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IMBALANCE OF THE HEMOCOAGULATION AND FIBRINOLYSIS SYSTEMS UNDER CONDITIONS OF DIABETES MELLITUS COMPLICATED BY ACUTE CIRCULATORY DISTURBANCE IN THE POOL OF CAROTID ARTERIES OF RATS

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Abstract

Introduction. Both cerebral ischemia and diabetes mellitus (DM) are evidenced to be associated with hypercoagulation state, though today the facts concerning hemocoagulation condition with diabetes mellitus complicated by cerebral ischemia-reperfusion are lacking.

The aim of the study. To examine the dynamics of interrelations between the pro-, anticoagulant and fibrinolytic parameters in rats with diabetes mellitus complicated by acute cerebral circulatory disturbance.

Results. At the early ischemic-reperfusion period activation of the pro-coagulant potential (decrease of prothrombin and thrombin time and increase of fibrinogen content) is found to be balanced by a reduced activity of XIII factor and an increase activity of antithrombin III in rats without diabetes mellitus. At the same period in rats with diabetes increase of the pro-coagulant potential is accompanied by the activation of XIII factor and a reduced activity of antithrombin III promoting clot formation. On the 12^{th} day of the observation the parameters of the coagulation chain in the hemostasis system and antithrombin III return to the level of the control animals in rats without diabetes, but in rats with diabetes the factors of intensification of a thrombotic risk remain activated.

At the early and late ischemic-reperfusion periods the parameters of blood fibrinolytic activity increase in rats without diabetes; in animals with diabetes mellitus the parameters of fibrinolytic activity remain without changes at the early period of observation (except decrease of a potential plasminogen activity), and they decrease on the 12th day of the post-ischemic period, which deteriorates conditions of thrombolysis.

Conclusions. Complication of diabetes mellitus by ischemic-reperfusion lesion of the brain results in imbalance in the hemocoagulation system at the expense of intensification of pro-coagulant mechanisms, and promotes inhibition of fibrinolytic processes with advanced changes in the dynamics of observation.

Key words: diabetes mellitus; cerebral ischemia-reperfusion; hemocoagulation; fibrinolysis.

Cerebral ischemia is a powerful stimulus for spontaneous coagulation resulting in reperfusion deficiency and death of the brain cells. The fact of hypercoagulation with diabetes mellitus is universally known as well. Nowadays a sufficient amount of studies confirm the role of disturbances in various chains of blood coagulation in pathogenesis of ischemic lesions of the brain [1, 2] and DM [3, 4]. Meanwhile, the facts concerning the state of hemocoagulation with DM complicated by cerebral ischemia-reperfusion are not sufficient.

Objective and tasks: to examine the dynamics of interrelations between the pro-, anticoagulant and fibrinolytic parameters in rats with diabetes mellitus complicated by acute cerebral circulatory disturbance.

Materials and methods of the study. The study is carried out on nonlinear albino laboratory male rats. DM was simulated by a single injection of Streptozotocin into the peritoneum («Sigma», USA, 60 mg/kg) of rats aged two months [5]. Duration of diabetes was 4 months. Incomplete global cerebral ischemia was simulated by bilateral clamping of the common carotid arteries during 20 minutes [6] under Calypsol narcosis (75 mg/kg). The early consequences of cerebral ischemia-reperfusion were studied 1 hour after reperfusion, and the remote ones – on the 12th day of the post-ischemic period. DM was confirmed by detection of glycemia level by means of glucose-oxidase method with the glucometer “One Touch Ultra Easy” (Life Scan, Deutschland) and destructive changes found in the islet apparatus of the pancreas. The experimental groups included rats with glycemia level 10 mmol/L and higher.

The state of hemostasis system was analyzed by the following parameters of the comprehensive hemostasiogram: coagulation hemostasis (prothrombin time (PT) and

fibrinogen content); anticoagulant blood potential (thrombin time and antithrombin III (ATIII) activity); fibrinolytic blood potential (Hageman-factor-dependent fibrinolysis (HFDF)), potential plasminogen activity (PPA), total, enzymatic and non-enzymatic fibrinolytic activity (TFA, EFA and NFA); post-coagulation phase (activity of XIII factor (fibrin stabilizing factor)) [7].

The results of the study are processed by means of the applied program package "Statistica ("Statsoft", USA). The groups of comparison were normally distributed according to Shapiro-Wilk test. Statistical significance of differences was evaluated by Student t-criterion for independent samples. The differences were considered reliable with probability of null hypothesis < 5 % ($p<0,05$).

