Distribution of antidepressants between plasma and red blood cells

Zdeněk Fišar [1], Květoslava Fuksová [2], Jan Sikora [3], Lucie Kališová [1], Marie Velenovská [1] & Michaela Novotná [3]

[1] Department of Psychiatry and [2] Institute of Nuclear Medicine, 1st Faculty of Medicine, Charles University, Prague 2, Czech Republic
[3] Psychiatric Outpatient Department, Prague 5, Czech Republic.

Correspondence to: Zdeněk Fišar, PhD
Department of Psychiatry, 1st Faculty of Medicine, Charles University
Ke Karlovu 11, 120 00 Prague 2, Czech Republic
TEL.: +420 224965313; FAX: +420 224923077
EMAIL: zfisar@lf1.cuni.cz

Submitted: March 27, 2006 *Accepted: April 10, 2006*

Key words: **antidepressants; distribution; blood; plasma; erythrocyte; ghosts**

Neuroendocrinol Lett 2006; **27**(3):307–313 **PMID:** 16816836 NEL270306A14 ©Neuroendocrinology Letters **www.nel.edu**

Abstract

OBJECTIVE: The distribution of different antidepressants between plasma and red blood cells (RBCs) or between water and erythrocyte membranes (ghosts) has not been sufficiently compared so far.

MATERIALS AND METHODS: Distribution of seven antidepressants (amitriptyline, nortriptyline, imipramine, desipramine, didesmethylimipramine, dothiepin, and citalopram) was measured *in vitro* in small volumes of blood or erythrocyte membrane suspension using radiolabeled drugs. Blood samples were taken from healthy subjects.

RESULTS: The distribution of antidepressants between plasma and RBCs is strongly affected by temperature; however, it does not depend on the antidepressant concentration in the range of their therapeutic concentrations. The data analysis proved that the ratio of RBCs to plasma volume concentrations is the suitable parameter characterizing antidepressant distribution in whole blood. Significantly higher ratios of RBCs to plasma concentrations were found for demethylated metabolites of tricyclic antidepressants and in the case of citalopram. Citalopram showed the highest accumulation in intact RBCs and at the same time the lowest binding to isolated membranes. The binding of drugs to isolated erythrocyte membranes was much higher than in whole blood.

CONCLUSION: The concentration ratio of antidepressant in RBCs and in plasma is sensitive not only to the binding properties of plasma proteins and cell membranes, but also to changes in drug molecule, both in aminopropyl chain and in aromatic rings. This ratio is to a large extent characteristic of a particular antidepressant.

Abbreviations:

RBC	– red blood cell,	DMI	– desipramine,
AD	– antidepressant,	DDMI	– didesmethylimipramine,
TCA	– tricyclic antidepressant,	CIT	– citalopram,
SSRI	– selective serotonin reuptake inhibitor,	TOT	– total drug concentration,
DOT	– dothiepin,	PLA	– plasma drug concentration,
AMI	– amitriptyline,	ERY	– drug concentration in RBCs sediment,
NOR	– nortriptyline,	SUP	– free drug,
IMI	– imipramine,	GHO	– drug concentration in ghost sediment

ORIGINAL ARTICLE

Introduction

Although the primary biochemical effects of antidepressants (ADs) are well known, they do not suffice to explain their therapeutic effects that occur only after a long-term treatment. Adaptive changes in neurotransmission play the key role in the medical effects of ADs [10,41,44] though their specification is complicated by the interrelation of intracellular processes on the one hand, and, on the other, by the fact that individuals with identical depressive symptoms often respond to one and the same AD in a distinct way.

Generally speaking, ADs are amphiphilic molecules, which dissolve in the aqueous phase, though they are equally soluble in the lipid part of cell membranes. As they easily pass through membranes [16], they occur in plasma as well as in cell membranes and cytoplasm in the state of equilibrium. Owing to the accumulation in the lipid part of cell membranes ADs can both indirectly affect the protein-protein or lipid-protein interactions and induce changes in composition or physical properties of the lipid bilayer [45]. The ADs in plasma occur either in a free form or bound to albumin, α_1-acid glycoprotein, and lipoproteins [38]. Equilibrium distributions of some ADs between red blood cells (RBCs) and plasma were studied both *in vitro* [2,5], and *in vivo* [13,27,42].

A long-term treatment with ADs results in setting their concentration in blood and an uneven distribution in the brain [33]. It was demonstrated that the brain concentrations of several ADs could be affected by expression and activity of P-glycoprotein which acts as an extrusion pump for many xenobiotic compounds [46]. Plasma ADs levels of different individuals are largely varied with respect to differences in drug metabolism, variability in distribution between plasma and tissues, a mode of the drug application, age, smoking, or other psychotropics [6,34,35]. Besides applied ADs, antidepressant metabolites are usually active as well [40]. Genetic factors contribute to the phenotype of antidepressant response [20,24]. Combined use of therapeutic drug monitoring and genotyping contributes to improve pharmacotherapy [11].

