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In Vitro Characterization of Chlorpromazine Hydrochloride Uptake by Human Erythrocytes: Effect of Concentration and Hematocrit

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Introduction

Many factors influence the kinetic events of compounds within the body including binding to blood and tissue components, enzymatic and cellular activity and membrane permeability[1]. One process, erythrocyte permeability, is generally not investigated as often compounds permeate this membrane so readily that it does not rate limit disposition of compounds. However, time to reach partitioning equilibrium between erythrocytes and plasma or plasma water ranges between a few seconds to several hours for different drugs[2]. Therefore, erythrocytes play an important role in the transport and disposition kinetics of compounds that accumulate in erythrocytes to an appreciable extent[2-4].

The primary binding sites of drugs in the erythrocytes are associated with haemoglobin (e.g. digoxin and derivatives, sulfonamides, mefloquine, phenytoin, phenothiazines, salicylic acid and congeners, imipramine and derivatives), plasma membranes (e.g. codeine, chlorpromazine, imipramine) or proteins[2,5]. Acetazolamide, methazolamide, chlorthalidone and dorzolamide are bound extensively to carbonic anhydrase which is present in cytosol of erythrocytes[6-11]. Cyclosporin A and tacrolimus are

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strongly bound to the cytosolic proteins in the erythrocytes[12,13]. It is commonly assumed that only unbound drug molecules in the plasma water of blood can leave the capillary bed in the liver and kidney for elimination. Therefore, the drugs that are bound to or partitioned into the erythrocytes are not immediately available for elimination as they cannot leave the capillary bed of eliminating organs. One implication of the red blood cell (RBC) distribution kinetics concerns the influence of route of administration on hepatic extraction of compounds. Because of the short transit time between the absorption site and liver[14] (1-2 s) orally absorbed compounds may have insufficient time to equilibrate within the blood before reaching the hepatic sinusoidal bed. In contrast, most drug molecules delivered into the liver after intravenous administration should have more time for equilibration than occurs after oral administration. Therefore, the degree of first-pass hepatic extraction of drugs, especially those with a high affinity for erythrocyte components, may be greater for intestinally absorbed than systemically circulating drug[15,16].

Chlorpromazine (as chlorpromazine hydrochloride, CPZ) is classified as a low-potency typical antipsychotic drug and in the past, it was used in the treatment of both acute and chronic psychoses including schizophrenia and the manic phase of bipolar disorder as well as amphetamine-induced psychoses. Due to side effects, the use of chlorpromazine has been largely replaced by newer generation of atypical antipsychotics which are generally better tolerated. Chlorpromazine has also been used in porphyria, short term management of severe anxiety and aggressive episodes, severe hiccups, severe nausea/emesis[17,18]. It has previously been shown that CPZ has an affinity for blood components including erythrocyes[19,20]. CPZ was reported to interact with erythrocyte membranes, membrane lipids and hemoglobin[20,21]. Although the mechanism of interaction is not known, it was reported that CPZ distributes asymmetrically between the inner and outer layers of the membrane[22]. At physiological pH, it is bound to the inner face of the membrane[23,24]. At low concentration (<1.0 mM), CPZ protects erythrocytes against hypotonic lysis[25], whereas at higher doses (>0.5-1.0 mM), CPZ causes loss of erythrocyte membrane integrity and cell lysis[24,26]. It was also reported that CPZ forms little pores on the membrane allowing transport of micro solids like Na^+ but not hemoglobin or the other ions[27]. Therefore, in the present study, CPZ was chosen as the model compound to investigate the effect of concentration and hematocrit on erythrocyte uptake.

Materials and Methods

Materials

Chlorpromazine hydrochloride (CPZ) was a generous gift from Eczacıbaşı (Turkey). All other chemicals were of analytical grade and used as received. The human erythrocyte suspension and whole blood were obtained from the Blood Bank of Hacettepe University Hospital (Ankara).

