Vascular Thrombogenicity and Thromboresistance during Postischemic Reperfusion

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Responsiveness, thrombogenicity, and thromboresistance of mesenteric microvessels were studied in rats during postischemic reperfusion of the intestine, brain, and hind limb. Irrespective to localization of the ischemized organ, the mesenteric microvessels increased responsiveness and decreased thromboresistance. Vascular thrombogenicity depended on the localization of ischemia. During cerebral reperfusion, thrombogenicity of some venules was decreased against the background of its general increase in arterioles. Microvascular disturbances are predominantly related to perturbations in nitric oxide synthesis.

Key Words: *ischemia; reperfusion; microcirculation; distant disturbances; nitric oxide*

The effect of ischemia and postischemic reperfusion on blood vessels and tissue in ischemized organ is studied in detail. However, both ischemia and reperfusion are often accompanied by various systemic disturbances, such as endogenous intoxication syndrome, blood hypercoagulation, *etc.* [3]. Many mechanisms leading to tissue damage during postischemic reperfusion are known, but no leading factor is established. One of the major damaging factors in reperfusion is oxygen radicals. Oxygen radicals can be produced by endotheliocytes during myocardial ischemia and reperfusion [2]. This mechanism is nonspecific and realized in all tissues during postischemic reperfusion [12]. There is evidence that the major factor of tissue damage during postischemic reperfusion is neutrophilic leukocytes [4] releasing oxygen radicals and proteolytic enzymes. Some authors believe that major source of free oxygen radicals, in particular, hydroxyl radical is parenchymatous cells in ischemized organ releasing biologically active substances, that promote leukocyte adhesion to the endothelium, thus aggravating the damage [8].

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MATERIALS AND METHODS

The study was carried out on mature male rats (body weight 200-250 g) anesthetized with nembutal (50 μg/kg intramuscularly). Ischemia of the brain, small intestine, and hind limb was modeled by complete clamping of both common carotid, mesenteric, and femoral arteries, respectively. In all experimental groups, the blood flow was restored after 1-h ischemia; the thrombogenic properties, thromboresistance, and vascular responsiveness of mesenteric vessels were studied for 1 h. The intestine was placed on a plate thermostabilized at 37°C and persistently rinsed with warm physiological solution.

The thrombogenicity and thromboresistance of arterioles and venules were studied on a model of laser-induced thrombosis [1]. Venules and arterioles (d=30-40 μ) were subjected to pulse laser irradiation (λ=337 nm). Laser beam was focused with the help of a microscope optic system, which produced a narrow beam in the object's plane with a diameter of about 10 μ. The energy of laser radiation at this wavelength is absorbed by hemoglobin, whereas plasma and protein components of the vascular wall are transparent in this optical range. Temperature of irradiated blood volume in microvessels increases, which produces damage to the endothelium lumen. Such damage could

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lead to rhexis or thrombus in the vessel. The data were recorded with a video tape recorder and analyzed off-line. The frame-to-frame image analysis determined the duration of thrombus development at the initial stage, its length along the vascular wall, and the character of embolism.

The responsiveness of rat mesenteric vessels was studied interactively by TV-biomicroscopy (MT-9 microscope, LOMO). Vessel reaction to application of various concenration norepinephrine was determined (0,2% norepinephrine hydrotartrate). The bloodflow and vessel diameter was evaluated on the monitor with the help of a visual marker.

The data were statistically analyzed using Student's *t* test.

RESULTS

Thrombogenicity and thromboresistance of blood vessels, as well as their responsiveness were studied during the 1st hour of reperfusion. The thrombogenicity of blood vessels was characterized by primary hemostasis time, thrombus growth rate, thrombus size, and specific features of the embolization period. In the case of rhexis of arterioles in the control group during modeling of laser-induced thrombosis, hemorrhage was arrested in all vessels during 1 min. The primary hemostasis time was 21 sec on average (Table 1), which was considered as effective hemostasis. In venules, primary hemostasis was effective in 80% cases, while the period of microhemorrhage arrest was significantly longer than in arterioles, which agrees with previous data [10]. In arterioles of the test animals the efficient primary hemostasis was observed during cerebral reperfusion (CR), intestinal reperfusion (IR), and hind limb reperfusion (HLR). In HLR, complete arrest of microvascular hemorrhage occurred in 98% venules during 90 sec (*vs.* 80% the control). It attests to increased thrombogenicity of venules during HLR, which may be related to thrombogenic factors of the vascular wall. The ischemic zone is characterized by increased production of thromboxane A_2 [7] and the release of Willebrand's factor [11] and tissue thromboplastin [5], which enhance the thrombogenic properties of the vessels. Simultaneously, venous thrombogenic properties decreased in 39% cases during CR and in 50% cases during IR, which can be explained by damage to venul wall by enzymes released from leukocytes [9]. In a vessels with complete hemostasis, its temporal characteristics did not differ from the control (Table 1).

