PHARMACOLOGY AND TOXICOLOGY

Effect of *Alchemilla vulgaris* Extract on the Structure and Function of Erythrocyte Membranes during Experimental Arterial Hypertension

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The course of treatment with *Alchemilla vulgaris* extract increased the concentrations of lipids and phospholipids in erythrocyte membranes, decreased the number of abnormal erythrocytes, and improved deformability of red blood cells in rats with arterial hypertension.

Key Words: *hemorheology; flavonoids; erythrocytes; lipids*

Changes in rheological properties of the blood underlie the pathogenesis of hypertension and contribute to disturbances in coronary and cerebral blood flow [11]. Extracts of flavonoid-containing plants hold much promise for the development of new drugs for the correction of hemorheological shifts. These extracts effectively prevent increased blood viscosity syndrome during experimental cardiovascular pathologies [8]. *Alchemilla vulgaris* extract (AVE) is most potent in this respect [10]. Erythrocytes account for approximately 50% blood volume. Therefore, blood viscosity is primarily determined by the structure and function of erythrocytes. Here we studied the effect of AVE on morphological characteristics of erythrocytes, erythrocyte deformability, and lipid composition of membranes in rats with arterial hypertension accompanied by increased blood viscosity syndrome [7].

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MATERIALS AND METHODS

Experiments were performed on 16 male SHR rats and 8 male Wistar rats weighing 230-280 g. Blood samples were taken from the common carotid artery under ether anesthesia. Heparin in a final concentration of 50 U/ml blood served as the anticoagulant. Dry AVE standardized by the flavonoid fraction [1] was dissolved in 1% starch gel, and administered intragastrically in a daily dose of 300 mg/kg for 10 days. Control animals received an equivalent volume of 1% starch gel.

Morphological characteristics of the erythrocyte surface were studied under a REM-200 electron microscope. We examined 1000 randomly selected erythrocytes in each sample. Erythrocytes were classified by shape [3]. The quantitative ratio between cells of various types was expressed in percents.

Erythrocyte ghosts were isolated for evaluation of lipid composition of membranes [13]. Protein concentration in the suspension of erythrocyte ghosts was measured by the microbiuret method [4]. The lipid extract was obtained as described

elsewhere [14]. Total lipid content was estimated in the colored reaction with phosphorus-vanillic reagent [4]. Phospholipid concentration was determined by the formation of complexes with ammonium ferrothiocyanate [6]. The concentration of individual lipid fractions was measured after one-way chromatography on Silufol UV 254 plates [9] and quantified by densitometry of colored spots on a BIAN-170 densitometer. Erythrocyte deformability was estimated by the method of ektacytometry [10].

The results were analyzed using Statistica software.

RESULTS

Study of erythrocyte surface architectonics by means of scanning electron microscopy showed that the number of discocytes in the peripheral blood of SHR rats is lower than in Wistar rats (Table 1). The decrease in the number of discocytes is typical of various disorders [5]. The number of transitional erythrocytes significantly increased (15.21±0.10%). However, these cells retain a discoid structure and undergo reverse transition to disc cells under certain conditions [5]. Transitional forms are thus a reserve replenishing discocyte pool upon pharmacological treatment. Arterial hypertension was accompanied by a significant increase in the ratio of irreversibly modified erythrocytes. The number of prehemolytic and degenerate cells in SHR rats was higher than in Wistar rats (by 1.8 and 2.1 times, respectively).

AVE treatment was followed by a significant increase in the number of disc cells in SHR rats (compared to control animals, Table 1). The number of transitional cells did not differ in treated and control rats. However, AVE prevented transition of discoid erythrocytes to irreversibly modified cells. The ratio of prehemolytic and degenerate erythrocytes 1.4-fold decreased in extract-treated rats.

The shape of erythrocytes mainly depends on the lipid composition and ratio of various lipid fractions in the membrane [15]. Bearing in mind the fact that AVE prevents changes in erythrocyte surface architectonics, we studied the effect of this extract on the lipid composition of erythrocyte membranes.