Methods

CPZ Uptake by Erythrocytes

Human erythrocyte suspension obtained from the Blood Bank was centrifuged (NF 615 Nuve) at 4000 rpm for 10 minutes. The supernatant and the Buffy coat were discarded, and then packed erythrocytes were washed three times with phosphate buffer saline (PBS, pH 7.4)[28]. After the final washing, the supernatant was removed and the erythrocytes were diluted to the required hematocrit (20, 30, 40%). However, the colour intensity of these suspensions was too high for reliable measurement of CPZ, these suspensions were further diluted with PBS (1/500 v:v). The number of erythrocytes in each suspension was determined using Coulter Counter (Coulter Max M, UK) and hematocrit value of the suspension was expressed as cell/mL. Also, after dilution with PBS (1/500), whole blood was used to investigate CPZ uptake by erythrocytes.

Whole blood or erythrocyte suspensions (4.06 - 7.76x10^6 cell/mL) were incubated with CPZ (5, 7.5, 10, 15 mg/mL) at 37°C, and concentration decrease in buffer was determined by measuring CPZ absorbance (254 nm) automatically at 2.5 min-intervals for 30 min, using a Shimadzu UV-160 A model spectrophotometer (Japan)[29,30].

CPZ Interaction with Erythrocyte membrane

After dilution (1/500 v:v) of erythrocyte suspension (20, 40%) with phosphate buffer, the suspension was centrifuged at 4000 rpm for 10 min and buffer phase was removed. Erythrocyte membranes were prepared by hemolysing packed erythrocytes in distilled water (5 mL) and centrifuging at 4000 rpm for 15 min. Post hemolytic residue was washed three times with PBS (pH 7.4), and then erythrocyte membrane was diluted with PBS.

A suspension of erythrocyte membranes (3 mL) was incubated with CPZ (5, 15 mg/mL) at 37 °C, and concentration decrease in buffer was determined by measuring CPZ absorbance (254 nm) automatically at 2.5 min-intervals for 30 min, using a Shimadzu UV-160 A model spectrophotometer (Japan).

CPZ Interaction with Hemolysate

After dilution (1/500 v:v) of erythrocytes suspension (20, 40%) with phosphate buffer, the suspension was centrifuged at 4000 rpm for 10 min and the buffer phase was removed. Erythrocytes were hemolysed in distilled water and then centrifuged at 4000 rpm for 15 min. The upper hemolysate layer was removed and used to investigate the possible interaction between CPZ and hemolysate.

Two different hemolysate (3 mL), obtained from the hemolysis of erythrocyte suspensions with hematocrit values of 20 and 40%, were incubated with CPZ (5, 15 mg/mL) and concentration decrease in buffer was determined by measuring CPZ absorbance (254 nm) automatically at 2.5 min-intervals for 30 min, using a Shimadzu UV-160 A model spectrophotometer (Japan).

Sample Analysis

Concentration of CPZ was determined by means of a spectrophotometric method. The calibration curve was constructed in PBS (pH 7.4), over the concentration range of 0 to 15 μg/mL. Calibration equation and corresponding determination coefficient (r^2) were obtained by least-square linear regression analysis. The method was validated as to linearity, specificity, precision (repeatability and reproducibility), and stability.

Data Analysis

Mean time to reach equilibrium (MET) and the area under the curve (AUC) values were estimated from the buffer concentration-time profiles using the following equations[28],

$$AUC = \int_0^{30} C(t)\,\mathrm{dt} \qquad (1)$$

$$MET = \frac{\int_0^{30} tC\,(t)dt}{AUC} \quad (2)$$

Erythrocyte-associated CPZ (f_{RBC}) was calculated indirectly from the hematocrit (H) and concentrations in blood (C_B) and buffer (C_P)[31].

$$f_{RBC} = 1\text{-}\,[Cp\,(1\text{-}H)\,/C_B] \quad (3)$$

Permeation coefficient (PC; cm/s) of CPZ across erythrocyte membrane was estimated from Equation 4.

$$PC = \int \frac{\Delta C/\Delta t \cdot V_{RBC}}{A_{RBC} \cdot C_o \cdot 60} \quad (4)$$

where $\Delta C/\Delta t$ is the slope of erythrocyte concentration- time profiles; V_{RBC} is the volume of erythrocytes (cm^3); A_{RBC} is surface area of erythrocytes (cm^2), and Co initial drug concentration (μg/mL).

