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RHEOLOGIC AND MORPHOMETRIC PARAMETERS OF THE MICROCIRCULATORY BED OF THE RABBIT EAR AFTER LOCAL ISCHEMIA

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UDC 616-005.4-031.84-092.9-07:616.16-008.1.-092:616.151.4

KEY WORDS: microcirculation; rheology; morphometry; ischemia.

The study of the adequate level of the blood supply at the microcriculatory levels requires evaluation not only of pathogenetic factors, but also of their correlations with one another, maintaining tissue homeostasis. Much remains uncertain about the role of blood viscosity in the microcirculation [9, 11], although negative correlation has been demonstrated between viscosity and velocity of the blood flow in the microcirculatory bed of the brain, both intact and ischemic [11]. Viscosity does depend on the hematocrit value, but in clinical practice this relationship is not always observed [5] and, in addition, the hematocrit index is not always the dominant factor responsible for the change in blood flow in the microvessles [1]. Positive correlation has been found between the viscosity of arterial blood and the diameter of microvessels [8], and this may evidently be taken as a manifestation of autoregulatory processes. Much attention is being paid to blood cells as a factor controlling the blood flow in microvessels. Some workers consider that the main role in microcirculatory disturbances in pathological states is played by changes in the rheologic properties of the blood and vessel wall [3, 6]. Postischemic disturbances at the microcirculatory level have mainly been studied when the blood flow is restricted in either the arterial or the venous section. A model of ischemia due to simultaneous occlusion of artery and vein has rarely been used. When the arterial blood flow is restricted, myogenic and metabolic mechanisms of autoregulation have been shown to act in the same direction, but when the venous outflow is limited, these mechanisms act in opposite directions [7, 10] and reactive hyperemia is depressed or absent altogether. The effects of simultaneous occlusion of artery and vein will therefore evidently depend on relations between these mechanisms in the region concerned.

The aim of the present investigation was to study correlations between rheologic and morphometric parameters of the microcirculatory bed in the rabbit ear during and after local ischemia.

EXPERIMENTAL METHODS

Experiments were carried out on 10 chinchilla rabbits weighing 2-2.5 kg, by a method using a transparent chamber [4] 7 weeks after implantation. Ischemia was produced by folding the pinna at the junction between its upper and middle thirds. Along the line of folding

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 407-409, April, 1988. Original article submitted April 28, 1987.

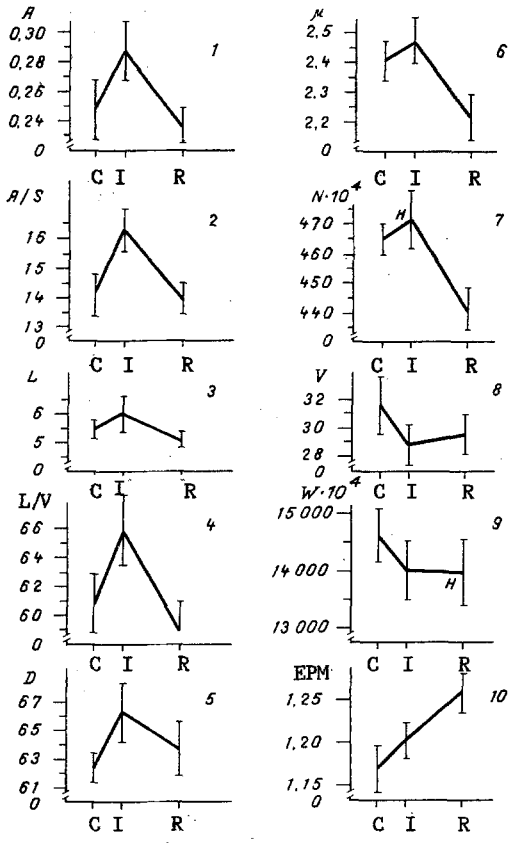

Fig. 1. Time course of rheologic and morphometric parameters of microvessels of rabbit ear after local ischemia. Abscissa: C) control (before clamping), I) immediately after ischemia, R) 1 h after ischemia. Ordinate: A) area of projection of perfused microvessels (in mm^2); A/S) relative area of microvessels (in %), L) length of microvessels (in mm); L/B) relative length (in mm/m^3); D) mean diameter (in μ); μ) apparent viscosity of blood (in cP); N) number of erythrocytes per unit volume of blood ($1/mm^3$); V) mean volume of erythrocyte (conventional units); W) erythrocyte mass (conventional units); EPM (in $\mu \cdot sec^{-1} \cdot V^{-1} \cdot cm$).

a soft clamp was applied for 30 min, so as not to crush the tissues. Blood was taken from the medial vein a few seconds after removal of the clamp. The apparent viscosity of whole blood (μ) was estimated by means of a "Copley" capillary viscometer, with a shear stress of 3.68 dynes/cm^2. The electrophoretic mobility (EPM) of the erythrocytes was determined in blood diluted 1:800 with phosphate buffer, pH 7.34, on an apparatus with a Goryaev's chamber, in our own modification [2]. The distributions of the erythrocytes by volume (V) and the number of cells (N) in unit volume of blood in a dilution of 1:80,000, in salt solution buffered with phosphate buffer to pH 7.34, were estimated by a conductometric method on the "Celloscope-101" instrument. The erythrocyte mass (W) was calculated as N. V. Morphometry of the microcirculatory bed was carried out with the "Leitz-TAS" television analyzing system [4]. The total area of projection of the perfused microvessels (A) and the degree of vascularization of part of the chamber (A/S, where S is the area of the part of the chamber in which the measurements were made) were calculated; the total length of the microvessels (L), the relative length (L/V), and the mean diameter (D) also were determined. The results were subjected to statistical analysis by "Olivetti" computer. The significance of differences was determined by Student's test for tied pairs, and crossed-correlation between variables was estimated by methods of correlation analysis.