Protein concentration in erythrocyte membranes practically did not differ in SHR and Wistar rats

TABLE 1. Effect of Repeated Intragastric Administration of AVE in a Dose of 300 mg/kg on Erythrocyte Surface Architectonics in SHR Rats (%, $M\pm m$)

Parameter	Wistar	SHR	
		control	AVE
Disc cells	85.03±0.15	80.60±0.24*	81.91±0.22*+
Transitional cells	12.76±0.12	15.21±0.10*	15.12±0.10
Prehemolytic cells	1.62±0.10	2.99±0.14*	2.12±0.08*+
Degenerate cells	0.56±0.07	1.21±0.07*	0.87±0.08*+

Note. Here and in Table 2: $p<0.05$: *compared to Wistar rats; +compared to control SHR rats.

TABLE 2. Effect of Repeated Intragastric Administration of AVE on the Concentrations of Protein, Lipids, Phospholipids, and Individual Phospholipids in Erythrocyte Membranes of SHR Rats

Parameter	Wistar	SHR	
		control	AVE
Protein, mg/ml erythrocyte ghost suspension	4.14±0.49	4.00±0.58	4.06±0.53
Total lipids, mg/mg protein	1.968±0.104	1.294±0.042*	1.543±0.081*+
Total phospholipids, mg/mg protein	0.960±0.050	0.587±0.029*	0.749±0.065*+
Phosphatidylcholine, mg/mg protein	0.354±0.020	0.110±0.018*	0.171±0.022*
Sphingomyelin, mg/mg protein	0.110±0.008	0.067±0.004*	0.096±0.007+
Phosphatidylethanolamine, mg/mg protein	0.226±0.024	0.180±0.018	0.225±0.026
Phosphatidylserine, mg/mg protein	0.231±0.015	0.181±0.017	0.223±0.021
Lysophospholipids, mg/mg protein	0.039±0.004	0.049±0.004	0.033±0.003+

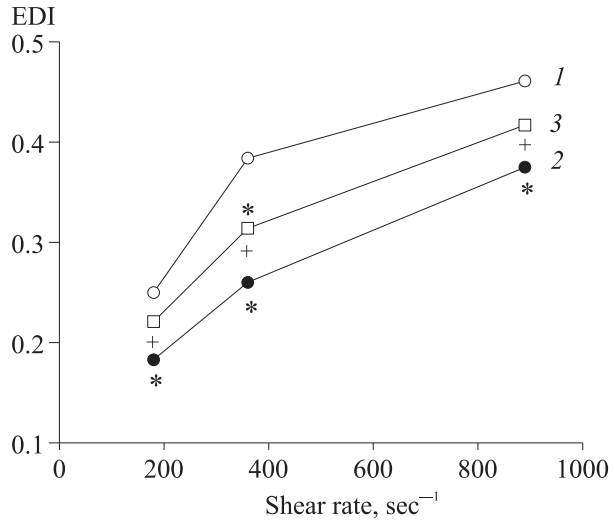

Fig. 1. Effect of repeated intragastric administration of *Alchemilla vulgaris* extract (AVE) on the erythrocyte deformability index (EDI) in SHR rats. Wistar (*1*); SHR (*2*); AVE (*3*). $p<0.5$: *compared to Wistar rats; +compared to SHR rats.

(Table 2). Total lipid content and phospholipid concentration in erythrocyte membranes of rats with arterial hypertension were much lower than in normotensive animals (by 34 and 39%, respectively). The concentrations of phosphatidylcholine and sphingomyelin in SHR rats decreased more significantly compared to normotensive animals. The decrease in the concentrations of phosphatidylethanolamine and phosphatidylserine was not statistically significant. The erythrocyte membrane is characterized by asymmetric distribution of phospholipids [2]. Phosphatidylcholine and sphingomyelin are mainly localized in the outer leaflet. Phosphatidylethanolamine and phosphatidylserine are localized in the inner leaflet. Significant decrease in the concentrations of phosphatidylcholine and sphingomyelin and slight decrease in the concentrations of phosphatidylethanolamine and phosphatidylserine are followed by a decrease in the area of the outer lipid leaflet (relative to the inner one). These changes contribute to variations in the shape of erythrocytes. The concentration of lysophospholipids in erythrocyte membranes tended to increase in rats with arterial hypertension.