All tabulated results were expressed as mean ± SE. The results were compared by means of Kruskall-Wallis and Mann-Whitney U tests. A p value less than 0.05 was considered significant.

Results and Discussion

CPZ was successfully determined using the spectrophotometric method and there were no interfering peaks at 254 nm, from phosphate buffer, blood and blood components. The method was linear in the concentration range of 0 to 15 μg/mL (r^2= 0.999). The coefficient of variation values estimated from repeatability and reproducibility studies were within the acceptable range (<2%), indicating that the precision of the method was satisfactory. CPZ was found to be stable during the time course of experiment.

Preliminary incubation studies indicated that distribution equilibrium between erythrocytes and buffer (or plasma) was achieved within 10 min. Therefore, 30 min incubation period was selected for all experiments. Mean time to reach equilibrium was estimated from buffer concentration-time profiles. Regardless of the conditions used, the equilibrium was achieved within 15 minutes. This observation was in agreement with the literature[32].

Buffer concentration-time profiles obtained from incubation of CPZ (5, 7.5, 10, 15 μ/mL) with erythrocyte suspensions were given in Figure 1. Area under the curve (AUC) values estimated from these profiles by means of linear trapezoidal method, and results were summarized in Table 1. For all conditions, AUC values increased significantly with an increase in CPZ dose ($p<0.05$). There was a linear correlation between the CPZ concentrations and corresponding AUC values ($r^2 > 0.997$), indicating that CPZ uptake by erythrocytes was linear within the concentration range used in this study. For a given concentration, AUC value determined from the incubation with whole blood suspension was slightly lower than those obtained with the erythrocyte suspension, however, the difference was not significant. In the case of erythrocyte suspension, although, the AUC values decreased slightly with an increase in the hematocrit value for all CPZ dose studied, there was no significant difference between them. On the other hand, almost identical AUC values were obtained from the studies undertaken with erythrocyte membrane and hemolysate (Table I).

Influence of hematoctit and dose on uptake of CPZ by erythrocytes was given in Figure 2. When washed erythrocyte suspension was used, the fraction of CPZ uptaken by erythrocytes was influenced by both CPZ dose and hematocrit value of suspension. For a given dose, CPZ uptake by erythrocytes was increased with an increase in hematocrit value (e.g. for 5 μg/mL CPZ, 11 and 24% for the hematocrit values of $4.06x10^6$ and $7.76x10^6$ cell/mL, respectively). For a given hematocrit value, erythrocyte uptake was decreased with an increase in CPZ dose (e.g. for hematocrit value of $4.06x10^6$ cell/mL, 11 and 6 % for 5 and 15 μg/mL, respectively). This observation could be due to 3-fold increase in CPZ dose compared to 2-fold increase in cells numbers in the suspension. On the other hand, when whole blood suspension was used, uptake was higher (about 34%) than those obtained with the erythrocyte suspensions, and was

