EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Hyperviscosity Syndrome in Ovariectomized Rats

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Ovariectomy reduces blood levels of sex hormones and considerably increases blood viscosity due to an increase in hematocrit and plasma fibrinogen content, disorders in viscoelastic characteristics of erythrocytes, and increase of their aggregation activity. Changes in the macrorheology are mainly responsible for the development of the hyperviscosity syndrome.

Key Words: *ovariectomy; estrogens; hyperviscosity syndrome*

Increased incidence of cardiovascular diseases in women with estrogen deficiency caused by natural or surgical menopause is an important medical and social problem. Postmenopausal women form a group at a high risk of coronary disease [9]. An important task of this period is prevention and treatment of arterial hypertension, detected in $^2/_3$ of women with the climacteric syndrome [5]. Unfavorable time course of cardiovascular diseases during postmenopause is in line with positive effects of estrogens on the myocardium, vascular wall, and lipid metabolism [6,11]. Disorders in blood rheology can be important for the pathogenesis of cardiovascular diseases under conditions of natural or surgical menopause [1,12]. However, a comprehensive analysis of hemorheological status of postmenopausal women in comparison with the premenopausal period is difficult, because in addition to hypoestrogenemia, blood rheology can depend on the age (the onset of the natural menopause fluctuates within a decade), body weight index, tobacco smoking, and presence (or absence) of concomitant diseases (diabetes, hyperlipemia, *etc.*) [10].

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We evaluated changes in hemorheological status of ovariectomized inbred rats in comparison with sham-operated animals of the same age and body weight.

MATERIALS AND METHODS

Experiments were carried out on 19 female Wistar rats (320-380 g). The animals were obtained from Laboratory of Experimental Biosimulation, Institute of Pharmacology. The ovaries were excised by the common method under ether narcosis. Sham-operated animals were subjected to laparotomy and wound suturing. On day 21 of the experiment, common carotid artery was mobilized in ether-narcotized rats and catheterized for collection of blood specimens. The concentrations of sex hormones and rheological parameters were evaluated in blood samples. Plasma estrogen and progesterone were measured by enzyme immunoassay using Progesterone EIA (Chema) and EIAgen Estradiol (Adaltis Italia S.p.A.) kits on a Picone device.

Blood viscosity was measured on an AKP-2 rotation viscosimeter at shear rates of 3-300 sec^{-1}, plasma viscosity at shear rates of 300 sec^{-1} [3]. Erythrocyte aggregation was measured by syllectometry [4]. The aggregation half-time $T_{1/2}$ (time

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during which the photometric signal decreased 2-fold) served as the criterion of erythrocyte aggregation activity. Hematocrit was evaluated by centrifugation in glass capillaries. Erythrocyte deformability was studied by ektacytometry [2,6]. Erythrocyte deformability index (EDI) reflecting erythrocyte deformation capacity under the effects of shear rates was calculated by the formula:

$$EDI=(L-H)/(L+H),$$

where L and H are the greater and lesser diameters of the ellipse, respectively.

Plasma fibrinogen concentration was evaluated by Klaus' clotting method on a KG-4 coagulometer (Cormay) using Fibrinogen-test kit of reagents for measurements of fibrinogen concentration (Tekhnologiya-standart).

The results were statistically processed using Statistica 5.0 software.

RESULTS

Blood from sham-operated females was collected during the estrus phase. Blood estrogen and progesterone levels in these animals were 42.5±3.1 pg/ml and 71.0±13.4 nmol/liter, respectively. Ovariectomy led to cessation of estrous cycles in the females; analysis of vaginal smears showed only leukocytes and epithelial cells [8]. By the end of the experiment, estrogen level dropped to 17.6±1.5 pg/ml, progesterone level to 22.7±4.8 nmol/liter (by 59 and 68%, respectively, in these animals in comparison with sham-operated females).

The decrease in the level of sex hormone in ovariectomized rats was paralleled by the formation of hyperviscosity syndrome (HVS) characterized by significant shifts of macro- and microrheological parameters in comparison with sham-operated animals. Hematocrit increased by 7%, plasma fibrinogen content increased by 38% (Table 1). Significant shifts in cell rheology parameters were observed. The increase of erythrocyte aggregation characteristics manifested by reduction of their aggregation half-period (1.7 times) in comparison with sham-operated animals. A decrease in EDI at shear rate of 180-890 sec^{-1} by 13-25%, respectively, was noted (Table 1). Changes in the main rheological parameters led to a statistically significant increase (by 5-25%) in blood viscosity for the entire range of shear rate values (Fig. 1).

Similar changes in some blood rheology parameters (increase of hematocrit, plasma fibrinogen, erythrocyte hyperaggregation) were noted in postmenopausal women [1,12]. This indicates similarity

TABLE 1. Effects of Ovariectomy on Blood Rheology in Rats

Group	PV, mPa×sec	Hematocrit, %	FG, g/liter	$T_{1/2}$, sec	EDI, arb. units			
					90 sec^{-1}	180 sec^{-1}	360 sec^{-1}	890 sec^{-1}
Sham-operated (n=8)	1.39±0.03	43±1	2.4±0.4	19.4±1.5	0.095±0.005	0.210±0.010	0.292±0.010	0.362±0.004
Ovariectomized (n=9)	1.41±0.01	46±1^{+}	3.3±0.2^{+}	11.5±0.9^{+}	0.086±0.008	0.158±0.001^{+}	0.224±0.009^{+}	0.316±0.001^{+}

Note. PV: plasma viscosity; FG: plasma fibrinogen level. $^{+}p<0.05$ compared to sham-operated animals.

