PHARMACOLOGY AND TOXICOLOGY

Effect of *Rhaponticum carthamoides* Extract on Structural and Metabolic Parameters of Erythrocytes in Rats with Cerebral Ischemia

M. B. Plotnikov, O. I. Aliev, A. S. Vasil'ev, V. Yu. Andreeva*, E. A. Krasnov*, and G. I. Kalinkina*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 7, pp. 50-53, July, 2008
Original article submitted August 14, 2007

Cerebral ischemia in rats was accompanied by an increase in erythrocyte degradation, which results from changes in lipid composition of their membranes. The content of lipids and phospholipid fraction (phosphatidylcholine, sphingomyelin, and phosphatidylserine) decreased, while the relative content of lysophospholipids increased in erythrocyte membranes. The course of treatment with *Rhaponticum carthamoides* extract (150 mg/kg perorally, 5 days) contributed to an increase in the contents of total lipids and phospholipids (primarily of sphingomyelin and phosphatidylserine) and decrease in the ratio of lysophospholipids in the erythrocyte membrane of rats with cerebral ischemia. Morphological characteristics of erythrocytes returned to normal, which manifested in an increase in the number of discocytes and decrease in the count of degenerated cells.

Key Words: *Rhaponticum carthamoides extract; cerebral ischemia; erythrocytes; erythrocyte membrane phospholipids*

Extracts of ecdysteroid-containing plants hold much promise for the development of new hemorheological drugs. These extracts prevent the syndrome of increased blood viscosity during experimental cardiovascular disorders [8]. *Rhaponticum carthamoides* extract (RCE) is one of the most potent plant ecdysteroid-containing preparations. The extract prevents changes in erythrocyte deformability on the model of cerebral ischemia [6]. However, the mechanism underlying the action of RCE on erythrocytes is poorly understood.

Here we studied the effect of RCE on morphological characteristics of erythrocytes and lipid composition of erythrocyte membranes on the model of increased blood viscosity in rats with cerebral ischemia.

MATERIALS AND METHODS

Experiments were performed on 18 male Wistar rats weighing 230-280 g. Cerebral ischemia in rats was induced by ligation of the left common carotid artery and 50% reduction of blood flow in the right common carotid artery under ether anesthesia [7]. RCE (daily dose 150 mg/kg) in 1% starch gel was administered intragastrically for 5 days. Control animals received an equivalent volume of 1% starch gel. Blood samples were taken from the carotid artery under ether anesthesia. Heparin in a final concentration of 50 U/ml blood served as the anti-

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; *Siberian State Medical University, Tomsk, Russia

0007-4888/08/1461-0045

coagulant. Ecdysteroid concentration in the extract was measured by chromatography and spectrophotometry [11]. Total ecdysteroid content was 2.7% of dry extract weight.

Morphological characteristics of the erythrocyte surface were studied by means of scanning electron microscopy on a REM-200 electron microscope. Erythrocytes were classified by shape [2]. We counted 1000 randomly selected erythrocytes in each sample. The quantitative ratio of various cells was expressed in percent.

Erythrocyte ghosts were isolated to study the lipid composition of membranes [12]. The lipid extract was obtained as described elsewhere [13]. Total lipid content was estimated in the color reaction with phosphovanillin reagent [3]. Total phospholipid content was determined in the reaction with ammonium ferrothiocyanate [5]. The concentration of individual phospholipid fractions (phosphatidylcholine, sphingomyelin, phosphatidylserine, phosphatidylethanolamine [PEA], and lysophospholipids [LPL]) was measured after separation by one-dimensional ascending chromatography on Silufol UV 254 plates [9].

Statistical treatment of the results involved Statistica software. We calculated the mean values and errors. Between-group differences were evaluated by Student's *t* test and nonparametric Mann—Whitney *U* test.

RESULTS

The number of discocytes in the peripheral blood from rats with cerebral ischemia was much lower than in intact animals. The number of prehemolytic erythrocytes and degenerated forms increased by 1.3 and 1.5 times, respectively (Table 1).

