blood cell is 75,000Å, and the thickness of the flat, tablet-shaped cell is 18,000Å. The membrane of the cell is considered to be 150-300Å thick. The size of the erythrocyte is much larger than that of albumin—it would require about 500 albumin molecules in a row to cover the diameter of the cell. The molecular weight of the major protein hemoglobin is 64,500, and thus compares well with the molecular weight of albumin.

DISTRIBUTION IN WHOLE BLOOD

Different drugs have different affinities for the blood cells, as has been shown by the degree of binding to the plasma proteins for

TABLE 10.1
Examples of Distribution of Drugs to Blood Cells

Drug	λbc	f(p)	C(bc)/C(pw)	Reference
Diphenylhydantoin	25%	90%	3.6	Ehrnebo and Odar-Cederlöf 1975
Pentazocine	49%	61%	3.1	Ehrnebo et al. 1974
Tetrahydro-cannabinol	9%	97%	30	Widman et al. 1974
Ampicillin	0%	20%	0	Ehrnebo 1978
Acetazolamide	n.i.	>90%	max. 60-80	Wallace and Riegelman 1977
Chlorthalidone	94-99%	75%	>77*	Beermann et al. 1975; Collste et al. 1976
Bumetanide	0%	95%	0	Pentikäinen et al. 1977

Notes:
λbc = fraction of amount in whole blood distributed to blood cells
f(p) fraction bound in plasma
C(bc)/C(pw) = ratio concentration in blood cells to concentration in plasma water
n.i. = not interpreted
*Calculated by the author assuming λbc > 94%, f(p) = 75%, and a hematocrit of 0.45.

various drugs. In fact, there is a competition between plasma proteins and blood cells for the free amount of drug available in the whole blood. Some typical whole blood distribution data are shown in Table 10.1. Diphenylhydantoin is distributed to about 25 percent of the amount in blood to the red blood cells and the ratio between concentration in cells to concentration in plasma water is about 3.6. That not more than 25 percent goes to the blood cells is due to the moderately high protein binding of the drug, about 90 percent. For a drug like pentazocine, more is in the blood cells, since the protein binding is lower. The very lipophilic substance tetrahydrocannabinol

has a high degree of incorporation into the blood cells. The ratio cell/water is about 30. Nevertheless, the distribution to the blood cells of the whole blood is only 9 percent due to the high degree of plasma protein binding of this substance. Some non-lipophilic drugs are not distributed at all to the erythrocytes, as exemplified in the table by the antibiotic ampicillin. Two diuretic drugs, acetazolamide and chlorthalidone, have among the highest affinities to the blood cells that have been published so far. On the contrary, another diuretic, bumetanide, does not distribute at all to the erythrocytes (Table 10.1). It should be pointed out that for a non-electrolyte, a concentration ratio cell/water significantly greater than 1 would indicate not only a distribution in the aqueous phase of the red cell, if the drug penetrates the cell wall, but also that some binding occurs to the cell. For an anionic drug, the discriminating ratio is about 0.7-0.8; for a cationic drug about 1.1-1.2, due to the Donnan equilibrium across the red cell membrane. Merely binding of drug to the membrane of the red cell, without diffusion into the cell, may of course complicate these general statements.

BINDING COMPONENTS IN THE RED BLOOD CELL

There are three major components in the erythrocyte capable of binding drugs—hemoglobin, carbonic anhydrase, and the cell membrane. For example, binding of phenothiazines, pentobarbital, and diphenylhydantoin to hemoglobin has been demonstrated (Wind, Berliner, and Stern 1973; Ehrnebo 1980). Binding of acetazolamide and chlorthalidone to carbonic anhydrase (Beermann et al. 1975; Wallace and Riegelman 1977) has also been demonstrated, and binding of chlorpromazine and imipramine to the membrane (Kwant and Seeman 1969; Bickel 1975) has been demonstrated.

