Pharmacological Correction of Hemostasis-Regulating Function of the Lungs in Rats with Uncomplicated Pregnancy and Experimental Gestosis

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Fetal growth retardation, hypercoagulation, and changes in pulmonary fibrinolytic activity were observed during experimental gestosis induced by long-term feeding of a high-sodium diet. The course of fraxiparine treatment to correct gestosis improved hemostasis-regulating lung function, decreased coagulation activity of the arterial blood, and increased the weights of the placenta and fetus.

Key Words: *hemostasis; lungs; fetoplacental complex; experimental gestosis; fraxiparine*

Placental dysfunction, vasospasm, and cytotrophoblast death with partial denudation of the basal layer accompany complicated pregnancy and are followed by a significant increase in coagulation activity of the blood outflowing from the placenta and impairment of the barrier function in the pulmonary fibrinolytic filter [2, 4,5]. Changes in rheological characteristics of the blood circulating in the intervillous space and hormonal imbalance can produce an adverse effect on the adaptive mechanisms, which promotes the development of gestosis, chronic placental insufficiency, and fetal growth retardation. Hemostasis, hemostasis-regulating function of the lungs, and efficiency of fraxiparine treatment for the correction of pulmonary fibrinolytic dysfunction in pregnant rats were studied on the model of alimentary gestosis [6] to evaluate pathophysiological changes during gestosis.

MATERIALS AND METHODS

Experiments were performed on pregnant albino rats weighing 170-190 g. The animals were quarantined for 7 days and then randomly divided into 4 groups (7 rats per group). Control group 1 included intact rats with uncomplicated pregnancy. Control group 2 included animals with uncomplicated pregnancy receiving intracutaneous injections of fraxiparine in a daily dose of 100 U/kg over 7 days starting from day 16 of gestation. Experimental group 1 included rats with experimental gestosis receiving no pharmacological correction. The animals with experimental gestosis receiving intracutaneous injections of fraxiparine (similarly to rats of control group 2) entered experimental group 2. Experimental gestosis was produced by feeding of a high-salt diet starting from day 7 of pregnancy [6]. The concentration of NaCl in drinking water was 1.8%. On day 21 of pregnancy the animals were narcotized with 60 mg/kg hexenal and fixed by the limbs and tail. The fetuses were removed after hysterotomy. The animals were killed. The blood was taken from the umbilical artery and vein during hysterotomy (before extraction of the fetus and placenta). We measured the weights of the placenta and fetus at birth. Biochemical studies were conducted. Plasma sodium concentration was measured by means of flame spectrophotometry with Vital Diagnostics kits. The blood clotting time (BCT) was measuring to study hemostasis. The concentrations of fibrinogen [1] and antithrombin III were

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estimated by immunodiffusion on Hoechst dishes using Nascola ecat fundation test system (Antithrombin Antigen). Progesterone concentration in the arterial and venous blood was determined by enzyme immunoassay with Immunotekh reagents. Cortisol concentration was measured by the radioimmunological method with Cea-Ire-Sorin test system. The data were processed using Statistica 5.5A software. The significance of differences and correlation indexes were estimated by parametric (Student) and nonparametric (Spearman and Wilcoxon tests).

RESULTS

The antithrombolytic effect of low-molecular-weight heparins is associated with antithrombin activity and release of extravascular coagulation inhibitor or antiaggregant compounds (prostacyclin). Therefore it can be used under condition of activated of coagulation and platelet hemostasis in patients with complicated pregnancy (gestosis and chronic fetoplacental insufficiency) [3, 7,8]. A hypocoagulant effect of the lungs was revealed in rats of control group 1. Arterial BCT was 11% higher compared to venous blood inflowing into the lungs ($p<0.05$, Table 1).

Administration of fraxiparine to animals of control group 2 increased BCT for the arterial and venous blood by 20 and 16%, respectively. It should be emphasized that after fraxiparine administration the difference between arterial and venous parameters was significant. After fraxiparine treatment the arteriovenous difference (AVD) in BCT increased by 3 times compared to animals of control group 1 (Table 1). Arterial BCT decreased by 11%, while venous BCT remained unchanged in rats with experimental gestosis (experimental group 1). A hypocoagulant effect of the lungs was not observed in animals with gestosis receiving 1.8% NaCl. A positive value of AVD in the clotting time was typical of all control animals. However, this parameter had a negative sign and approached zero in animals with gestosis (Table 1, Fig. 1).