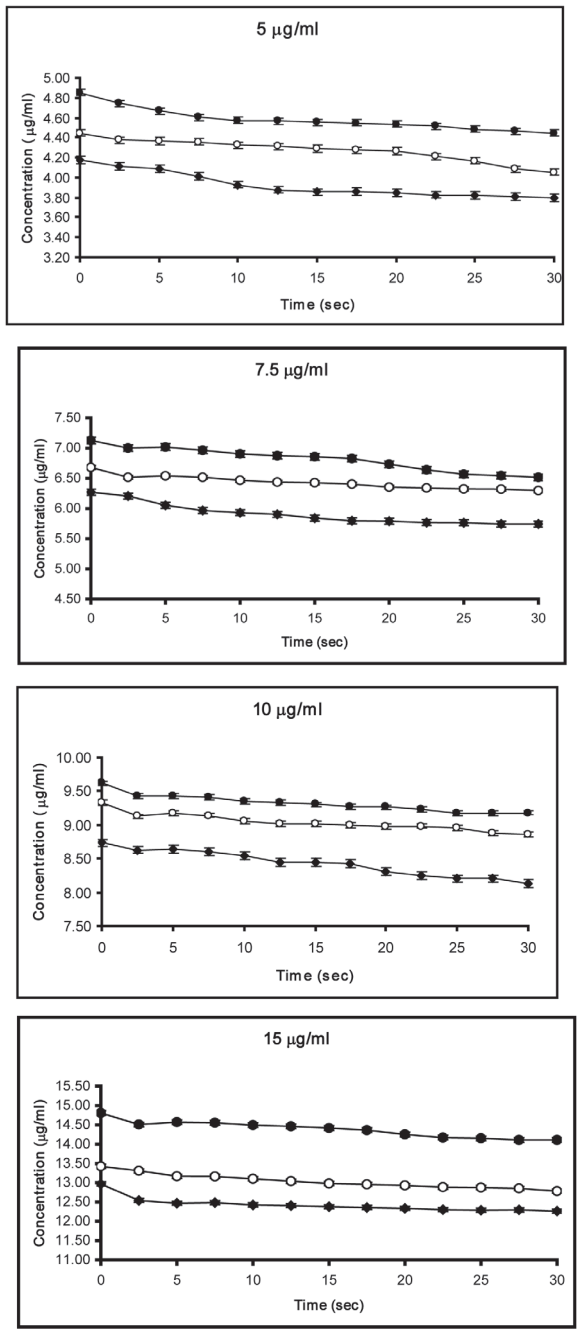

Figure 1

Buffer concentration time profiles of chlorpromazine hydrochloride (5, 7.5, 10, 15 mg/mL), obtained from erythrocyte suspensions with hematocrit values of 4.06×10^6 cell/ml (•), 5.74×10^6 cell/ml (♦) and 7.76×10^6 cell/ml (¨) (mean ± S.E, n=4).

TABLE I

Area under the curve (AUC) values estimated from buffer concentration time profiles (mean ±SE; n= 4).

Concentration (mg/mL)	AUC (μg.min/mL)							
	Whole Blood	Erythrocyte Suspension			Erythrocyte Membrane		Hemolysate	
		4.06 x 10^6 [a]	5.74 x 10^6	7.76 x 10^6	H_{20} [b]	H_{40} [b]	H_{20} [b]	H_{40} [b]
5	112.8 ± 4.60	137.3± 3.48	128.2 ± 8.10	117.6 ± 2.29	147. 3 ± 0.53	146.3 ± 1.30	146.0 ± 0.51	146.9 ± 0.16
7.5	157.9 ± 3.85	204.4 ± 5.17	192.8 ± 2.76	176.9 ± 4.31	-	-	-	-
10	214.1 ± 3.43	279.4 ± 2.51	271.0 ± 5.31	252.8 ± 4.58	-	-	-	-
15	322.8 ± 1.42	431.3± 2.72	391.1 ± 3.75	372.3 ± 7.65	446. 4 ± 0.50	443.5 ± 1.40	446. 5 ± 0.24	446.1 ± 0.25

[a]Hematocrit values (cell/mL)

[b]H_{20} and H_{40} hematocrit values of the erythrocyte suspension used for the preparation of erythrocyte membrane and hemolysate

