Leukocytes as a Cause of Microcirculatory Dysfunction

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Massive leukocyte adhesion to the endothelium of pial veins in rats with ischemic injury of brain tissue was studied by the method of vital microscopy.

Key Words: *ischemia; leukocytes; erythrocytes; microcirculation*

A rigid structure of mammalian leukocytes is related to the presence of the nucleus and high viscosity of the protoplasm. These cells can adhere to the wall of microvessels. Leukocytes have a spherical shape and their volume 1.5-2.2-fold surpasses that of erythrocytes. Because of these specific features, leukocytes decrease blood flow velocity in microvessels and contribute to constant variations in the rate of microcirculation under normal conditions [1,2]. It was hypothesized that leukocytes impair microcirculation and promote the development of cerebral hypoxia during changes in blood supply to the brain [3-5].

Here we studied adhesion of leukocytes to the vascular wall and other leukocytes under conditions of cerebral ischemia. The influence of massive leukocyte adhesion on the course of cerebral ischemia was evaluated by means of vital microscopy.

MATERIALS AND METHODS

Vital study of circulation of erythrocytes and leukocytes in pial microvessels was performed on narcotized rats. We used a Lyumam-1 microscope with a contact objective, TS-6020 PSC miniature color-video camera, Intel Pentium 4 computer, and Pinnacle system (image processing). Final monitor magnification was ×1000 or 2000. The measurements were calibrated using a standard OSh-1 micrometer (scale interval 10 μ).

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Adherent leukocytes were counted in pial venules and smallest veins (diameter 10-30 μ) of 9 male Wistar rats weighing 200-250 g. The adhesion rate was calculated per unit vessel length (1000 μ) at various stages of ischemia. Cerebral ischemia was induced by ligation of the common carotid artery. The severity of cerebral ischemia was estimated by blood pressure in the circle of Willis. Blood pressure was measured by the direct method using a polyethylene catheter. The catheter was inserted above the site of carotid artery ligation. Systemic blood pressure (SBP) and blood pressure in the circle of Willis were measured with mercury barometers. The heart rate (HR) was determined by ECG. We measured the respiration rate and core body temperature (5 cm from the anus). The results were analyzed by Student's *t* test.

RESULTS

After common carotid artery ligation, SBP was 106 mm Hg (Table 1). Blood pressure in the circle of Willis decreased to ~41 mm Hg. SBP was disproportionate to tissue blood supply. However, our findings suggest that vertebrate arteries provide 30-40% blood supply to the brain under conditions of common carotid artery ligation (compared to normal). This value is similar to blood supply in intact brain tissue near the necrotic focus during cerebral stroke (*penumbra*). Published data show that common carotid artery ligation in rats is the most suitable model to study human cerebral stroke and determine the cause of impaired blood supply to the penumbra and extension of the necrotic area [5].

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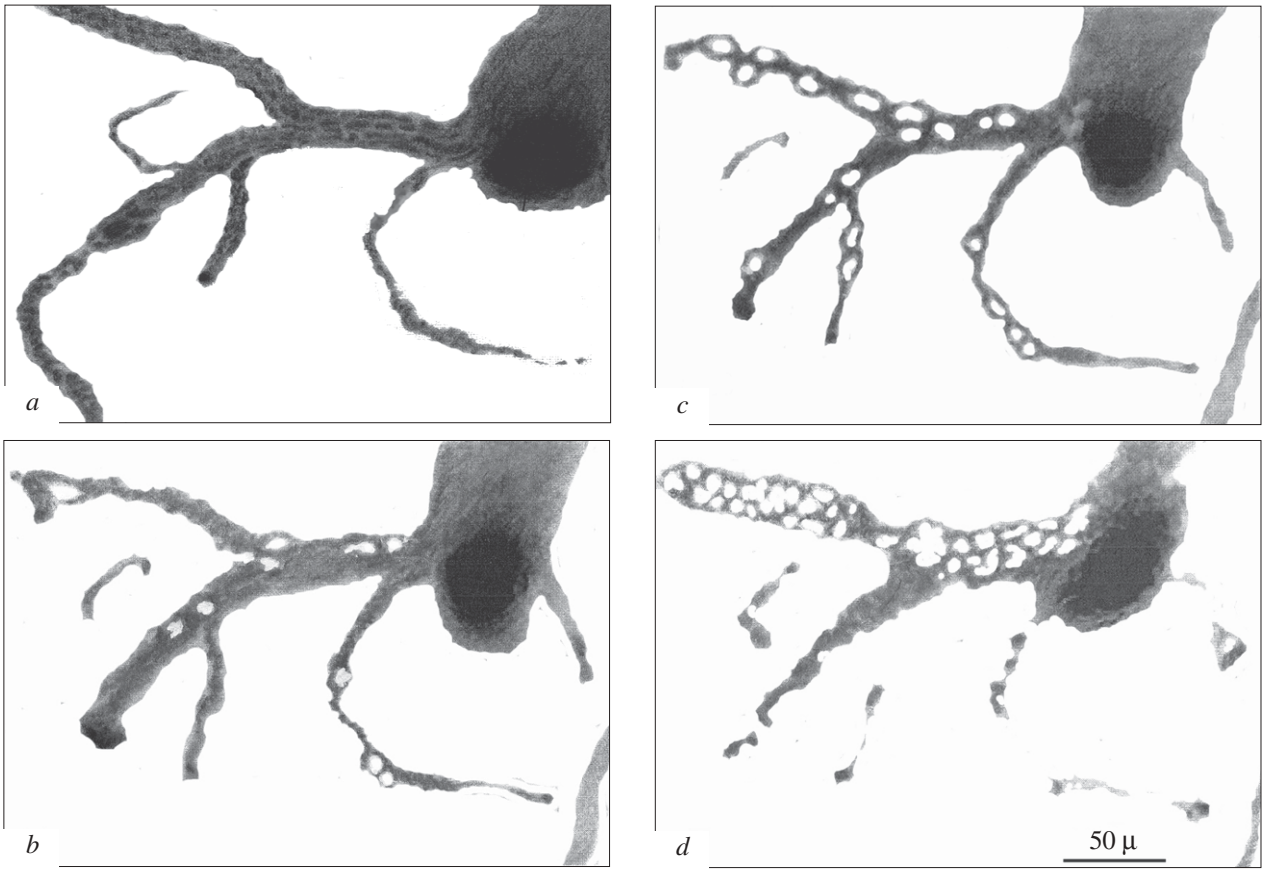