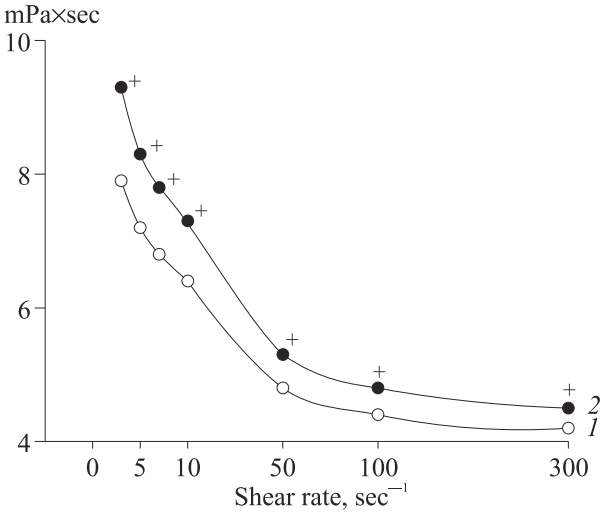

Fig. 1. Whole blood viscosity in rats on day 21 after ovariectomy and in sham-operated animals. *1*) sham-operated animals; *2*) ovariectomized rats. $^{+}p<0.05$ compared to sham-operated rats.

of the processes underlying blood rheology disorders in women with estrogen insufficiency and in ovariectomized animals. The experimental conditions (ovariectomy in inbred animals) ruling out the effects of age, body weight index, concomitant diseases, or tobacco smoking on blood rheology indicate that hypoestrogenemia is the main factor causing the detected shifts in blood rheology and forming HVS.

Analysis of correlations revealed a relationship between blood viscosity and some hemorheological parameters in sham-operated rats and confirmed known regularities [9]. Significant negative correlations were detected: between blood viscosity at high shear rate (300-100 sec^{-1}) and erythrocyte deformability (r=-0.856; $p<0.05$), between erythrocyte aggregation half-period, on the one hand, and hematocrit (r=-0.789, $p<0.05$) and plasma fibrinogen level (r=-0.937; $p<0.05$), on the other. Medium negative correlation was detected between blood viscosity at low shear rate (3-10 sec^{-1}) and erythrocyte aggregation half-period (r=-0.656; $p<0.05$). Blood viscosity was in positive correlation with plasma fibrinogen concentration (r=0.856; $p<0.05$) and hematocrit (r=0.726; $p<0.05$; Fig. 2). The relationship between blood viscosity and cell rheology parameters (erythrocyte aggregation and deformability) at the corresponding shear rate was lost in ovariectomized animals. These animals retained a significant positive correlation between blood viscosity at all shear rate values and hematocrit (r= 0.789; $p<0.05$) and negative correlation between plasma fibrinogen concentration and erythrocyte aggregation half time (r=-0.853; $p<0.05$). Hence, a specific feature of HVS model induced by ovariectomy is the predominance of changes in macrorheological parameters in the mechanism of blood viscosity increase (Fig. 2).

Hence, ovariectomy in rats causes a reduction of sex hormone level, an appreciable increase in blood viscosity as a result of increased hematocrit and plasma fibrinogen level, disorders in erythrocyte viscous elastic characteristics, and increase in their aggregation activity. Changes in macrorheological parameters make the main contribution to the formation of HVS after ovariectomy.

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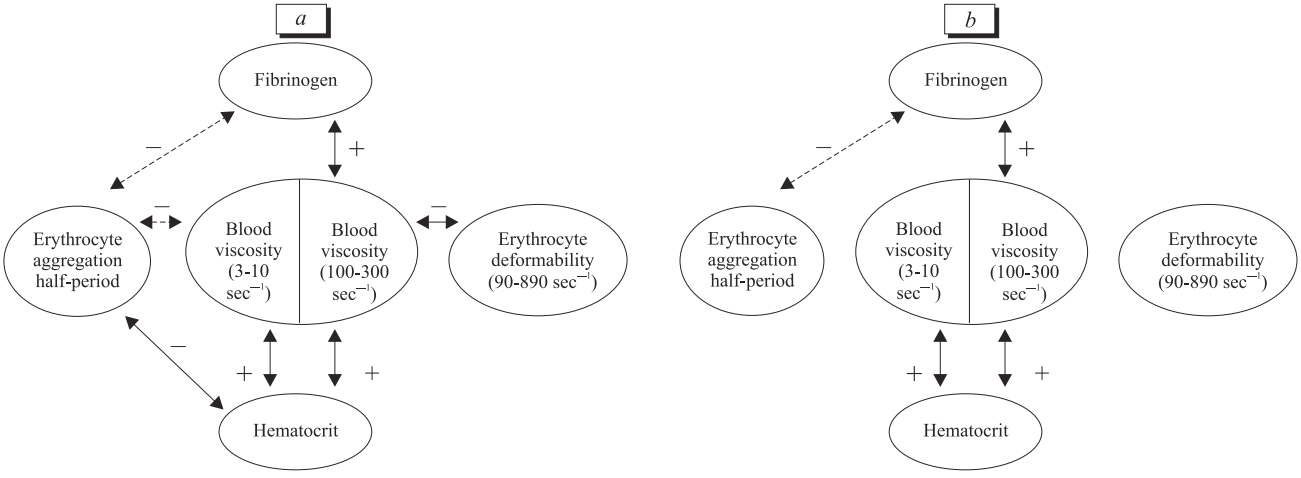

Fig. 2. Correlations between blood rheology in sham-operated (*a*) and ovariectomized rats (*b*). Arrow shows strict correlation, dotted arrow shows medium-strength correlation.

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