The therapeutic ranges of plasma concentrations of ADs stretch from tens to hundreds of ng/mL; however, no definitive therapeutic concentrations have been demonstrated for some antidepressants [32]. Different studies concluded that the maximum therapeutic effects shown by many ADs with specific groups of patients can be reached in a special range of invariable plasma levels, though the interrelation between plasma concentrations and clinical effectiveness is not straightforward [22,30,35,36,39]. It is assumed that the RBCs/plasma ratios of ADs may correlate with the treatment outcome better than the total drug concentration in plasma [21,29]. When we quantified the relation between concentrations of imipramine+desipramine in blood and in the brain tissue of rats a close correlation among concentrations measured in the brain homogenate, plasma, and erythrocyte membranes was proved [13].

Recently, an interest in the relation of the lithium distribution between plasma and RBCs has been restored, in particular with respect to its possible role as a diagnostic indicator of bipolar disorder [8], or as a possible predictor of lithium therapeutical effects or overdosing [7,28]. Mechanisms of desipramine toxicity and its dosage recommendations were also related to erythrocyte/plasma ratios [3]. A correct interpretation of results requires distinguishing the free (unbound) ADs in the aqueous phase, the ADs bound to the plasma proteins, and those accumulated in the lipid part of cell membranes or in the intracellular milieu.

Our study was focused on determination of *in vitro* distribution of ADs both in whole blood and isolated erythrocyte membranes (ghosts), i.e. without plasma and cytoplasm proteins. These studies were performed in man, and in particular, in healthy subjects without medication. We studied the most often prescribed ADs both from the group of so-called tricyclic antidepressants (TCAs) and from the group of selective serotonin reuptake inhibitors (SSRIs).

Materials and methods

We used our own measurement technique that afforded us to specify ADs distribution between RBCs and plasma in small volumes of blood. The tritiated ADs were prepared in our laboratories (see below) and used in these experiments. The following ADs were tested: dothiepin (DOT), amitriptyline (AMI) and its main active metabolite nortriptyline (NOR), imipramine (IMI) and its demethylated metabolites desipramine (DMI) and didesmethylimipramine (DDMI). Furthermore, we tested citalopram (CIT) as a representative of SSRI [4]. The experiments comply with the current laws of Czech Republic in which they were performed, and were approved by the Ethical Committee of Human Experimentation of the 1st Faculty of Medicine of the Charles University of Prague, and were in accordance with the Declaration of Helsinki.

Human subjects

Blood samples were taken from healthy volunteers at the Blood Transfusion Unit of the Faculty of General Hospital. The group consisted of 128 subjects (woman and men, age spread 18–55) who had not been treated by any drugs.

Preparation of tritiated antidepressants

CIT was obtained from Lundbeck (H. Lundbeck A/S, DK-2500 Copenhagen-Valby, Denmark), DDMI from the VÚFB (Prague, CZ), DOT from Léčiva (Prague, CZ). All other chemicals were purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

[^{3}H]dothiepin ([^{3}H]DOT) was obtained by catalyzed exchange in the solution-gas system (the CESG method). This method is generally applicable if we need to prepare compounds tritiated in the methylene position of benzyl [12,27]. With regard to the danger of simultaneous hydro-

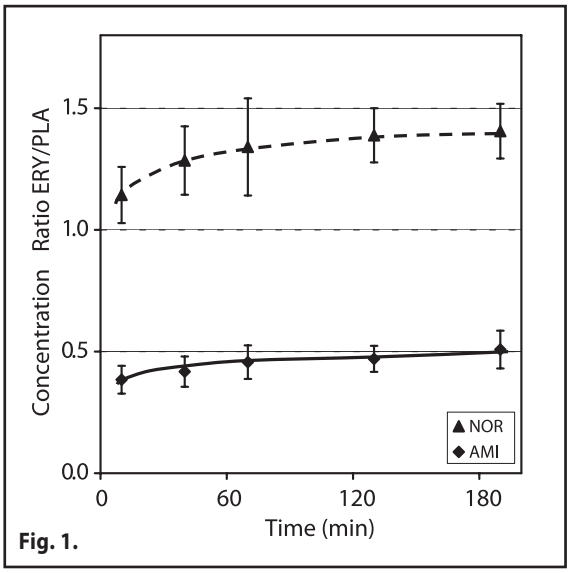

Fig. 1.