RATE OF EQUILIBRATION

Generally, the passage of lipophilic substances into the red blood cell is very rapid, but for hydrophilic substances, the time for equilibration may be prolonged (Schanker, Johnson, and Jeffrey 1964). For diphenylhydantoin, the equilibration takes place in less than 5 minutes (Ehrnebo and Odar-Cederlöf 1977), but for the more hydrophilic drug chlorthalidone, it takes about 90 minutes before a steady state is achieved in vitro (Beermann et al. 1975). For diphenylhydantoin, the distribution to the red cells is completely reversible, that is, if the plasma water concentration of drug is lowered, a rapid redistribution occurs (Ehrnebo and Odar-Cederlöf 1977).

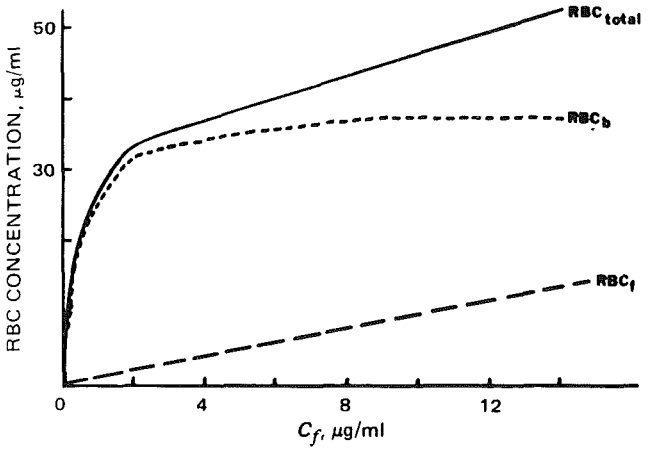

FIGURE 10-1
Total (RBC total, obtained experimentally), bound (RBCb), and free (RBCf) erythrocyte concentrations of acetazolamide as a function of the free drug concentration in plasma.
Source: Wallace and Riegelman (1977).

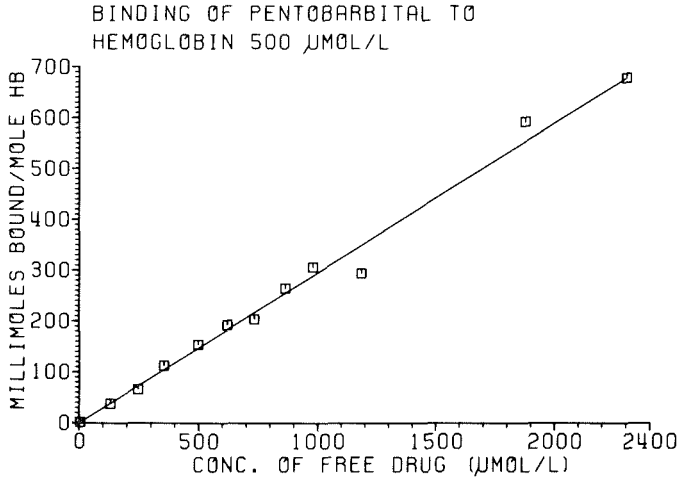

FIGURE 10-2
Binding of pentobarbital to human hemoglobin (500 µMol/L). Y-axis: millimoles of bound drug per mole hemoglobin. X-axis: concentration of free drug in µMol/L.
Source: Ehrnebo (to be published).

DEPENDENCE OF CONCENTRATION

The distribution of pentobarbital to the erythrocytes, expressed as the cell/water distribution ratio, is not dependent on the concentration of pentobarbital up to 60 μg/ml of whole blood concentration. Diphenylhydantoin is also constantly distributed to the erythrocytes within the therapeutic range (Ehrnebo and Odar-Cederlöf 1977). On the other hand, acetazolamide binding to the red blood cells is saturable as concentration of the drug increases (Fig. 10-1). The non-saturable distribution of pentobarbital to the erythrocytes in in accordance with the linear binding of the drug to human hemoglobin (Fig. 10-2). Acetazolamide, on the contrary, is considered to have saturable binding sites at the human carbonic anhydrase in the red blood cells (Wallace and Riegelman 1977). Recently, saturable binding of clofibrate to the human blood cells has been reported (Altmayer and Garrett 1983).