Results and discussion. The changes in the parameters of the hemostasis system coagulation chain in rats of different experimental groups are presented in Table 1.

Table 1

Dynamics of the parameters of the hemostasis system coagulation chain in rats with diabetes mellitus complicated by cerebral ischemia-reperfusion (M±m, n=11)

Group of observation	Prothrombin time (PT), sec	Thrombin time (TT), sec	Fibrinogen content (g/L)
Control	20,51±0,72	15,39±0,18	2,283±0,048
Ischemia-reperfusion 20 min / 1 hour	18,48±0,68 $p<0,05$	14,21±0,11 $p<0,005$	2,575±0,127 $p<0,05$
Ischemia-reperfusion 12 days	21,26±1,01 $p_1<0,05$	14,86±0,16	2,317±0,143
Diabetes	18,06±0,81 $p<0,05$	13,28±0,13 $p<0,001$	2,200±0,055
Diabetes and ischemia-reperfusion 20 min / 1 hour	16,02±0,64 $p_2<0,05$	11,80±0,19 $p_2<0,001$	2,878±0,092 $p_2<0,001$
Diabetes and ischemia-reperfusion 12 days	17,68±0,89	12,39±0,15 $p_2<0,001$ $p_3<0,01$	2,594±0,128 $p_2<0,01$

Notes: here and in the following Tables: p – probability of differences compared with the control; p_1 – probability of differences compared with ischemia-reperfusion (20 min / 1 hour) in the control animals; p_2 – probability of differences compared with diabetes; p_3 – probability of differences compared with ischemia-reperfusion (20 min / 1 hour) in animals with diabetes.

As one can see, that 20-minute ischemia-an hour reperfusion in rats without DM resulted in 10 and 8% decrease of PT and TT respectively, and 13% increase of fibrinogen content. In general it is indicative of the inclination to hypercoagulation. Meanwhile, on the

12th day of the observation these parameters returned to the parameters of the control animals, and PT became 15% higher in comparison with the parameter at the early period, which is evidence of hemostasis normalization.

Though we have not determined prothrombin and thrombin levels directly, but decrease of PT and TT parameters is an indirect sign indicative of increasing the content and/or activity of these hemocoagulation cascade enzymes. In this respect, to interpret the results obtained we consider it reasonable to mention those scientific ideas that have been formed recently concerning a direct role of these enzymes in the brain in case of acute cerebral circulatory disturbances.

In recent years certain information has been accumulated concerning the local (extrahepatic) expression of prothrombin and thrombin in the central nervous system, and in the neurons and astrocytes in particular. In spite of the fact that it constitutes 1% only out of the hepatic expression, it plays an important role both in the processes of the CNS maturation, its normal functioning, and ischemic lesion of the brain [8]. Prothrombin of the hepatic origin after conversion into thrombin is able to penetrate through the damaged hematoencephalic barrier into the brain in spite of its substantial molecular weight, and together with thrombin of the cerebral origin can reach high concentrations there [9]. Increase of thrombin and its precursor prothrombin was demonstrated in the brain of rats after focal ischemia [10, 11]. Cerebral effects of thrombin with cerebral ischemia depend on its concentration – in low doses it produces a neuroprotective effect, in high doses – neurotoxic one [11-13]. Thus, intracerebral infusion of a low dose of thrombin activates endogenous neuroprotective mechanisms and increases tolerance to cerebral ischemia. In case of a considerable increase thrombin intensifies edema and secondary lesion of the brain after ischemia and hemorrhages [8, 14, 15]. Instillation of thrombin *in vitro* in nanomolar and micromolar concentrations induces death of the cells in the culture of the hippocampus and motor neurons [16]. Within the picomolar concentrations it protects neurons and astrocytes of the hippocampus against simulated focal ischemia or cellular strokes by means of hypoglycemia, glucose deprivation or the action of reactive oxygen species (ROS) [10, 11, 13, 14]. Thrombin inhibitors (PN-1, AT III, α_2-macroglobulin, α_1-antitripsin, C1-inhibitor and thrombomodulin) expressed locally in the brain [8] possess neuroprotective action.

During cerebral ischemia thrombin is found to act by several ways including direct cellular toxicity, disturbance of vascular state, intensification of OC and inflammatory reaction [17, 18].