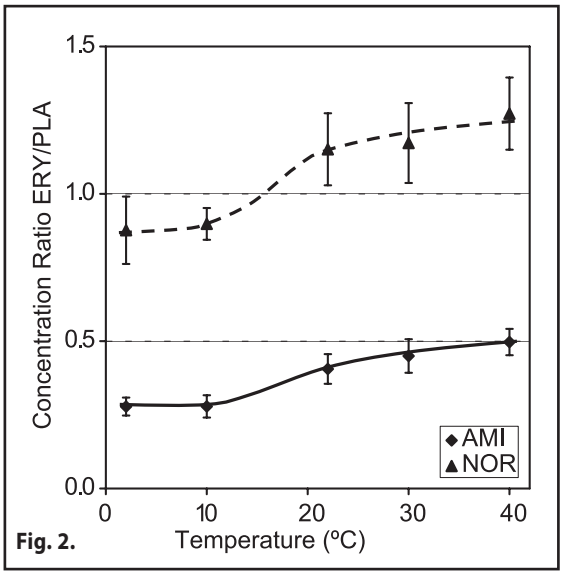

Fig. 2.

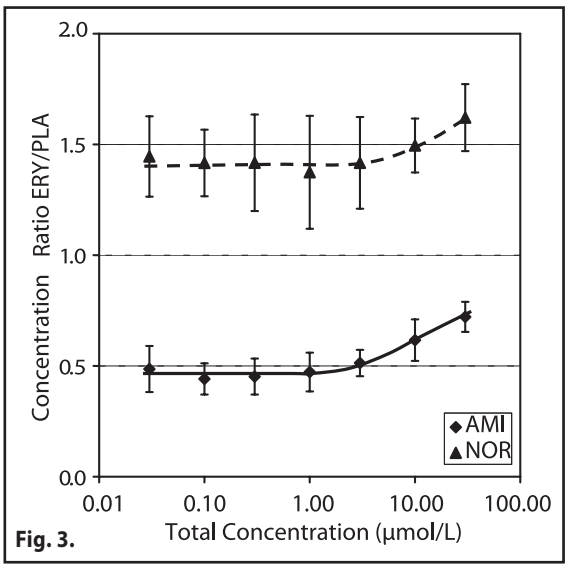

Fig. 3.

Figure 1 (above left). The time dependence of the amitriptyline (AMI) and nortriptyline (NOR) distribution in blood characterized by concentration ratio in red blood cells to the plasma concentration (ERY/PLA). The mean ± SD out of four measurements is indicated.

Figure 2 (above right). The temperature dependence of the amitriptyline (AMI) and nortriptyline (NOR) distribution in blood characterized by concentration ratio in red blood cells to the plasma concentration (ERY/PLA). The mean ± SD out of four measurements is indicated.

Figure 3 (left). The concentration dependence of the amitriptyline (AMI) and nortriptyline (NOR) distribution in blood characterized by concentration ratio in red blood cells to the plasma concentration (ERY/PLA). The mean ± SD out of four measurements is indicated.

genation of the multiple bond in the DOT molecule, the catalyst employed (10% $PdO/BaSO_4$) was prereduced with gaseous hydrogen. The exchange reaction was carried out in the dioxan : water (9 : 1) solvent system with approximately 5% tritium. After the reactive mixture was treated the product was purified by means of preparative chromatography on the thin layer with the chlorophorm : ethanol : ammonia (60 : 40 : 0.5) system, leaving the [^{3}H]DOT with the specific activity 6.8 GBq/mmol and radiochemical purity > 98%.

The technique used for the preparation of [^{3}H]citalopram ([^{3}H]CIT) involved reductive alkylation of the correspondent desmethylderivative with formaldehyde [27]. The following reactive conditions were used: five-molar formaldehyde abundance, methanol, 10% $Pd/BaSO_4$ catalyst, and approximately 5% tritium. When the reactive compound was processed the product was purified with preparative chromatography on the thin layer with the ethyl acetate : *n*-propanol : ammonia (40 : 30 : 3) solvent system, leaving the [^{3}H]CIT with the specific activity 25.9 GBq/mmol and radiochemical purity > 98%.

The procedure of preparation of other tritiated ADs was described before [26,27]. The following tritiated ADs were used: [^{3}H]amitriptyline ([^{3}H]AMI, specific activity 4.07 TBq/mmol), [^{3}H]nortriptyline ([^{3}H]NOR, specific activity 80 GBq/mmol), [^{3}H]imipramine ([^{3}H]IMI, specific activity 2.85 TBq/mmol), [^{3}H]desipramine ([^{3}H]DMI, specific activity 2.04 TBq/mmol) and [^{3}H] didesmethylimipramine ([^{3}H]DDMI, specific activity 1.81 TBq/mmol); all in the radiochemical purity within 94–98%.