The course treatment with AVE had no effect on protein content in rats with arterial hypertension (compared to the control, Table 2). Total lipid content in these rats significantly increased compared to control animals (by 19%). Total phospholipid content exceeded the control by 28%. The study of phospholipid fractions revealed a tendency to an increase in phosphatidylcholine concentration. Sphingomyelin concentration in AVE-treated rats significantly increased compared to control animals. No differences were revealed in sphingomyelin concentration in AVE-treated SHR rats and normotensive animals.

An important component of the effect of AVE is a decrease in the concentration of lysophospholipids, obligatory elements of various membranes playing an important role in membrane function [4]. Excess of lysophospholipids in the membrane leads to rearrangement of lipid packing, promotes transition of the lipid bilayer into monolayer, and impairs membrane permeability for Na^+, K^+, and Ca^{2+}. These changes contribute to transformation of erythrocytes [12].

Taking into account the positive effect of AVE on surface architectonics and lipid composition of erythrocyte membranes, we studied the influence of this extract on erythrocyte deformability.

At a shear rate of 180-890 sec^{-1}, erythrocyte deformability index in SHR rats was much lower than in Wistar rats (Fig. 1). The course treatment with AVE significantly improved erythrocyte deformability at a shear rate of 180-890 sec^{-1} (by 11-21% compared to the control). It should be emphasized that erythrocyte deformability index in SHR and Wistar rats was similar at 180 and 890 sec^{-1}.

Our results indicate that the course of AVE treatment increases lipid content and normalizes phospholipid composition of erythrocyte membranes. These changes contribute to a decrease in the ratio of irreversibly modified erythrocytes, increase in the number of disc cells in the peripheral blood, and improvement of erythrocyte deformability.

REFERENCES

1. V. Yu. Andreeva, M. B. Plotnikov, G. I. Kalinkina, and O. I. Aliev, *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 3, 32-36 (2001).
2. R. Gennis, *Biomembranes: Molecular Structure and Functions* [in Russian], Moscow (1997).
3. G. I. Kozinets and Yu. A. Simovart, *Surface Architectonics of Peripheral Blood Cells under Normal Conditions and during Blood Diseases* [in Russian], Tallinn (1984).
4. V. G. Kolb and V. S. Kamyshnikov, *Handbook of Clinical Biochemistry* [in Russian], Minsk (1982).
5. V. V. Novitskii, N. V. Ryazantseva, and E. A. Stepovaya, *Physiology and Pathophysiology of the Erythrocyte* [in Russian], Tomsk (2004).
6. A. A. Pentyuk, V. I. Gutsol, O. A. Yakovleva, *et al*., *Lab. Delo*, No. 6, 457-460 (1987).
7. M. B. Plotnikov, O. I. Aliev, A. A. Koltunov, and M. Yu. Maslov, *Byull. Eksp. Biol. Med*., No. 8, 150-151 (1998).
8. M. B. Plotnikov, O. I. Aliev, M. Yu. Maslov, *et al*., *Tromboz Gemostaz Reologiya*, No. 3, 32-35 (2000).
9. J. Findley and W. Evans, *Biological Membranes* [in Russian], Moscow (1990).

10. M. Bessis, N. Mohandas, and S. Feo, *Blood Cells*, **6**, 315-327 (1980).
11. L. Bogar, *Clin. Hemorheol. Microcirc*., **26**, No. 2, 81-83 (2002).
12. L. M. Chi, W. G. Wu, K. L. Sung, and S. Chien, *Biochim. Biophys. Acta*, No. 2, 163-171 (1990).
13. J. T. Dodge, C. Mitchell, and D. J. Hanahan, *Arch. Biochem. Biophys*., **100**, 119-130 (1963).
14. J. Folch, M. Lees, and G. Sloane-Stanley, *J. Biol. Chem*., **226**, 497-509 (1957).
15. J. Gimsa, *Biophys. J.*, **75**, No. 1, 568-569 (1998).