DOI 10.1007/s10517-015-3048-9
Bulletin of Experimental Biology and Medicine, Vol. 159, No. 5, September, 2015

METHODS

Brattleboro Rats as the Model of Blood Hyperviscosity Syndrome for Testing Substances with Hemorheological Activity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 159, No. 5, pp. 653-655, May, 2015
Original article submitted June 17, 2014

Hyperviscosity syndrome was described in Brattleboro rats. The aim of this study was to investigate the possibility of Brattleboro rats using, as a test system for the study of agents with hemorheological activity. Under conditions of this model of high blood viscosity syndrome in Brattleboro rats, *Lychnis chalcedonica L.* extract (150 mg/kg) administered intragastrically for 10 days exhibited hemorheological activity by modulating macro- (plasma viscosity, fibrinogen concentration) and microrheological (erythrocyte aggregation and deformability) parameters. Hence, Brattleboro rats are an adequate model of hyperviscosity syndrome that can be used for search and testing of substances with hemorheological activity.

Key Words: *hereditary hypothalamic diabetes insipidus; Brattleboro rats; Lychnis chalcedonica extract; high blood viscosity syndrome; erythrocyte aggregation and deformability*

Brattleboro rats reproduce the main pathogenetic factors of hereditary hypothalamic diabetes insipidus. In Brattleboro rats, significant and pronounced shifts in macro- and microrheological parameters were found compared to Wistar rats: high values of whole blood and plasma viscosity, erythrocyte aggregation, fibrinogen concentration, hematocrit, and erythrocyte deformability low [6]. Analysis of the hemorheological profile attests to the formation of a pathological condition, high blood viscosity syndrome (HBVS) in Brattleboro rats.

The aim of this work was to evaluate the possibility of using Brattleboro rats as the test systems for evaluation of substances with hemorheological activity.

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MATERIALS AND METHODS

Experiments were carried out on 2.5-3.0-month-old male Brattleboro rats (n=16) and male Wistar rats (n=8) weighing 200-250 g. Brattleboro rats were obtained from the Research Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, and Wistar rats from the Department of Experimental Biological Models, E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine. The experimental animals are conventional by their microbiological status.

The rats were kept in standard Velaz plastic cages (5 per cage) on a bed of fine wood shavings under open regimen and on standard vivarium ration. The animals were kept in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986). The air temperature in the vivarium was 20-23°C, hu-

midity did not exceed 50%, air exchange 8:10 outflow-to-inflow, light conditions (day:night) 1:1.

After a 14-day quarantine, Brattleboro rats were divided into experimental and control groups. Dry *Lychnis chalcedonica* extract (LCE) exhibiting distinct hemorheological activity in various *in vitro* and *in vivo* models of blood hyperviscosity [1-4] was obtained from the aerial parts of *Lychnis chalcedonica L*. by repercolation with 40% ethanol solution, followed by lyophilization. LCE was standardized by ecdysterone content (0.74%). Experimental animals intragastrically received LCE suspension in 1% starch mucus in a dose of 150 mg/kg for 10 days; control rats received the same volume of 1% starch mucus.

At the end of experiment, the rats were anesthetized with ether, blood samples were taken through a Teflon catheter introduced into the common carotid artery, stabilized with 3.8% sodium citrate (1:9), and used for measuring whole blood viscosity, plasma viscosity, hematocrit, erythrocyte aggregation, and erythrocyte deformability. The viscosity of the whole blood and plasma were recorded on a rotary viscometer AKR-2. Hematocrit was determined by centrifugation in glass capillaries in a MHz-8 centrifuge. Fibrinogen content in the plasma was determined on a KG-IV coagulometer (Cormay) by Clauss chronometric method using a Tekh-Fibrinogen-test diagnostic kit (Technologiya-standart). Erythrocyte aggregation and deformability were measured using a RheoScan AnD-300 instrument (RheoMeditech Inc.). Aggregation halftime served as the measure of aggregation activity of erythrocytes (the decrease in this parameter corresponded to an increase in erythrocyte aggregation capacity). Erythrocyte deformability was evaluated by the elongation index of erythrocytes (EIE) at a shear stress of 20 Pa. Oxygen availability for tissues was calculated by the hematocrit/viscosity ratio at 300 sec^{-1} [8]. The animals were sacrificed in a CO_2-chamber.