Distribution of antidepressants

The distribution of ADs between plasma and RBCs was measured *in vitro* in the following way. Small samples of non-coagulable blood were taken (0.1% EDTA was used as an anticoagulant). 250 µL of blood were shaken with 2.5 µL of ADs with the resulting concentration 1 µmol/L; the sample was incubated for 2 hours while being

Table 1: The distribution of antidepressants in blood characterized by concentration ratios in plasma to whole blood (PLA/TOT), in red blood cells to whole blood (ERY/TOT), and in red blood cells to the plasma concentration (ERY/PLA)

	PLA/TOT			n	ERY/TOT			n	ERY/PLA			n
AMI	1.45	±	0.14	106	0.61	±	0.09	106	0.43	±	0.07	128
NOR	0.86	±	0.06	25	1.19	±	0.17	25	1.38	±	0.21	25
IMI	1.25	±	0.10	13	0.83	±	0.08	13	0.68	±	0.09	13
DMI	0.91	±	0.05	13	1.08	±	0.08	13	1.19	±	0.13	13
DDMI	0.86	±	0.08	13	1.10	±	0.08	13	1.29	±	0.17	13
DOT	1.26	±	0.13	25	0.87	±	0.14	25	0.70	±	0.13	25
CIT	0.74	±	0.04	25	1.26	±	0.12	25	1.70	±	0.18	25

AMI, amitriptyline; NOR, nortriptyline; IMI, imipramine; DMI, desipramine; DDMI, didesmethylimipramine; DOT, dothiepin; CIT, citalopram. The mean ± SD is indicated.

Table 2: Post-hoc (after obtaining a statistically significant *F* test from the ANOVA) comparisons between the means of concentration ratios in red blood cells to the plasma concentration (ERY/PLA, see Table 1)*

	NOR	IMI	DMI	DDMI	DOT	CIT
AMI	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NOR		<0.0001	0.0034	0.6196	<0.0001	<0.0001
IMI			<0.0001	<0.0001	0.9998	<0.0001
DMI				0.6324	<0.0001	<0.0001
DDMI					<0.0001	<0.0001
DOT						<0.0001

*Post-hoc *p*-levels for the Scheffé's test.
AMI, amitriptyline; NOR, nortriptyline; IMI, imipramine; DMI, desipramine; DDMI, didesmethylimipramine; DOT, dothiepin; CIT, citalopram.

mixed at 37 °C. The following tritiated ADs were used as a radiotracer: [^{3}H]AMI, [^{3}H]NOR, [^{3}H]DOT, [^{3}H]CIT, [^{3}H]IMI, [^{3}H]DMI, and [^{3}H]DDMI. The incubation was followed by withdrawing 10 μL of the sample by help of a positive displacement digital micropipette (Nichiryo Co., Tokyo, Japan) to read the whole blood activity (TOT), the blood was centrifuged at 14000 g for 10 min; 10 μL of plasma (PLA) and 10 μL of sediment (ERY) were withdrawn to measure the radioactivity. After adding the scintillation cocktail the samples were assayed on LS 6000IC scintillation counter (Beckman Instruments Inc, Fullerton, CA, USA). The samples were measured in doublets.

The method was selected on the basis of measuring time (Fig. 1), temperature (Fig. 2), and concentration (Fig. 3) dependencies of [^{3}H]AMI and [^{3}H]NOR distribution between plasma and RBCs sediment. Trapped plasma in the erythrocyte sediment (about 7%) was determined by comparison of filtration and centrifugation technique to separate bound and free [^{3}H]AMI. However, to avoid data error accumulation we did not correct drug concentration in RBCs sediment on the antidepressant localized in the intercellular space of the sediment.

Measurements of AD distribution between erythrocyte membranes (GHO) and the aqueous phase (SUP) followed the same basic steps, with a difference, that erythrocyte membranes (ghosts) prepared in the standard way [9] were used instead of whole blood.

Data processing

The distribution of ADs in whole blood was characterized with concentration ratios (v:v) in plasma to the total concentration (PLA/TOT), in RBCs sediment to the total concentration (ERY/TOT), and in RBCs to the plasma concentrations (ERY/PLA). With ghosts we examined concentration ratios of the free ligand in the aqueous phase to its total concentration (SUP/TOT), in the ghost sediment to the total concentration (GHO/TOT), and in ghosts to the aqueous phase (GHO/SUP).

All the values are presented as mean ± standard deviation (SD). Hypothesis testing was performed using analysis of variance (ANOVA), followed by post hoc Scheffé's test. Spearman R was used to quantify relation between two quantitative parameters; pairwise deletion of missing data in correlation matrix was used. Statistical analyses were performed with the statistical package Statistica (StatSoft Inc, Tulsa, USA).