Administration of fraxiparine to rats with experimental gestosis was followed by lengthening of the clotting time in the arterial (by 20%, $p<0.05$ compared to experimental group 1) and venous blood (by 15%, $p<0.05$). Under these conditions the lungs produced a normal hypocoagulant effect. AVD in the clotting time increased by 2.5 times after fraxiparine treatment (Fig. 1).

Fibrinogen concentration did not differ in the arterial and venous blood from animals of control group 1. Administration of fraxiparine to rats with uncomplicated pregnancy (control group 2) decreased fibrinogen concentration in the arterial blood by 2.3% ($p<0.05$ compared to control group 1), but had little effect on the venous blood. AVD was negative only in 57% animals of control group 1 (fibrinogen concentration tended to decrease). However, negative AVD was observed in 100% animals receiving fraxiparine (control group 2). These data illustrate strong fibrinolytic effect of the lungs (Fig. 2).

Hyperfibrinogenemia in the arterial and venous blood was found in rats with experimental gestosis receiving 1.8% NaCl. The pulmonary fibrinolytic effect was not observed in these animals. The mean AVD in fibrinogen concentration had a negative sign and approached zero (Table 1).

Administration of fraxiparine to rats with gestosis decreased the degree of hyperfibrinogenemia in the arterial and venous blood by 8 ($p<0.05$ compared to experimental group 1) and 5.5%, respectively. However, this treatment had no effect on pulmonary hypocoagulant activity. AVD was negative and close to zero (Table 1).

TABLE 1. Effect of Fraxiparine on Homeostasis Parameters in the Arterial and Venous Blood from Rats with Uncomplicated Pregnancy and Experimental Gestosis ($M\pm m$)

Parameter		Control		Experiment	
		group 1	group 2	group 1	group 2
BCT, sec	artery	322.45±6.51	388.42±10.02^{+}	285.75±7.98^{x}	341.75±11.86^{+}
	vein	290.82±10.39*	336.28±19.54^{+}	276.00±5.59^{x}	333.50±14.45^{+}
	AVD	17.28±5.27	52.14±12.07^{+}	9.75±9.61^{x}	24.50±7.83^{x}
Fibrinogen, g/liter	artery	4.38±0.03	4.27±0.03^{+}	4.95±0.06^{x}	4.55±0.12
	vein	4.39±0.05	4.31±0.03*	4.85±0.15	4.58±0.06^{+}
	AVD	-0.01±0.05	-0.030±0.006	0.15±0.18	-0.03±0.10
Antithrombin III, mg/dl	artery	8.87±0.37	8.04±0.16	7.76±0.47^{x}	8.00±0.57
	vein	9.62±0.39*	8.25±0.21^{+}	8.37±0.31*	8.72±0.15
	AVD	-0.75±0.15	-0.40±0.07^{+}	-0.67±0.25	-0.72±0.46

Note. AVD, arteriovenous difference. $p<0.05$: *compared to the artery; $^{+}$compared to group 1; ^{x}compared to the corresponding control group.

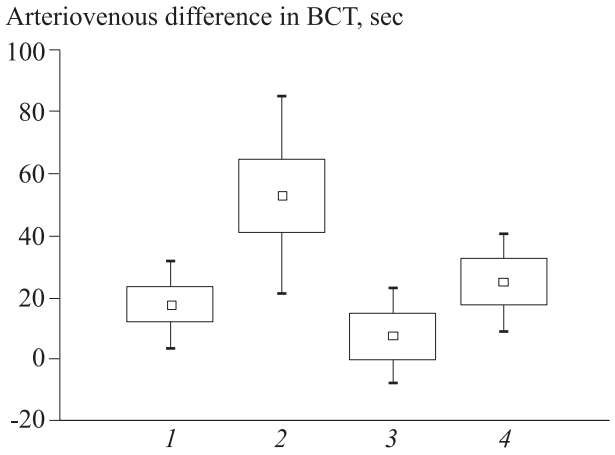

Fig. 1. Arteriovenous difference in blood clotting time (AVD) in rats with uncomplicated pregnancy and experimental gestosis (*M*±*m*). Here and in Fig. 2: control group 1 (*1*), control group 2 (*2*), experimental group 1 (*3*), and experimental group 2 (*4*).

Examination of rats with uncomplicated pregnancy showed that antithrombin III concentration decreases in the blood flowing from the lungs by 10%. Antithrombin III is synthesized in the lungs. Therefore, antithrombin III concentration in the umbilical vein reflects its initial concentration. A decrease in antithrombin III concentration in the arterial blood is associated with its consumption in the pulmonary endothelium.