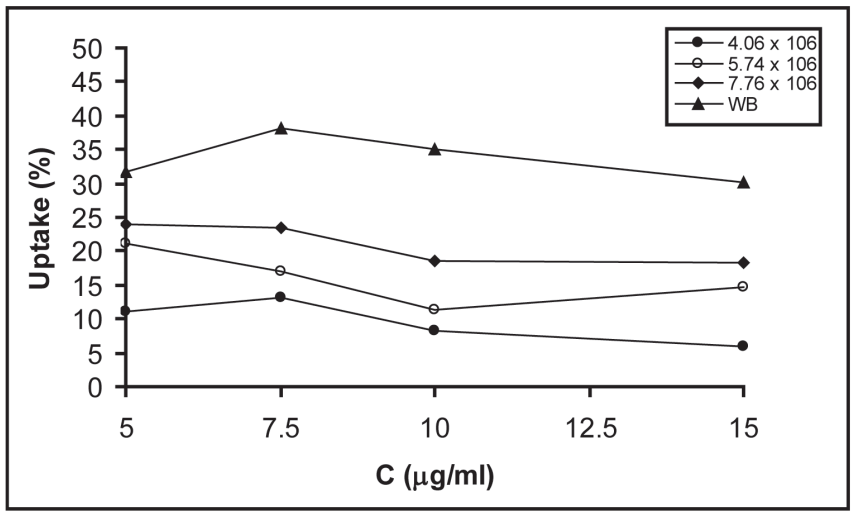

Figure 2
Uptake (%) of chlorpromazine hydrochloride (5, 7.5, 10, 15 mg/ml) by erythrocytes [hematocrit 4.06x10^6 cell/ml (•), 5.74 x10^6 cell/ml (o) and 7.76 x10^6 cell/ml (♦)] and whole blood [WB (▲)].

not influenced by CPZ dose. Higher uptake with whole blood suspension could be attributed to possible interaction of CPZ with other blood components such as plasma proteins and other cells present in blood. When erythrocyte membrane was used, for a given concentration, interaction between CPZ and membrane was less than erythrocytes suspensions (e.g. for 5 μg/mL CPZ and hematocrit value of 4.06x10^6 cell/mL, 11% for erythrocyte suspension and 4% for erythrocyte membrane), and was very similar for different hematocrit values (e.g. for 15 μg/mL CPZ and hematocrit values of 4.06x10^6 and 7.76x10^6 cell/mL, about 2%). Similar observation was made with hemolysate (e.g. for 5 μg/mL CPZ and hematocrit value of 4.06x10^6 cell/mL, 11% for erythrocyte suspension and 4% for erythrocyte membrane; for 15 μg/mL CPZ and hematocrit values of 4.06x10^6 and 7.76x10^6 cell/mL, about 1%).

The permeability coefficients of CPZ across erythrocyte membranes were summarized in Table II. When washed erythrocytes were used, for a given concentration, the permeability values were decreased with an increase in hematocrit value. For a given hematocrit value, permeability coefficient decreased with an increase in CPZ dose. However, the differences in permeability values obtained as a function of dose and hematocrit values were not significant ($p>0.05$). Similarly, when whole blood suspension

TABLE II

Permeability coefficients of chlorpromazine hydrochloride across erythrocyte membrane (mean ±SE; n= 4).

Concentration (mg/mL)	Permeability Coefficient (x10^{-6}; cm/s)					
	Whole Blood	Washed Erythrocytes			Erythrocyte membrane	
		4.06 x 10^{6} [a]	5.74 x 10^{6}	7.76 x 10^{6}	H_{20} [b]	H_{40} [b]
5	1.03 ± 0.74	5.50 ± 1.53	4.08 ± 1.52	3.21 ± 1.52	2.13 ± 0.45	1.10 ± 0.23
7.5	1.36 ± 0.30	6.73 ± 0.76	2.09 ± 0.69	2.92 ± 1.00	-	-
10	1.20 ± 0.11	3.15 ± 0.41	2.17 ± 0.76	2.61 ± 1.06	-	-
15	0.48 ± 0.17	3.56± 0.38	2.50 ± 0.74	1.34 ± 0.35	1.13 ± 0.12	0.59 ± 0.06

[a]Hematocrit values (cell/mL)

[b]H_{20} and H_{40} hematocrit values of the erythrocyte suspension used for the preparation of erythrocyte membrane and hemolysate

was used, there was no significant difference in permeability values estimated for different CPZ dose ($p>0.05$). On the other hand, when erythrocyte membrane suspension was used, permeability values were found to be significantly influenced both by hematocrit and CPZ dose ($p<0.05$).