Results

We introduced a straightforward technique for *in vitro* measurement of tritiated AD distribution in small volumes of the sample. Time courses of the ERY/PLA ratio determined for [^{3}H]AMI and [^{3}H]NOR (Fig. 1) proves that the parameter changed very little after two-hour incubation at 37 °C. A strong dependence on temperature was observed (Fig. 2). In the range of ADs concentrations overlapping their therapeutic values (0.03–3 μmol/L) no changes of the ERY/PLA ratio were observed (Fig. 3);

Table 3: The distribution of antidepressants in erythrocyte membrane suspension characterized by concentration ratios of the free ligand in the aqueous phase (supernatant) to its total concentration (SUP/TOT), in the ghost sediment to the total concentration (GHO/TOT), and in ghosts to the aqueous phase (GHO/SUP) (n = 9)

	SUP/TOT	GHO/TOT	GHO/SUP
AMI	0.392 ± 0.078	2.81 ± 0.38	7.4 ± 1.3
NOR	0.209 ± 0.058	3.41 ± 0.77	16.9 ± 2.9
IMI	0.403 ± 0.089	3.04 ± 0.64	7.7 ± 1.3
DMI	0.342 ± 0.068	2.98 ± 0.58	8.9 ± 1.6
DDMI	0.338 ± 0.064	3.32 ± 0.77	9.9 ± 1.7
DOT	0.288 ± 0.066	3.49 ± 0.84	12.3 ± 1.8
CIT	0.584 ± 0.098	2.24 ± 0.33	3.9 ± 0.8

AMI, amitriptyline; NOR, nortriptyline; IMI, imipramine; DMI, desipramine; DDMI, didesmethylimipramine; DOT, dothiepin; CIT, citalopram. The mean ± SD is indicated.

Table 4: Post-hoc comparisons between the means of concentration ratios in ghosts to the aqueous phase (GHO/SUP, see Table 3)*

	NOR	IMI	DMI	DDMI	DOT	CIT
AMI	<0.0001	0.9999	0.7856	0.2200	0.0002	0.0229
NOR		<0.0001	<0.0001	<0.0001	0.0004	<0.0001
IMI			0.9172	0.3776	0.0006	0.0089
DMI				0.9686	0.0289	0.0001
DDMI					0.2808	<0.0001
DOT						<0.0001

*Post-hoc *p*-levels for the Scheffé's test.
AMI, amitriptyline; NOR, nortriptyline; IMI, imipramine; DMI, desipramine; DDMI, didesmethylimipramine; DOT, dothiepin; CIT, citalopram.

however, the ratio is growing at high concentrations. The correlations between concentrations in plasma and in RBCs were high both for AMI (R = 0.974, n = 28) and for NOR (R = 0.969, n = 28; calculated from the source data of Fig. 3).

We examined distribution of seven ADs between RBCs and plasma and sought to find which of the ERY/PLA, ERY/TOT, and PLA/TOT ratios (Table 1) would best characterize differences between particular ADs. The data analysis (ANOVA and post hoc Scheffé's test) proved that the number of significant differences for AMI, NOR, IMI, DMI, DDIMI, DOT, and CIT was for the ratio ERY/PLA > ERY/TOT = PLA/TOT. From this point of view the most suitable parameter characterizing ADs distribution between RBCs and plasma is the ratio of the ERY/PLA volume concentrations. Statistically, the ERY/PLA values were significantly different for all the tested ADs pairs with the exception NOR and DDMI, DMI and DDMI, IMI and DOT (Table 2). The highest ERY/PLA ratios were observed for CIT, NOR, DMI, and DDMI (Table 1).

The binding of ADs to isolated erythrocyte membranes, i.e. without plasma and cytoplasm proteins, was much higher than binding to RBCs in whole blood ($p < 0.0001$). In the experiments involving erythrocyte ghosts (Table 3) the ADs concerned showed significant differences much more frequently for the GHO/SUP ratios than for the GHO/TOT or the SUP/TOT ratios. Significant differences in the GHO/SUP ratios were not found between AMI, IMI, DMI and DDMI (Table 4). The lowest GHO/SUP ratio was found for CIT. We found that the drug demethylation affects TCAs distribution in the suspension of erythrocyte membranes in an analogous way as in whole blood, i.e. higher GHO/SUP ratios for demethylated derivatives (Table 3).

The GHO/SUP ratios were used to draw comparison with the ERY/PLA ratios in whole blood. For TCAs (AMI, NOR, IMI, DMI, DDMI) a significant correlation between ERY/PLA and GHO/SUP ratios was detected ($R = 0.604$, $p < 0.0001$, $n = 45$).

Discussion

Our project resumes our previous findings concerning the fact that the concentrations of TCAs in brain significantly correlate with their concentrations in plasma as well as erythrocyte membranes [13]. Therefore, if a significant change in ADs distribution between plasma and erythrocytes occurs, the brain concentrations need not correlate properly with plasma concentrations. This effect probably complicates the search for the relation between plasma concentrations of ADs and their therapeutic effects. ADs distribution between RBCs and plasma is a result of relatively complex procedures occurring in the interface between the membrane and the aqueous phase, which might be affected not only by changes of the free ADs concentration but also by local changes of pH or membrane lipid composition [14,15,17,31].