EFFECT OF TEMPERATURE ON BINDING

Several authors have shown that the binding of drugs to plasma proteins generally increases at lower temperatures. This is the case for diphenylhydantoin, where the fraction unbound in plasma decreased by about 38 percent when the temperature was lowered from 37° to 25°C. On the other hand, the distribution and binding to the blood cells were unchanged by temperature. When a whole blood sample is allowed to reach room temperature, the plasma concentration of diphenylhydantoin will increase about 10 percent as an effect of this in vitro redistribution from the blood cells (Ehrnebo and Odar-Cederlöf 1977). A possible influence of this kind should therefore be taken into account in drug monitoring studies.

DISPLACEMENT OF DRUG

It is well known that under certain circumstances an acidic compound like salicylic acid may displace acidic drugs from plasma protein binding sites. For example, both pentobarbital and diphenylhydantoin are displaced by salicylic acid, and free fractions of these drugs in plasma are increased. When the influence of salicylic acid on the cell to water distribution ratio of diphenylhydantoin and pentobarbital was studied, no effect was seen at all (Ehrnebo and Odar-Cederlöf 1977). Thus, in the whole blood sample in vitro, drug bound to plasma proteins will tend to accumulate in the plasma water and blood cells in the presence of salicylic acid.

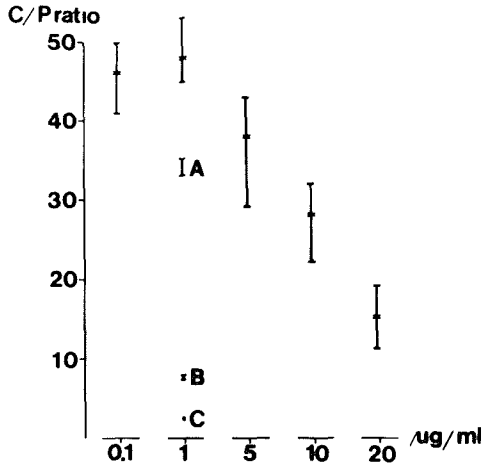

FIGURE 10-3
Blood cell to plasma concentration ratio (C/P ratio; mean, range) of radioactive material recovered in blood specimens incubated for 2 hr with various concentrations of (14)C-chlorthalidone (x-axis; in µg/ml). In some experiments, (14)C-chlorthalidone (1 µg/ml) was incubated in blood preincubated for 30 min with acetazolamide in a concentration of 4 µg/ml (A), 40 µg/ml (B), and 80 µg/ml (C).
Source: Beermann et al. (1975).

An interesting interaction phenomenon in the red blood cell has been published by Beermann et al. (1975). They found that acetazolamide drastically decreased the cell to plasma concentration ratio of chlorthalidone in a concentration dependent way (Fig. 10-3). It was suggested that the two drugs competed for the same binding sites at the carbonic anhydrase of the erythrocyte. In vivo, a decreased elimination half-life of chlorthalidone was seen in humans when acetazolamide was co-administered. It is, however, uncertain if this shortening of half-life in fact depended on the interaction in the red blood cell, since acetazolamide also causes an alkalinization of the urine. The renal clearance of the weak acid chlorthalidone may well have been increased by this change in urinary pH. Further studies are needed to investigate this and similar interactions of drugs bound to the carbonic anhydrase of the red blood cell.

INTERINDIVIDUAL DIFFERENCES

Several reports have shown that acidic drugs have decreased binding to plasma proteins from patients with uremia as compared to