The results of this study suggest that CPZ interacts with erythrocytes irrespective of the conditions used. The site for interaction was reported to be hemoglobin[25,33] and erythrocyte membrane[27,34]. Recognizing that only unbound drug molecules are capable of passing through the membranes, such an interaction limits not only the distribution but also elimination CPZ within the body. This aspect can be further investigated in the absence and presence of erythrocytes using *in situ* perfused liver preparation.

Summary

Although erythrocytes are not expected to constitute any barrier effect for rapidly penetrating substances, they may have profound effects on the distribution and elimination of compounds that slowly equilibrates and/or extensively partition into erythrocytes. Chlorpromazine hydrochloride (CPZ) was chosen as a model compound to investigate the effect of concentration and hematocrit on erythrocyte uptake. Suspensions of erythrocyte with hematocrit values of $4.06\text{-}7.76\text{x}10^{6}$ cell/mL and whole blood were incubated with CPZ solutions (5, 7.5, 10, 15 μg/mL). Suspensions of erythrocyte membranes and hemolysates were also incubated with CPZ (5, 15 μg/mL). Absorbance of CPZ in buffer phase was automatically measured at 2.5 min. intervals for 30 min. Mean time for equilibration and the area under curve values (AUC) were determined from buffer concentration time profiles, whereas degree of drug uptake and permeation coefficient across erythrocyte membranes were estimated from the erythrocyte concentration time profiles. For all conditions, equilibrium between buffer and erythrocytes was achieved within 15 min, and there was a linear correlation between the AUC values and CPZ dose. When erythrocyte suspension was used, uptake was found to be influenced by both concentration and hematocrit. On the other hand, CPZ uptake (about 34%) was not influenced by drug concentration when whole blood suspension was used. In addition, in all conditions, the permeation coefficients for CPZ was found to be in the range of $0.34\text{-}6.75\text{x}10^{-6}$ cm/s.

Keywords: Chlorpromazine Hydrochloride, Erythrocytes, Hematocrit, Permeation Coefficient

Özet

Klorpromazin Hidroklorürün İnsan Eritrositleri Tarafından Tutulumunun *in Vitro* Karakterizasyonu: Konsantrasyon ve Hematokritin Etkisi

Eritrositlerin hızla penetre olan maddeler için herhangi bir bariyer etkisi oluşturması beklenmese de eritrositlerle yavaş dengelenen ve/veya aşırı partisyon gösteren bileşiklerin dağılımı ve eliminasyonu üzerinde önemli etkileri olabilir. Klorpromazin hidroklorür (CPZ), eritrosit tutulumu üzerine konsantrasyon ve hematokritin etkisini incelemek amacıyla model ilaç olarak seçilmiştir. Hematokrit değerleri 4.06-7.76x10^6 hücre/mL olan eritrosit süspansiyonu ve tam kan değişik CPZ konsantrasyonları (5, 7.5, 10, 15 μg/mL) ile inkübe edilmiştir. Eritrosit membranı süspansiyonu ve hemolizat da CPZ (5, 15 μg/mL) ile inkübe edilmiştir. Tampon fazındaki CPZ'nin absorbansı 30 dakika süreyle 2.5 dakika aralıklarla otomatik olarak ölçülmüştür. Ortalama dengeye ulaşma zamanı ve eğri altında kalan alan (AUC) tampon konsantrasyon zaman profillerinden tayin edilirken ilaç tutulum derecesi ve eritrosit membranından permeasyon katsayısı eritrosit konsantrasyon zaman profillerinden hesaplanmıştır. Bütün şartlarda tampon ve eritrositler arasındaki dengeye 15 dakika içinde erişilmiş ve AUC ile CPZ dozu arasında lineer bir korelasyon bulunmuştur. Eritrosit süspansiyonu kullanıldığında tutulum hem konsantrasyon hem de hematokritten etkilenmiştir. Buna karşılık tam kan süspansiyonu kullanıldığında CPZ tutulumu (%34 civarı) ilaç konsantrasyonundan etkilenmemektedir. Ek olarak bütün koşullarda CPZ için permeasyon katsayısı 0.34-6.75x10^{-6} cm/s sınırları arasındadır.