In our study we introduced a straightforward technique affording *in vitro* measurement of drug distribution between plasma and RBCs or between water and membranes. Our method was based on measurement of time, temperature, and concentration dependence of AMI and NOR distribution in human blood. We found that it took a relatively long time (hours) till the equilibrium in whole blood was established compared to the rate at which balance was reached in the model of phospholipid membranes (liposomes) [14]. Obviously, this can be caused both by lower concentrations of free ADs in plasma and by a more difficult accessibility of the lipid part of cell membranes in the presence of integral and peripheral proteins. Our experiments supposed that two-hour incubation at 37 °C is sufficient to reach equilibrium distribution of ADs between plasma and RBCs in whole blood (Fig. 1). The temperature dependence showed an increase in ADs accumulation in erythrocytes with increasing temperature (Fig. 2), which points to the role of the lipid part of cell membranes in ADs distribution between plasma and RBCs. On the other hand, no significant influence of various AD concentrations on the ERY/PLA ratio was observed, as far as these concentrations were less than 3 μmol/L (Fig. 3), which is fulfilled under the physiological conditions. An increase in the ERY/PLA ratio with high ADs concentrations can be explained by gradual saturation of their binding to plasma proteins, in particular to α_1-acid glycoprotein [2], and it proves the previously described TCAs redistribution in the case of an acute overdose [1]. For AMI and NOR we found virtually identical distribution between RBCs and plasma as published before [2].

We measured distribution of seven ADs between RBCs and plasma in human blood or between ghosts and water (Tables 1 and 3). Significantly lower ERY/PLA ratios, i.e. smaller accumulation in RBCs, were observed for tertiary amines in comparison to their demethylated metabolites, i.e. for AMI versus NOR, IMI versus DMI, or IMI versus DDMI (Table 2). On the other hand, the ERY/PLA ratio was virtually identical for IMI and DOT (both being tertiary amines). These results imply an important function of the aminopropyl chain in the ADs distribution in blood. Besides the aminopropyl group all the tested ADs include a non-polar part composed of the dibenzazepine group for IMI, DMI, and DDMI, the dibenzothiepine group for DOT, the dibenzocycloheptane group for AMI and NOR, and phenylizobenzofuran for CIT. These groups are responsible for binding to hydrophobic binding sites in proteins and the lipid bilayer. AMI, IMI, and CIT have an identical dimethylaminopropyl chain bound to different cyclical parts of the molecule; therefore, these non-polar parts of molecules are evidently responsible for different ERY/PLA ratios of these drugs.

The comparison of the ERY/PLA and GHO/SUP ratios proved that the increased accumulation in RBCs for DMI and DDMI in comparison to IMI could not be explained by their increased binding into cell membranes resulting from the aminopropyl chain demethylation. This outcome corresponds with our previous findings, when we observed no significant differences in binding to model phospholipid membranes for these TCAs [14]. We suppose that the higher ERY/PLA ratios for demethylated IMI metabolites probably originate in their altered binding to plasma proteins [2]. The increased binding to RBCs for NOR in comparison with AMI is, in particular, due to the high NOR binding to a membrane that is enabled by the aminopropyl chain demethylation. The significantly higher binding to ghosts for DOT in comparison with IMI can be explained by the higher DOT binding into membranes, enabled by the distinct features of its tricyclic group.

In comparison with TCAs, CIT showed the highest accumulation in intact RBCs and at the same time the lowest binding to isolated membranes. Such a behaviour of CIT can be explained by its lower binding to plasma proteins, because for TCAs the concentrations of the free (unbound) molecules in plasma are presented within the range of 4–16% [5,19,23,25,43], whereas for CIT within 20–50% [18,37,47].

Considering the measurement of distribution of seven distinct ADs in whole blood and in the erythrocyte membrane suspension we can conclude that the ERY/PLA ratios are to a large extent characteristic of a particular AD. The measurement of ADs distribution between ghosts and water proved that the binding to isolated membranes is much higher without plasma and cytoplasm proteins than that to the intact RBCs in whole blood. This can be explained by the fact that ADs in blood are to a large extent bound to proteins and their transfer to the lipid part of cell membranes is determined by a relatively low concentration of a free AD. It can be speculated that RBCs/plasma concentration ratio can be used as one of parameters to study interindividual differences in response to pharmacotherapy.

Acknowledgements

This research was supported by grants No 27/2000/C given by the Granting Agency of the Charles University (GA UK) and No NR8408-3/2005 given by the Internal Grant Agency of the Ministry of Health of the Czech Republic (IGA MH CR). The author would like to thank Mr. Zdeněk Hanuš for his technical support.