In rats with DM much lower values of PT and TT are found than in animals without diabetes (12 and 14 % respectively), which coordinates with the existing views concerning hypercoagulation state with diabetes [19, 20].

20-minute ischemia-an hour reperfusion with diabetes caused 11 and 11% decrease of PT and TT and 31% increase of fibrinogen content in comparison with the parameters with diabetes without cerebral ischemia. On the 12^{th} day of the post-ischemic period compared with the parameters of rats with diabetes without acute cerebral circulatory disturbance (ACCD) TT remained 7% reliably decreased, and fibrinogen content –18 % increased. At this period TT increased reliably (5 %) concerning the parameter at the early period of the observation. PT returned to the parameters of animals with diabetes uncomplicated by ischemia-reperfusion of the brain.

In addition to fibrinogen, III phase of coagulation hemostasis is characterized by the activity of XIII factor (fibrin stabilizing factor), which 22% decreased (Table 2) at the early ischemic-reperfusion period in animals without DM concerning the parameter of the control animals. Meanwhile, on the 12^{th} day this parameter 29% has increased in comparison with the previous period and returned to the control level.

Table 2

Dynamics of antithrombin III and XIII factor in the blood of rats with diabetes mellitus complicated by cerebral ischemia-reperfusion (M±m, n=11)

Group of observation	Antithrombin III, %	XIII factor, %
Control	90,200±6,460	83,700±5,736
Ischemia-reperfusion 20 min / 1 hour	118,583±5,551 $p<0,005$	65,583±4,112 $p<0,01$
Ischemia-reperfusion 12 days	98,480±5,122 $p_1<0,05$	84,762±4,161 $p_1<0,01$
Diabetes	115,400±4,245 $p<0,005$	66,300±3,401 $p<0,01$
Diabetes and ischemia-reperfusion 20 min / 1 hour	103,222±3,093 $p_2<0,01$	74,222±3,201 $p_2<0,05$
Diabetes and ischemia-reperfusion 12 days	108,241±3,401	75,709±3,118 $p_2<0,05$

Much lower activity of fibrin stabilizing factor is found in rats with diabetes (21% decreased) than in the absence of the pathology. The activity of fibrin stabilizing factor was 12% higher at the early post-ischemic period in rats with diabetes compared with the same parameter in case of diabetes uncomplicated by ACCD, and on the 12^{th} day it was 14 %

higher.

Anticoagulation blood potential according to the activity of antithrombin III in rats without diabetes at the early ischemic-reperfusion period 31% increased (Table 2). On the 12th day of the observation the activity of antithrombin III 17% decreased concerning the early period and returned to the control level.

The activity of antithrombin III in rats with diabetes appeared to be 28% higher than in animals without the pathology.

The activity of antithrombin III 11% decreased at the early post-ischemic period in animals with DM, and at the late period this parameter did not differ from that of rats with diabetes without cerebral ischemia-reperfusion.

Fibrinolytic activity of the blood of rats from the experimental groups is characterized in Tables 3-4. The activity of HFDF, TFA and EFA 11, 16 and 32 % increased in rats without diabetes after 20-minute ischemia-one hour reperfusion in comparison with the control group; on the 12th day TFA and EFA remained elevated concerning the control (13 and 23 % respectively), HFDF returned to the control value, but PPA 9% increased.

Table 3

Effect of cerebral ischemia-reperfusion on the parameters of plasma fibrinolysis in rats with diabetes mellitus (M±m, n=11)

Group of observation	Total fibrinolytic activity (mkg azofibrin/g of tissue per hour)	Non-enzymatic fibrinolytic activity (mkg azofibrin/g of tissue per hour)	Enzymatic fibrinolytic activity (mkg azofibrin/g of tissue per hour)
Control	1,592±0,076	0,744±0,054	0,848±0,056
Ischemia-reperfusion 20 min / 1 hour	1,848±0,084 $p<0,05$	0,727±0,061	1,121±0,071 $p<0,01$
Ischemia-reperfusion 12 days	1,795±0,072 $p<0,05$	0,751±0,068	1,044±0,082 $p<0,05$
Diabetes	1,837±0,091 $p<0,05$	0,856±0,071	0,981±0,092 $p<0,01$
Diabetes and ischemia-reperfusion 20 min / 1 hour	1,678±0,086	0,846±0,097	0,832±0,074
Diabetes and ischemia-reperfusion 12 days	1,468±0,089 $p_2<0,01$	0,745±0,065	0,723±0,078 $p_2<0,05$

Fibrinolysis in rats with DM was characterized by higher parameters of TFA and EFA (15 and 16% respectively) and lower activity of HFDF and PPA (15 and 5% respectively) than in animals without the pathology.