Anahtar kelimeler: Klorpromazin Hidroklorür, Eritrosit, Hematokrit, Permeasyon Katsayısı.

REFERENCES

1. Rowland, M., Evans, A.M. (1991). Physiological models of hepatic elimination. In: (Rescigno, A., Thakur, A.K. (Eds.), New Trends in Pharmacokinetics. Plenum Press, New York, pp. 83-102.
2. Hinderling P.H. (1997). Red blood cells: a neglected compartment in pharmacokinetics and pharmacodynamics. Pharmacol. Rev., 49(3), 279-295.
3. Highley M.S., DeBruijin E.A. (1996). Erythrocytes and the transport of endogenous compounds. Pharm. Res., 13,186-195.
4. AbuTarif M.A., Taft D.R. (2002). Simulation of the pharmacokinetic profile of methazolamide in blood: Effect of erythrocyte carbonic anhydrase binding on drug disposition. Pharm. Res., 19(4), 551-555.
5. Vaidyanathan S., Boroujerdi M. (2000). Interaction dexrazoxane with red blood cells and hemoglobin alters pharmacokinetics of doxorubicin. Cancer Chemother. Pharmacol., 46, 93-100.
6. Colleste P., Garle M., Rawlins M.D., Sjoeqvist F. (1976). Interindividual difference is chlorthalidone concentration in plasma and red cells of man after single and multiple doses. Eur.J. Clin. Pharmacol., 9, 319-325.
7. Fleuren H.L.J., Van Rossum J.M. (1977). Nonlinear relationship between plasma and red blood cells pharmacokinetics of chlorthalidone in man. J. Pharmacokinet. Biopharm., 5, 359-375.
8. Wallace S.M., Riegelman S. (1977). Uptake of acetazolamide by human erythrocytes. J Pharm. Sci., 66, 729-731.
9. Bayne W.F., Tao T.F., Rogers G., Chu L.C., Theeuwes F. (1981). Time course and disposition of methazolamide in human plasma and red blood cells. J. Pharm. Sci., 70,75-80.
10. Lin J.H., Lin T.H., Cheng H. (1992). Uptake and stereoselective binding of enantiomers of MK-927, a potent carbonic anhydrase inhibitor, by human erythrocytes in vitro. Pharm. Res., 9. 339-344.
11. Biollaz J., Munafo A., Buclin T., Gervasoni J-P., Magnin J-L., Jaquet F., Brunner-Ferber F. (1995). Whole blood pharmacokinetics and metabolic effects of the topical carbonic anhydrase inhibitor dorzolamide. Eur. J. Clin. Pharmacol., 47, 453-460.
12. Agarwal R.P., McPherson R.A., Threatte G. A. (1986). Evidence for a cyclosporin-binding protein in human erythrocytes. Transplantation, 42, 627-632.
13. Hooks M.A, (1994). Tacrolimus, a new immunosuppressant-a review of the literature. Ann. Pharmacother., 28, 501-511.
14. Chiou, W.L. (1984). A new model-independent physiological approach to study hepatic drug clearance and its applications. Int. J. Clin. Pharmacol. Ther. Toxicol., 22, 577-590.
15. Lee, H.J., Chiou, W.L. (1989). Erythrocytes as barriers for drug elimination in the isolated rat liver. I. Doxorubicin. Pharm. Res., 6, 833-839.
16. Lee, H.J., Chiou, W.L. (1989). Erythrocytes as barriers for drug elimination in the isolated rat liver. II. Propranolol. Pharm. Res., 6, 840-843.
17. Martindale The Extra Pharmacopoeia (28th Ed.), The Pharmaceutical Press, 1989, S.1509-17.
18. Kayaalp, O. (1997). 'Rasyonel Tedavi Yönünden Tıbbi Farmakoloji', Feryal Matbaacılık, Ankara.
19. Bickel, M. H. (1975). Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and other blood components. J. Pharm. Pharmacol., 27, 733-738.