Lipid content in erythrocyte membranes significantly decreased in rats with cerebral ischemia. These changes were probably related to a decrease in the phospholipid fraction and other lipids (Table 2). Lipid bilayer stability depends on the ratio between constituent phospholipids. The erythrocyte membrane is characterized by asymmetric distribution of phospholipids [14]. Phosphatidylcholine and sphingomyelin are mainly localized in the outer monolayer, while phosphatidylserine and PEA occur in the inner monolayer. We revealed a decrease in phospholipid content in the outer layer of erythrocyte membrane from rats with cerebral ischemia. The contents of phosphatidylcholine and sphingomyelin decreased by 60 and 48%, respectively. Phospholipid content decreased less significantly in the outer layer (Table 3, Fig. 1). Significant decrease in the contents of phosphatidylcholine and sphingomyelin, as well as minor decrease in the content of phosphatidylserine in membranes resulted in a decrease in the area of the outer lipid monolayer. The observed changes contribute to bilayer instability and dysfunction of several membrane-bound enzymes, whose activity depends on the phospholipid environment [4,10]. These processes are followed by changes in morphological characteristics of erythrocytes during cerebral ischemia. Significant increase in the fraction of LPL in

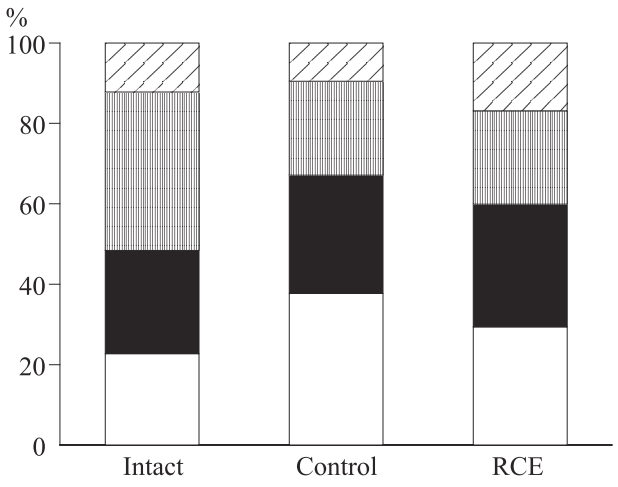

Fig. 1. Effect of the course of RCE administration (150 mg/kg) on the phospholipid ratio in erythrocyte membranes from rats with cerebral ischemia. Light area, PEA; dark area, phosphatidylserine; horizontal shading, phosphatidylcholine; vertical shading, sphingomyelin.

TABLE 1. Effect of Course Administration of RCE (150 mg/kg) on Erythrocyte Structure in Rats with Cerebral Ischemia (%)

Form of erythrocytes	Intact animals	Cerebral ischemia	
		control	RCE
Discocytes	85.03±0.15	83.35±0.16*	84.98±0.26^{+}
Transitional cells	12.76±0.12	13.70±0.22*	11.67±0.25*$^{+}$
Prehemolytic cells	1.62±0.10	2.08±0.10*	2.87±0.12*$^{+}$
Degenerated cells	0.56±0.07	0.86±0.07*	0.48±0.05^{+}

Note. Here and in Tables 2 and 3: $p<0.05$: *compared to intact animals; $^{+}$compared to the control.

TABLE 2. Effect of Course Administration of RCE (150 mg/kg) on the Content of Protein, Total Lipids, and Total Phospholipids in Erythrocyte Membranes from Rats with Cerebral Ischemia

Parameter	Intact animals	Cerebral ischemia	
		control	RCE
Protein, mg/ml erythrocyte ghost suspension	4.14±0.49	3.97±0.58	4.05±0.41
Total lipids, mg/mg protein	1.968±0.104	1.362±0.084*	1.667±0.043*+
Total phospholipids, mg/mg protein	0.960±0.050	0.630±0.020*	0.772±0.048*+

TABLE 3. Effect of Course Administration of RCE (150 mg/kg) on Phospholipid Content in Erythrocyte Membranes from Rats with Cerebral Ischemia (mg/mg protein)

Parameter	Intact animals	Cerebral ischemia	
		control	RCE
Sphingomyelin	0.110±0.008	0.057±0.002*	0.124±0.011+
Phosphatidylcholine	0.354±0.020	0.140±0.011*	0.170±0.021*
PEA	0.204±0.015	0.226±0.024	0.216±0.018
Phosphatidylserine	0.231±0.015	0.176±0.012*	0.224±0.011+
LPL	0.039±0.002	0.052±0.003*	0.039±0.003+

erythrocyte membranes also contributes to destabilization of erythrocyte membranes during cerebral ischemia (Table 3). LPL are an essential component of cell membranes. However, excessive accumulation of these compounds decreases phase transition temperature. These changes are accompanied by an increase in membrane fluidity and lipid packing [1]. The increase in membrane LPL content promotes transition of the lipid bilayer to monolayer, elevation of membrane permeability for Na^+ and K^+, and formation of hydrophilic channels followed by vesiculation of erythrocyte membrane and discocyte-echinocyte transformation [1].