Fig. 1. Increase in the rate of leukocyte adhesion to the wall of venous microvessels in the pia mater of rats with cerebral ischemia. Basal level (*a*); 2 h after artery ligation (*b*); after 3 h (*c*); 4.5 h after ligation, leukocyte agglutination and occlusion of venules.

Massive leukocyte adhesion to the wall of venules and smallest veins in the brain (diameter 10-30 μ) was observed 1 h after common carotid artery ligation. The adhesion rate per 1000 μ vessel length increased by 10 times compared to the basal level and continued to increase over the next 3 h (Fig. 1). The terminal stage of ischemia developed over the 5th hour (infrequent spasmodic breathing, sharp decrease in HR and SBP, and cessation of lung breathing). During this period we revealed a sharp increase in the adhesion rate in venous vessels of the brain (Table 1). Leukocyte agglutination in the venule and smallest vein of the brain was observed several minutes after cessation of breathing (Fig. 2).

TABLE 1. Physiological Parameters before and after Common Carotid Artery Ligation (*M*±*m*)

Period		Adhesion rate per 1000 μ vessel length	SBP, mm Hg	Blood pressure in the circle of Willis, mm Hg	HR, min^{-1}	Respiration rate, min^{-1}	Core body temperature, °C
Before ligation		2±1	115±5	115±5	485±20	45±3	37.0±0.7
After ligation, h	1	25±4***	106.3±4.7	41.3±1.9***	480±19	53±5	35.5±0.5
	1.5	30±5	98.9±6.2	36.7±0.9	488±18	57±5	36.1±0.7
	2	24±2	106.9±4.7	40.5±4.3	498±19	63±3	36.2±0.7
	3	29±4	95.4±9.7	36.8±8.1	475±14	59±5	35.6±0.5
	4	32±5	99.3±3.1	33.5±0.5	480±30	54±2	35.6±0.9
Before cessation of breathing		60±5*	47.5±9.8*	8.0±4.0*	393±46**	29±3*	36.2±0.6
After cessation of breathing		0	30.3±5.6**	3.3±3.3	186±54*	0	34.6±0.6**

Note. *p<0.01, **p<0.05, and ***p<0.001 compared to the previous period.

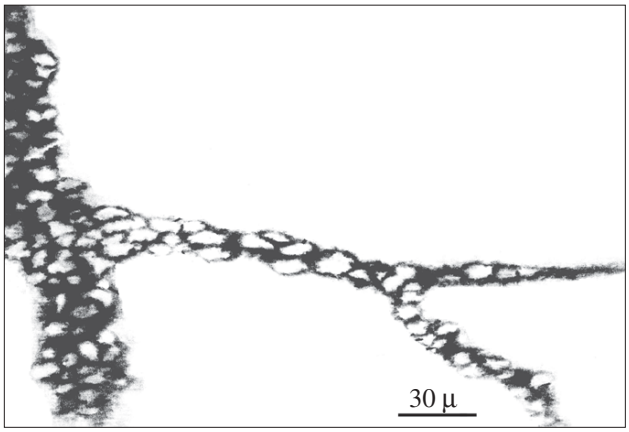

Fig. 2. Massive leukocyte adhesion to the wall of venules after cessation of lung breathing. Widening and deformation of venules.

A new pathogenetic phenomenon of ischemia suggests massive blockade of venous microvessels with leukocytes. These data explain the fact that it is difficult or impossible to restore microcirculation in the brain after profound and long-term cerebral hypoxia (no-reflow phenomenon). Massive leukocyte adhesion and microcirculatory disturbances contribute to an extension of the necrotic area after cerebral stroke. Leukocytes contribute to blockade of venous microvessels in the brain, which causes death of the organism during ischemia.

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