REFERENCES

1 Amitai Y, Erickson T, Kennedy EJ, Leikin JB, Hryhorczuk DO, Noble J, et al. Tricyclic antidepressants in red cells and plasma: correlation with impaired intraventricular conduction in acute overdose. Clin Pharmacol Ther 1993; **54**:219–227.
2 Amitai Y, Kennedy EJ, DeSandre P, Frischer H. Distribution of amitriptyline and nortriptyline in blood: role of alpha-1-glycoprotein. Ther Drug Monit 1993; **15**:267–273.
3 Amitai Y, Frischer H. Excess fatality from desipramine and dosage recommendations. Ther Drug Monit. 2004; **26**(5):468–473.
4 Brøsen K, Naranjo CA. Review of pharmacokinetic and pharmacodynamic interaction studies with citalopram. Eur Neuropsychopharmacol 2001; **11**:275–283.

5 Burch JE, Roberts SG, Raddats MA. Binding of amitriptyline and nortriptyline in plasma determined from their equilibrium distributions between red cells and plasma, and between red cells and buffer solution. Psychopharmacology 1981; **75**:262–272.
6 Burke MJ, Preskorn SH. Therapeutic drug monitoring of antidepressants: cost implications and relevance to clinical practice. Clin Pharmacokinet 1999; **37**(2):147–165.
7 Camus M, Hennere G, Baron G, Peytavin G, Massias L, Mentre F, et al. Comparison of lithium concentrations in red blood cells and plasma in samples collected for TDM, acute toxicity, or acute-on-chronic toxicity. Eur J Clin Pharmacol 2003; **59**:583–587.
8 Clarke WB, Guscott R, Downing RG, Lindstrom RM. Endogenous lithium and boron red cell-plasma ratios: normal subjects versus bipolar patients not on lithium therapy. Biol Trace Elem Res 2004; **97**:105–116.
9 Dodge JT, Mitchell C, Hanahan DJ. The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch Biochem Biophys 1963; **100**:119-130.
10 Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. Arch Gen Psychiatry 1997; **54**:597–606.
11 Eap CB, Jaquenoud SE, Baumann P. Therapeutic monitoring of antidepressants in the era of pharmacogenetics studies. Ther Drug Monit 2004; **26**(2):152–155.
12 Evans EA. Tritium and it's compounds. 2nd ed. London: Butterworth; 1974.
13 Fišar Z, Krulík R, Fuksová K, Sikora J. Imipramine distribution among red blood cells, plasma and brain tissue. Gen Physiol Biophys 1996; **15**:51–64.
14 Fišar Z, Fuksová K, Velenovská M. Binding of imipramine to phospholipid bilayers using radioligand binding assay. Gen Physiol Biophys 2004; **23**:77–99.
15 Fišar Z. Interactions between tricyclic antidepressants and phospholipid bilayer membranes. Gen Physiol Biophys 2005; **24**:161–180.
16 Gulyaeva N, Zaslavsky A, Lechner P, Chlenov M, McConnell O, Chait A, et al. Relative hydrophobicity and lipophilicity of drugs measured by aqueous two-phase partitioning, octanol-buffer partitioning and HPLC. A simple model for predicting blood-brain distribution. Eur J Med Chem 2003; **38**:391–396.
17 Heirwegh KPM, De Smedt H, Vermeir M. Analysis of membrane-bound acceptors. A correlation function for non-specific accumulation of poorly water-soluble hydrophobic or amphipathic ligands based on the ligand partition concept. Biochem Pharmacol 1992; **43**:701–704.
18 Hiemke C, Härtter S. Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacol Ther 2000; **85**:11–28.
19 Hursting MJ, Clark GD, Raisys VA, Miller SJ, Opheim KE. Measurement of free desipramine in serum by ultrafiltration with immunoassay. Clin Chem 1992; **38**:2468–2471.
20 Ishikawa T, Onishi Y, Hirano H, Oosumi K, Nagakura M, Tarui S. Pharmacogenomics of drug transporters: a new approach to functional analysis of the genetic polymorphisms of ABCB1 (P-glycoprotein/MDR1). Biol Pharm Bull 2004; **27**(7):939–948.
21 Javaid JI, Davis JM, Maiorano M. Uptake and/or binding of tricyclic antidepressants in human red cells. Life Sci 1985; **36**:1761–1769.
22 Jerling M. Dosing of antidepressants-the unknown art. J Clin Psychopharmacol 1995; **15**:435–439.
23 Juarez-Olguin H, Jung-Cook H, Flores-Perez J, Asseff IL. Clinical evidence of an interaction between imipramine and acetylsalicylic acid on protein binding in depressed patients. Clin Neuropharmacol 2002; **25**:32–36.