Administration of fraxiparine to rats with uncomplicated pregnancy (control group 2) significantly decreased antithrombin III concentration in the arterial and venous blood (by 9 and 14, respectively, $p<0.05$ compared to control group 1). These changes reflect consumption of antithrombin III after heparin treatment. AVD in antithrombin III concentration was negative.

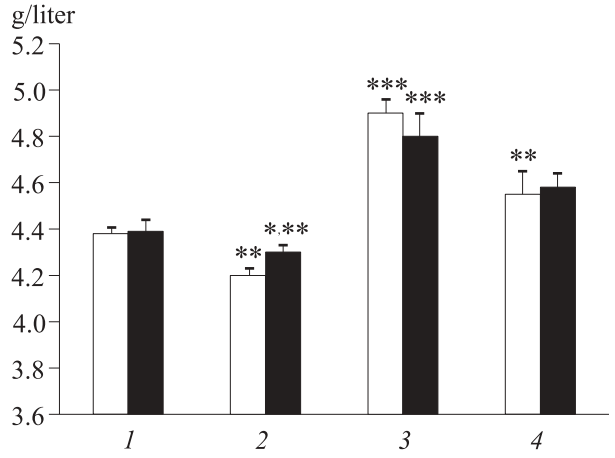

Fig. 2. Concentration of fibrinogen in arterial (light bars) and venous blood (dark bars) from rats with uncomplicated pregnancy and experimental gestosis. $p<0.05$: *compared to the artery; +compared to group 1; ×compared to the control group.

Gestosis is accompanied by pathological changes in various organs, including the endothelium of the lungs and liver. It contributes to a significant decrease in antithrombin III concentration in the arterial and venous blood (by 12.5 and 13.5%, respectively). Hence, antithrombin III concentration in animals with experimental gestosis is much lower compared to that observed in physiological pregnancy. These differences are probably associated with inhibition of antithrombin III synthesis in the liver, consumption of antithrombin III in the microcirculatory bed during hypercoagulation, and excretion of antithrombin III by the kidneys. Experimental animals were characterized by negative AVD in antithrombin III concentration. Under these conditions AVD was 2 times higher compared to uncomplicated pregnancy. Our findings demonstrate intensive consumption of the anticoagulant in pulmonary vessels during hypercoagulation.

Fraxiparine increased antithrombin concentration in the arterial (by 5%) and venous blood from rats with gestosis (experimental group 2). Table 1 shows that AVD in antithrombin III concentration was negative (85% animals) and below the corresponding value in the experimental group 1.

In rats with gestosis receiving 1.8% NaCl, the increase in plasma Na^+ concentration and fetal growth retardation correlated with changes in homeostasis parameters. A correlation was revealed between antithrombin III concentration in the venous blood and plasma Na^+ content (r=-0.73, $p<0.05$). The time of venous blood clotting linearly correlated with the weight of the placenta (r=0.83, $p<0.05$). Blood fibrinogen concentration negatively correlated with the weight of the placenta (r=-0.81, $p<0.05$), fibrinogen content, and weight of the fetus (r=-0.72, $p<0.05$). We found a positive correlation between antithrombin III concentration and weight of the fetus (r=0.69, $p<0.05$).

Pathological changes in the fetoplacental complex produced by 1.8% NaCl (decrease in the weight of the placenta and fetal growth retardation) were accompanied by an increase in coagulation activity of the blood outflowing from the placenta (decrease in BCT and increased production of fibrinogen) and reduction of anticoagulant activity (decrease in plasma antithrombin III concentration). During uncomplicated pregnancy the increase in blood coagulation activity is compensated by function of the pulmonary fibrinolytic filter (positive AVD in the clotting time and increased consumption of antithrombin III in the pulmonary endothelium). This mechanism is impaired during gestosis.

Our results indicate that administration of fraxiparine to animals with experimental gestosis not only decreases coagulation activity of the blood, but also improves function of the pulmonary fibrinolytic filter.

The increase in AVD in the clotting time reflects a hypocoagulant effect of the lungs. The improvement of rheological characteristics in placental blood inflow contributes to the correction of fetoplacental dysfunction during gestosis, which is confirmed by the increase in the weights of the placenta and fetuses in animals with experimental gestosis receiving fraxiparine.

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