Reliable changes of TFA, NFA, EFA and HFDF were not found after 20-minute ischemia-one hour reperfusion in rats with diabetes, and PPA 5% decreased in comparison with the parameter with diabetes uncomplicated by cerebral ischemia-reperfusion. On the 12th day of the observation the parameters of TEA and EFA 20 and 26% decreased respectively in animals with diabetes, and PPA remained on the level of the previous period of the observation.

Table 4

Peculiarities of dynamics of the hemostasis system fibrinolytic chain parameters in rats with diabetes mellitus complicated by cerebral ischemia-reperfusion (M±m, n=11)

Group of observation	Hageman-factor-dependent fibrinolysis, min	Potential plasminogen activity, min
Control	35,000±1,044	16,265±0,063
Ischemia-reperfusion 20 min / 1 hour	31,400±1,359 p<0,05	16,250±0,167
Ischemia-reperfusion 12 days	35,500±1,112	14,861±0,16 p<0,005 p_1<0,005
Diabetes	38,100±1,001 p<0,05	16,965±0,076 p<0,005
Diabetes and ischemia-reperfusion 20 min / 1 hour	36,000±0,707	17,867±0,104 p<0,001
Diabetes and ischemia-reperfusion 12 days	35,900±0,801	17,991±0,150 p<0,001

Analysis of the facts given enables to suggest that hypercoagulation signs in rats without DM during an acute phase of cerebral circulation disturbance are compensated by an increased activity of ATIII and decreased activity of XIII factor controlling clot formation, which is indirectly confirmed by the absence of fibrinolytic processes reaction according to the parameters of EFA, NFA and TFA, and reduced activity of HFDF that characterizes the internal way of fibrinolysis (kinin-kallikrein system). Returning of PT, activity of ATIII, HFDF to the control values on the 12th day of the observation is indicative of normalization of hemocoagulation processes, and increase of XIII factor activity at this period is compensated by intensification of EFA and TFA in the blood. Therefore, till the 12th day of the observation pro- and anticoagulation/fibrinolytic mechanisms in animals without diabetes become more or less balanced.

In rats with diabetes the response of these parameters at the early ischemic-reperfusion period resembles that in animals without DM, but their absolute values under conditions of diabetes were considerably lower and the state of hypercoagulation – deeper. It is confirmed

by a reduced activity of ATIII and increase of fibrin stabilizing factor. Moreover, considering the above data concerning cerebral effects of high thrombin concentrations a certain role of this enzyme could be suggested in deterioration of acute cerebral circulation disturbance with diabetes.

In addition such kind of pro-coagulation changes are accompanied by certain decrease of fibrinolytic activity, which is evidenced by a reduced potential plasminogen activity, that is, though compensatory mechanisms intended for maintenance of normal coagulation work in some measure with DM, they are absent when DM is complicated by cerebral ischemia-reperfusion.

The signs of hypercoagulation advance on the 12th day of the observation in rats with DM in the form of more considerable decrease of fibrinolytic activity at the expense of further inhibition of potential plasminogen activity and EFA of plasma. Thus, if at this period the majority of hemocoagulation parameters return to the normal values in rats without DM, with diabetes hypercoagulation aggravates.

Conclusions:

1. In rats without diabetes mellitus at the early ischemic-reperfusion period activation of pro-coagulation potential (decrease of prothrombin and thrombin time and increase of fibrinogen content) is balanced by a reduced activity of XIII factor and increase of antithrombin III activity. In rats with diabetes increase of pro-coagulation potential at this period is accompanied by XIII factor activation and reduced activity of antithrombin III promoting clot formation. On the 12th day of the observation the parameters of the hemostasis system coagulation chain and antithrombin III in animals without diabetes return to the control values, and in animals with diabetes the factors intensifying clot formation risk remain activated.

2. The parameters of blood fibrinolytic activity increase at the early and late ischemic-reperfusion periods in rats without diabetes; in animals with diabetes mellitus at the early period of the observation the parameters of fibrinolytic activity remain unchanged (except decrease of potential plasminogen activity), they decrease on the 12th day of the post-ischemic period which deteriorates the terms of thrombolysis.

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