In control rats, the length of developed thrombus in venules was larger than in arterioles, which attests to a higher thromboresistance of the arterial endothelium. In all experimental rats, thromboresistance of the vascular wall was markedly decreased: the mean thrombus length (up to the moment of abruption) increased in arterioles and, to a lesser degree, in venules. Maximum disturbances in thromboresistance were observed during IR, i.e. in vessels where 1-h hemostasis was modeled, and in vessels located in the immediate proximity to the ischemized organ. Less pronounced disturbances were observed during CR and HLR, i.e. during reperfusion of the organs located distantly in respect to the examined area. The embolization time in arterioles and venules of the test rats also increased, the changes in arterioles being more pronounced (Table 1).

The study of vascular responsiveness revealed general increase in sensitivity of mesenteric arterioles and venules to norepinephrine in rats subjected to CR and IR. In the control group, norepinephrine in a concentration of 10^{-11} M produced a significant vasoconstriction (no less than 20%) in almost all vessels. At a concentration of 10^{-12} M vasoconstriction was observed in less than 50% vessels. A high concentration (10^{-8} M) of norepinephrine produced reversible hemostasis. The threshold of mesenteric vascular sensitivity to norepinephrine was increased in rats subjected to CR and IR (most arterioles responded to norepinephrine in a concentration of 10^{-12} M). In addition, vascular constriction was significantly enhanced

TABLE 1. Thrombogenicity of Mesenteric Arterioles and Venules During Postischemic Reperfusion in Rats (*M±m*)

Group	Mean period of primary hemostasis, sec		Thrombus formation			
			thrombus length, μ		mean time of abruption, sec	
	arterioles	venules	arterioles	venules	arterioles	venules
Control (*n*=15)	21±2.7	43±1.3	25±3.2	30±2.0	15±0.9	14±1.3
CR (*n*=10)	24±2.3	45±2.5	40±7.5*	38±4.5*	39±13*	18±8.6
IR (*n*=9)	24±1.2	41.4±6	42±2.5*	40±4.0*	32±0.5*	15±10
HLR (*n*=8)	25±3.1	43.1±4	30±2.5	40±2.6*	36±0.3*	13±1.2
CR+L-arginine(*n*=9)	24.6±3.7	37.7±5	33.4±3.5	35.5±2.0	32.3±10	18±5.5

Note. Here and in Table 2: *$p<0.05$ compared with the control.

TABLE 2. Indices of Responsiveness (Percentage of Constriction) of Mesenteric Vessels to Norepinephrine (*M±m*)

Group		Norepinephrine concentration, M	
		10^{-12}	10^{-11}
Control	arterioles	23.0±1.7	31.4±1.2
	venules	24.1±1.3	33.0±0.6
CR	arterioles	34.1±1.2*	36.3±1.5*
	venules	32.3±1.9*	40.8±2.9*
IR	arterioles	34.9±1.9*	40.0±1.6*
	venules	42.0±3.4*	43.0±1.3*
HLR	arterioles	27.1±1.5	37.2±2.0
	venules	27.3±1.4	39.1±2.0*
CR+L-arginine	arterioles	27.3±0.9	34.4±3.3
(*n*=9)	venules	34.5±1.2*	38.7±2.6*

in comparison with the control (Table 2). Hemostasis in rats subjected to CR and IR was produced by norepinephrine in concentration of 10^{-10} M. Similar but less pronounced changes in the responsiveness of mesenteric vessels were observed in the rats subjected to HLR.

Therefore, postischemic reperfusion produces a systemic effect on microvessels and disturbs their thrombogenicity and responsiveness to norepinephrine.

To evaluate the role of disturbances in nitric oxide (NO) synthesis in vascular reactions, we studied responsiveness, thrombogenicity, and thromboresistance of vessels during CR against the background of intravenous injection of L-arginine (300 mg/kg), the substrate for NO synthesis, immediately prior to reperfusion. In this case, thromboresistance and vascular responsiveness (mostly of arterioles) returned to the control level in contrast to analogous experiments without injection of L-arginine (Tables 1 and 2). Thus, our data indicate that NO synthesis is moderated during postischemic reperfusion. The disturbances in NO synthesis occur not only in the ischemized area, but also in distant regions irrespective on the localization of the ischemized organ. Although the mechanism of distant alterations of the vascular wall is not completely understood, experimental data suggest that plasma collected from ischemic limb decreases NO production in intact vessels [6].

The distant disturbances of thrombogenicity and thromboresistance of blood vessels, as well as alterations in their responsiveness are evidently related to disturbances in ischemized organs during their reperfusion. Bioactive substances released from the ischemized area during postischemic reperfusion produce a systemic effect on vessels, for example, affect the intensity of NO synthesis. Thus, ischemia and postischemic reperfusion modify vascular properties not only in the ischemized organ, but in distant organ, due to the effects of biologically active substances released from the ischemized tissue. This effect is manifested in changed thrombogenicity, thromboresistance, and vascular responsiveness, which can produce microcirculatory disturbances in various organs and tissues.

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