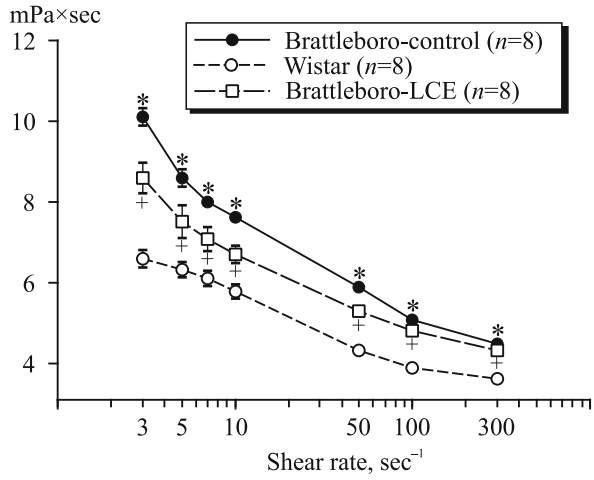

Fig. 1. Effect of course intragastrical administration of LCE on whole blood viscosity at different shear rates. *p<0.05 in comparison with *Wistar rats, +Brattleboro rats (control).

The data were processed statistically using Student's t test.

RESULTS

In Brattleboro rats, the whole blood viscosity in the entire range of shear rates was higher that in Wistar rats by 25-53% (Fig. 1), plasma viscosity by 20%, the concentration of fibrinogen by 51%, hematocrit by 7%, erythrocyte aggregation by 38%, and erythrocyte deformability by 21% (Table 1). Thus, the hemorheological profile in Brattleboro rats differed significantly from that in Wistar rats. Based on the analysis of certain hemorheological parameters and correlations between them, we concluded that blood hyperviscosity in these animals is not their biological characteristics,

TABLE 1. Effect of Course Treatment with LCE on Macro- and Microrheological Parameters in Wistar and Brattleboro Rats (M±m)

Parameter	Wistar rats (n=8)	Brattleboro rats	
		control (n=8)	LCE (n=8)
Plasma viscosity, mPa×sec	1.4±0.1	1.8±0.1*	1.6±0.1+
Hematocrit, %	45±1	49±1*	49±1
Fibrinogen concentration, g/liter	2.15±0.12	3.25±0.26*	2.33±0.14+
Erythrocyte aggregation halftime, sec	10.1±0.5	5.9±0.2*	9.2±0.7+
EIE	0.535±0.019	0.420±0.014*	0.500±0.011+
O_2 availability for tissues, arb. units	12.5±0.2	10.9±0.3*	11.6±0.3+

Note. p<0.05 in comparison with *Wistar rats, +control.

but a pathological condition, HBVS [6], caused by shifts in both macro- and microrheological parameters of the blood.

Course treatment with LCE significantly improved both the macro- and microrheological parameters of the blood in experimental rats: deformability of erythrocytes was improved in comparison with the control, EIE increased by 19% (Table 1), which can be due to antioxidant and antiradical activity of LCE [1]. Other substances with antiradical and antioxidant activity were also reported to increase erythrocyte deformability [5,7,9]. Erythrocyte aggregation was also influenced by LCE (Table 1). In Brattleboro rats of the experimental group, a significant (56%) increase in erythrocyte aggregation halftime was observed, which to a certain extent could be caused by reduced fibrinogen content (28%). Plasma viscosity also decreased due to the decrease in fibrinogen content. Due to cumulative effect of LCE on the basic macro- and microrheological parameters, viscosity of the whole blood significantly decreased by 4-15% in a wide range of shear rates (Fig. 1), which improved oxygen delivery to tissues by 6-12% in the studied range of shear rates (3-300 sec^{-1}) without changes in hematocrit.

Thus, under conditions of HBVS model in Brattleboro rats, course treatment with LCE (150 mg/kg intragastrically for 10 days) exhibited hemorheological activity and modulated macro- (plasma viscosity, fibrinogen concentration) and microrheological (erythrocyte aggregation and deformability) parameters. These findings suggest that Brattleboro rats are a convenient model of HBVS for the search and testing of substances with hemorheological activity.

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