24 Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, et al. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. Mol Psychiatry 2004; **9**(5):442–473.
25 Kristensen CB. Imipramine serum protein binding in healthy subjects. Clin Pharmacol Ther 1983; **34**:689–694.
26 Krulík R, Slíva D, Sikora J, Farská I, Fuksová K. Tricyclic antidepressant binding to lymphocyte membranes and changes during depression. Eur J Pharmacol 1988; **149**:357–361.
27 Krulík R, Exner J, Fuksová K, Píchová D, Beitlová D, Sikora J. Radioimmunoassay of dibenzazepines and dibenzcycloheptanodienes in body fluids and tissues. Eur J Clin Chem Clin Biochem 1991; **29**:827–832.
28 Layden BT, Minadeo N, Suhy J, Abukhdeir AM, Metreger T, Foley K, et al. Biochemical and psychiatric predictors of Li^+ response and toxicity in Li^+-treated bipolar patients. Bipolar Disord 2004; **6**:53–61.
29 Linnoila M, Dorrity F Jr, Jobson K. Plasma and erythrocyte levels of tricyclic antidepressants in depressed patients. Am J Psychiatry 1978; **135**:557–561.
30 Mann K, Hiemke C, Schmidt LG, Bates DW. Appropriateness of therapeutic drug monitoring for antidepressants in routine psychiatric inpatient care. Ther Drug Monit 2006; **28**(1):83–88.
31 Mason RP, Rhodes DG, Herbette LG. Reevaluating equilibrium and kinetic binding parameters for lipophilic drugs based on a structural model for drug interaction with biological membranes. J Med Chem 1991; **34**:869–877.
32 Mitchell PB. Therapeutic drug monitoring of non-tricyclic antidepressant drugs. Clin Chem Lab Med 2004; **42**(11):1212–1218.
33 Miyake K, Fukuchi H, Kitaura T, Kimura M, Sarai K, Nakahara T. Pharmacokinetics of amitriptyline and its demethylated metabolite in serum and specific brain regions of rats after acute and chronic administration of amitriptyline. J Pharmaceutical Sci 1990; **79**:288–291.
34 Nagy A, Johansson R. Plasma levels of imipramine and desipramine in man after different routes of administration. Naunyn-Schmiedeberg's Arch Pharmacol 1985; **290**:145–160.
35 Perel JM, Stiller RL, Glassman AH. Studies on plasma level/effect relationships in imipramine therapy. Commun Psychopharmacol 1978; **2**:429–439.
36 Perry PJ, Zeilmann C, Arndt S. Tricyclic antidepressant concentrations in plasma: an estimate of their sensitivity and specificity as a predictor of response. J Clin Psychopharmacol 1994; **14**:230–240.
37 Plenge P, Mellerup ET. Pindolol and the acceleration of the antidepressant response. J Affect Disord 2003; **75**:285–289.
38 Riant P, Urien S, Albengres E, Renouard A, Tillement JP. Effects of the binding of imipramine to erythrocytes and plasma proteins on its transport through the rat blood-brain barrier. J Neurochem 1988; **51**:421–425.
39 Risch SC, Leighton YH, Janowsky DS. Plasma levels of tricyclic antidepressants and clinical efficacy: review of the literature - part I. J Clin Psychiatry 1979; **40**:9–21.
40 Sanchez C, Hyttel J. Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. Cell Mol Neurobiol 1999; **19**:467–489.
41 Schloss P, Henn FA. New insights into the mechanisms of antidepressant therapy. Pharmacol Ther 2004; **102**:47–60.
42 Sikora J, Krulík R, Beitlová D, Příhoda P. Plasma levels of antidepressants after their withdrawal. Endocrinol Exp 1990; **24**:221–227.
43 Slordal L, Lundgren TI, Bessesen A, Sager G. Distribution of nortriptyline in human blood: effects of temperature, pH, and drug concentration. Ther Drug Monit 1987; **9**:67–71.
44 Stahl SM. Essential Psychopharmacology. Neuroscientific Basis and Practical Applications. 2nd ed. Cambridge: Cambridge University Press; 2000.
45 Toplak H, Zuehlke R, Loidl S, Hermetter A, Honegger UE, Wiesmann UN. Single and multiple desipramine exposures of cultured cells. Changes in cellular anisotropy and in lipid composition of whole cells and of plasma membranes. Biochem Pharmacol 1990; **39**:1437–1443.
46 Uhr M, Grauer MT, Holsboer F. Differential enhancement of antidepressant penetration into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption. Biol Psychiatry 2003; **54**(8):840-846.
47 van Harten J. Clinical pharmacokinetics of selective serotonin reuptake inhibitors. Clin Pharmacokinet 1993; **24**:203-220.