The total lipid content in rats with cerebral ischemia receiving RCE for 5 days was much higher than in control animals, but lower than in intact rats. The total phospholipid content in these rats increased by 23% compared to the control ($p<0.05$, Table 2). The contents of sphingomyelin and phosphatidylserine in RCE-receiving rats increased by 117 and 27%, respectively. No differences were found in these fractions of phospholipids in erythrocyte membranes from treated and intact animals. Phosphatidylcholine content in rats of the RCE group was slightly higher than in control animals (Table 3). LPL content in treated rats significantly decreased and did not differ from that in intact animals. Course treatment with RCE decreased LPL content and normalized the concentrations of sphingomyelin (outer layer component) and phosphatidylserine (inner layer) in erythrocyte membranes from rats with cerebral ischemia, which probably contributed to morphological recovery of red blood cells. The number of discocytes in the peripheral blood in treated rats was much higher than in control animals, but did not differ from that in intact rats. The number of transitional cells in treated rats was lower than in the control and intact animals. RCE had a strong effect on the number of degenerated erythrocytes. The percentage of these cells in treated rats (0.48±0.05%) was 1.8-fold lower than in control animals, but did not differ from that in intact specimens (Table 1). Our results suggest that RCE has a protective effect on transitional forms of erythrocytes. These cells serve as discocyte reserve. On the other hand, RCE prevents cell degradation due to a decrease in transformation of transitional erythrocytes to irreversibly degenerated cells and inhibition of this process at the stage of prehemolytic cells. On the other hand, this extract contributes to recovery of a normal discoid shape in erythrocytes with reversible transitional changes. RCE increases the number of disc cells in the peripheral blood, which improves erythrocyte deformability (key parameter of blood rheology) [6]. This characteristic is typical only of disc cells.

We conclude that course administration of RCE prevents erythrocyte degradation in rats with cerebral ischemia, which is related to a positive effect on the lipid composition of membranes in red blood cells.

REFERENCES

1. G. A. Gribanov, *Vopr. Med. Khimii*, **37**, No. 4, 2-10 (1991).
2. G. I. Kozinets and Yu. A. Simovart, *Surface Architectonics of Peripheral Blood Cells under Normal Conditions and during Blood Diseases* [in Russian], Tallinn (1984).
3. V. G. Kolb and V. S. Kamyshnikov, *Handbook of Clinical Biochemistry* [in Russian], Minsk (1982).
4. V. V. Novitskii and N. V. Ryazantseva, *Dysregulation Pathology*, Ed. G. N. Kryzhanovskii [in Russian], Moscow (2002), pp. 395-405.
5. A. A. Pentyuk, V. I. Gutsol, O. A. Yakovleva, *et al*., *Lab. Delo*, No. 6, 457-460 (1987).
6. M. B. Plotnikov, O. I. Aliev, A. S. Vasil'ev, *et al*., *Byull. Eksp. Biol. Med*., Suppl. 1, 58-60 (1999).
7. M. B. Plotnikov, O. E. Vaizova, and N. I. Suslov, *Ibid*., **118**, No. 12, 565-567 (1994).
8. M. B. Plotnikov, L. N. Zibareva, A. A. Koltunov, *et al*., *Rastit. Resursy*, No. 1, 91-96 (1998).
9. J. Findley and W. Evans, *Biological Membranes: Methods* [in Russian], Moscow (1990).
10. E. A. Chernitskii and A. V. Vorobei, *Structure and Functions of Erythrocyte Membranes* [in Russian], Minsk (1986).
11. M. R. Yakubova, G. L. Genkina, T. T. Shakirov, and N. K. Abubakirov, *Khim. Prirod. Soed*., No. 6, 737-740 (1978).
12. J. T. Dodge, C. Mitchell, and D. J. Hanahan, *Arch. Biochem. Biophys*., **100**, 119-130 (1963).
13. J. Folch, M. Lees, and G. Sloane-Stanley, *J. Biol. Chem*., **226**, 497-509 (1957).
14. V. V. Kumar, *Ind. J. Biochem. Biophys*., **30**, 135-138 (1993).