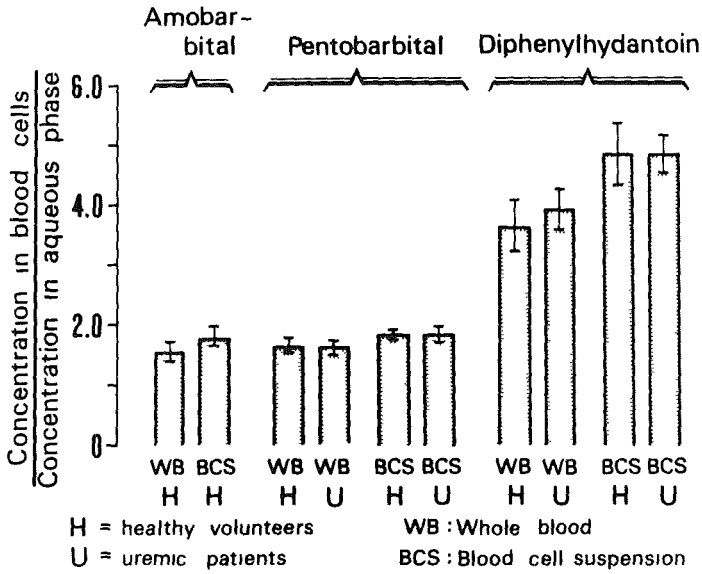

FIGURE 10-4
Ratio between concentrations in blood cells and plasma water in whole blood, or concentration in buffer phase of blood cell suspension. Mean values and S.D. of healthy volunteers (n = 10-17) and uremic patients (n = 6-8).
Source: Ehrnebo and Odar Cederlöf (1975).

normal healthy subjects. There are no significant differences, however, in the affinity of the blood cells between uremic and healthy subjects, since the cell to water distribution ratio is essentially the same between the subject groups (Fig. 10-4).

There are large differences between newborn infants, mothers, and control subjects in the unbound fraction in plasma of the acidic drugs cloxacillin and flucloxacillin, and of the basic drug alprenolol (Herngren, Ehrnebo, and Borgus 1982; 1983). However, no significant differences in the cell to water distribution ratio for cloxacillin or flucloxacillin could be seen when the different subject groups were compared.

In the elderly, there seem to be some conflicting reports in the literature. Chan et al. (1975) reported that the red cell binding of pethidine was greater in young than in old patients. However, Holmberg et al. (1982) were unable to confirm these findings. The latter reported that the blood cell to plasma water concentration ratio was on average 2.0 for pethidine in both subject groups. No significant age related difference in the plasma protein binding of the

DISTRIBUTION IN THE BODY

The binding of drugs to erythrocytes will only be important for the total body distribution, if the binding to the cells is extremely strong and/or the distribution volume of the drug is low. We must also keep in mind that in the circulating blood, the erythrocytes are an isolated pharmacokinetic compartment. Equilibration to various organs does not take place against free concentrations in whole blood, but rather to free concentrations in plasma. Nevertheless, it is possible that the red cell may serve as an interesting model cell in the pharmacokinetic analysis of a drug. For example, the antibiotic ampicillin has a limited intracellular distribution in the human body based on distribution volume estimates of unbound drug (Ehrnebo, Nilsson, and Boréus 1979). In agreement with this, practically no ampicillin enters or binds to the red blood cells (Table 10.1). Another antibiotic, flucloxacillin, differs from ampicillin, and unbound drug distribution volume suggests an in vivo intracellular distribution and substantial binding of flucloxacillin to extravascular tissues. In vitro experiments on human red blood cells with flucloxacillin demonstrates also substantial distribution and/or binding to the cell, in accordance with the in vivo data (Anderson et al. 1985). Future studies may show whether similar agreements can be seen between body distribution and red cell distribution for drugs within the same chemical class.

CONCLUSIONS

So far, no significant differences between normal subjects and other subject groups in the cell to water drug concentration ratio seem to have been reported for the erythrocyte. Clinical implications are few; however, the diuretics chlorthalidone and acetazolamide with high affinity to the carbonic anhydrase are important exceptions. Nevertheless, future knowledge may bring other important drugs with similar affinity to our attention. Interesting information on the distribution properties of a drug may be collected from blood cell binding experiments, especially when comparison is made with in vivo free distribution volumes. Thus, the drug-erythrocyte interaction is worthwhile to study in the complete pharmacokinetic analysis of a drug substance.

REFERENCES

Altmayer, P., and Garrett, E. R. (1983) "Plasmolysis, red blood cell partitioning, and plasma protein binding of etofibrate, clofibrate, and their degradation products," J. Pharm. Sci. 72: 1309-1318.

Anderson, P., Bluhm, G., Ehrnebo, M., Herngren, L., and Jacobson, B. (1985) "Pharmacokinetics and distribution of flucloxacillin in pacemaker patients," Eur. J. Clin. Pharmacol., 27: 713-719.