20. Owen, N. E., Gunn, R. B. (1983). Kinetic mechanism of chlorpromazine inhibition erythrocyte 3-o-methylglucose transport. Biochim. Biophys. Acta., 727, 213-216.
21. Benga, G., Ionescu, M., Popescu, O., Pop, I. V. (1983). Effect of chlorpromazine on proteins in human erythrocyte membranes as inferred from spin labeling and biochemical analyses. Mol. Pharmacol., 23, 771-778.
22. Sheetz, M.P., Singer, S. J. (1974). Biological membranes as bilayer couples a molecular mechanism of drug-erythrocyte interactions. Proc. Nat. Acad. Sci., 71(11), 4457-4461.
23. Elferink, J. G. R. (1977). The asymmetric distribution of chlorpromazine and its quaternary analogue over the erythrocyte membrane. Biochem. Pharmacol., 26, 2411-2416.
24. Ahyayaucha H., Gallego M., Casis O. and Bennouna M. (2006). Changes in erythrocyte morphology induced by imipramine and chlorpromazine. J. Physiol. Biochem., 62 (3), 199-206.
25. Bhattacharryya, M., Chaudri, U., Poddar R.K. (1990). Studies on the interaction of chlorpromazine with haemoglobin. Int. J. Biol. Macromol., 12, 297-301.
26. Cornelius, A. S., Reilly, M. P., Suzuki, M., Asakura, T., Horiuchi, K., (1994). The mechanism of chlorpromazine-induced red blood cell swelling. Gen. Pharmacol., 25 (1), 205-210.
27. Lieber, M. R., Lange, Y., Weinstein, R. S., Steck, T. L. (1984). Interaction of chlorpromazine with the human erythrocyte membrane. J. Biol. Chem., 259, 9225-9234.
28. Sahin S, Rowland M. (2007). Influence of erythrocytes on the hepatic distribution kinetics of urea and thiourea. Eur J Pharm Sci., 31(3-4),180-189.
29. Luxnat, M., Galla, J.H. (1986). Partition of chlorpromazine into lipid bilayer membranes: the effect of membrane structure and composition. Biochim. Biophys. Acta., 856, 274-282.
30. Bai, A. S., Abramson, F. P. (1984). Effects of chlorpromazine on the disposition and beta-adrenergic blocking activity of propranolol in the dog. J. Pharmacokin. Biopharm., 12(3), 333-349.
31. Ehrnebo M., Agurell S., Boreus L.O,, Gordon E., Lonroth U. (1974). Pentazocine binding to blood cells and plasma proteins. Clin. Pharmacol. Ther., 16(3), 424-429.
32. Tamura A., Sujimoto K., Sato T., Fujii T (1990). Effect of haematocrit, plasma protein concentration and temperature of drug-containing blood in vitro on the concentration of the drug in plasma. J. Pharm. Pharmacol., 42, 577-580.
33. Bhattacharyya J., Bhattacharyya M., Chakraborti A.S., Chaudhuri U., Poddar R.K.(1998). Structural organisations of hemoglobin and myoglobin influence their binding behaviour with phenothiazines. Int. J. Biol. Macromol., 23(1):11-18.
34. Chen J.Y., Brunauer L.S., Chu F.C.. Helsel C.M., Gedde M.M., Huestis W.H. (2003). Selective amphipathic nature of chlorpromazine binding to plasma membrane bilayers. Biochim. Biophys. Acta., 1616(1), 95-105.