Beermann, B., Hellström, K., Lindström, B., and Rosén, A. (1975) "Binding-site interaction of chlorthalidone and acetazolamide, two drugs transported by red blood cells," Clin. Pharmacol. Therap. 17: 424-432.

Bickel, M. H. (1975) "Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and other blood components," J. Pharm. Pharmacol. 27: 733-738.

Chan, K., Kendall, M. J., Mitchard, M., Wells, W. D. E., and Vickers, M. D. (1975) "The effect of ageing on plasma pethidine concentration," Br. J. Clin. Pharmacol. 2: 297-302.

Collste, P., Garle, M., Rawlins, M. D., and Sjöqvist, F. (1976) "Interindividual differences in chlorthalidone concentrations in plasma and red cells of man after single and multiple doses," Eur. J. Clin. Pharmacol. 9: 319-325.

Ehrnebo, M. (1978) "Distribution of ampicillin in human whole blood," J. Pharm. Pharmacol. 30: 730-731.

Ehrnebo, M. (1980) "Influence of drug binding to blood cells on pharmacokinetics," Acta Pharm. Suec. 17: 81-82.

Ehrnebo, M., Agurell, S., Boréus, L. O., Gordon, E., and Lönroth, U. (1974) "Pentazocine binding to blood cells and plasma proteins," Clin. Pharmacol. Ther. 16: 424-429.

Ehrnebo, M., Nilsson, S.-O., and Boréus, L. O. (1979) "Pharmacokinetics of ampicillin and its prodrugs bacampicillin and pivampicillin in man," J. Pharmac. Biopharm. 7: 429-451.

Ehrnebo, M., and Odar-Cederlöf, I. (1975) "Binding of amobarbital, pentobarbital and diphenylhydantoin to blood cells and plasma proteins in healthy volunteers and uraemic patients," Eur. J. Clin. Pharmacol. 8: 445-453.

Ehrnebo, M., and Odar-Cederlöf, I. (1977) "Distribution of pentobarbital and diphenylhydantoin between plasma and cells in blood: Effect of salicylic acid, temperature and total drug concentration," Eur. J. Clin. Pharmacol. 11: 37-42.

Herngren, L., Ehrnebo, M., and Boréus, L. O. (1982) "Drug distribution in whole blood of mothers and their newborn infants. Studies of cloxacillin and flucloxacillin," Eur. J. Clin. Pharmacol. 22: 351-358.

Herngren, L., Ehrnebo, M., and Boréus, L. O. (1983) "Drug binding to plasma proteins during human pregnancy and in the perinatal period. Studies on cloxacillin and alprenolol," Dev. Pharmacol. Ther. 6: 110-124.

Holmberg, L., Odar-Cederlöf, I., Nilsson, J. L. G., Ehrnebo, M., and Boréus, L. O. (1982) "Pethidine binding to blood cells and plasma proteins in old and young subjects," Eur. J. Clin. Pharmacol. 23: 457-461.

Kwant, W. O., and Seeman, P. (1969) "The membrane concentration of a local anesthetic (chlorpromazine)," Biochim. Biophys. Acta 183: 530-543.

Pentikäinen, P. J., Penttilä, A., Neuvonen, P. J., and Gothoni, G. (1977) "Fate of (14)C-bumetanide in man," Br. J. Clin. Pharmacol. 4: 39-44.

Schanker, L. S., Johnson, J. M., and Jeffrey, J. J. (1964) "Rapid passage of organic anions into human red cells," Amer. J. Physiol. 207: 503-508.

Wallace, S. M., and Riegelman, S. (1977) "Uptake of acetazolamide by human erythrocytes," J. Pharm. Sci. 66: 729-731.

Widman, M., Agurell, S., Ehrnebo, M., and Jones, G. (1974) "Binding of (+)- and (-)-delta-1-tetrahydrocannabinols and (-)-7-hydroxy-delta-1-tetrahydrocannabinol to blood cells and plasma proteins in man," J. Pharm. Pharmacol. 26: 914-916.

Wind, M., Berliner, A., and Stern, A. (1973) "The binding of phenothiazines to oxyhemoglobin A and S," Res. Commun. Chem. Pathol. Pharmacol. 5: 759-766.