EXPERIMENTAL RESULTS

The time course of the rheologic and morphometric parameters is illustrated in Fig. 1. It must be noted that the initial level of viscosity of the outflowing blood was lower

Table 1. Correlation between Rheologic and Morphometric Parameters (correlation matrix) of Microvessels of Rabbit Ear after Local Ischemia

Exptl. conditions	Parameter	1	2	3	4	5	6	7	8	9	10
Control	1	1,000	0,999	0,843	0,843	—0,021	—0,095	—0,002	0,416	0,392	0,662
	2	0,999	1,000	0,839	0,839	—0,017	—0,094	—0,004	0,410	0,385	0,665
	3	0,843	0,839	0,839	1,000	—0,519	—0,460	0,211	0,640	0,686	0,719
	4	0,843	0,839	1,000	1,000	—0,520	—0,461	0,212	0,640	0,686	0,720
	5	—0,021	—0,017	—0,519	—0,520	1,000	0,779	—0,367	—0,559	—0,660	—0,485
	6	—0,095	—0,094	—0,460	—0,461	0,779	1,000	—0,225	—0,353	—0,399	—0,236
	7	—0,002	—0,004	0,211	0,212	—0,367	—0,225	1,000	—0,041	—0,317	0,121
	8	0,416	0,411	0,640	0,640	—0,559	—0,353	—0,041	1,000	0,932	0,591
	9	0,392	0,385	0,686	0,686	—0,660	—0,399	—0,317	0,932	1,000	0,586
	10	0,662	0,665	0,719	0,720	—0,485	—0,236	0,121	0,591	0,586	1,000
Ischemia	1	1,000	1,000	0,927	0,927	—0,089	0,103	—0,117	0,611	0,227	0,575
	2	1,000	1,000	0,928	0,928	—0,090	0,102	—0,119	0,612	0,227	0,574
	3	0,927	0,928	1,000	1,000	—0,453	—0,075	—0,001	0,769	0,406	0,635
	4	0,927	0,928	1,000	1,000	—0,453	—0,075	—0,001	0,769	0,406	0,635
	5	—0,089	—0,090	—0,453	—0,453	1,000	0,466	—0,284	—0,609	—0,564	—0,327
	6	0,103	0,102	—0,075	—0,075	0,466	1,000	0,563	—0,194	0,208	0,331
	7	—0,117	—0,119	—0,001	—0,001	—0,284	0,563	1,000	0,185	0,775	0,602
	8	0,611	0,612	0,769	0,769	—0,609	—0,194	0,185	1,000	0,731	0,586
	9	0,227	0,227	0,406	0,406	—0,564	0,208	0,775	0,731	1,000	0,649
	10	0,575	0,574	0,635	0,635	—0,327	0,331	0,602	0,586	0,649	1,000
Recovery	1	1,000	1,000	0,921	0,921	—0,342	—0,404	0,267	0,305	0,190	0,368
	2	1,000	1,000	0,921	0,921	—0,341	—0,403	0,268	0,307	0,192	0,368
	3	0,921	0,921	1,000	1,000	—0,675	—0,386	0,393	0,406	0,296	0,449
	4	0,921	0,921	1,000	1,000	—0,675	—0,386	0,393	0,406	0,297	0,449
	5	—0,342	—0,341	—0,675	—0,675	1,000	0,203	—0,495	—0,398	—0,358	—0,474
	6	—0,404	—0,403	—0,386	—0,386	0,203	1,000	0,013	—0,165	—0,105	0,041
	7	0,267	0,268	0,393	0,394	—0,495	0,013	1,000	0,388	0,707	0,935
	8	0,305	0,307	0,406	0,406	—0,398	—0,165	0,388	1,000	0,827	0,248
	9	0,190	0,192	0,296	0,297	—0,358	—0,105	0,707	0,827	1,000	0,484
	10	0,368	0,368	0,449	0,449	—0,474	0,041	0,935	0,248	0,484	1,000

Legend. Significance of parameters, respectively: 1) A; 2) A/S, 3) L; 4) L/V, 5) D; 6) μ, 7) N, 8) V, 9) W, 10) EMP.

in the animals with diffusion chambers than in those without (2.41 ± 0.16 and 3.14 ± 0.15 cP respectively, $p < 0.05$).

It follows from Fig. 1 that ischemia for 30 min led to a clearly defined state of reactive hyperemia. The parameters of the blood microvessels changed significantly ($p < 0.01$) in the same direction: the total area of the vascular bed increased by 16% (Fig. 1: 1, 2), the length of the vessels increased by 9% (Fig. 1: 3, 4), and the mean diameter of the vessels increased by 6.7% (Fig. 1: 5). The significant decrease in parameters 1-4 by 4% of the initial level ($p < 0.01$) was observed 1 h after ischemia but their diameter remained increased by 2% ($p < 0.01$).

The rheologic parameters immediately after ischemia indicate a small but significant increase in the apparent viscosity of blood flowing from the ischemic region (Fig. 1: 6) accompanied by a small increase (not significant) in the number of cells per unit volume of blood (Fig. 1: 7) and by a decrease in the mean volume of an erythrocyte (Fig. 1: 8) and the erythrocyte mass (Fig. 1: 9; $p < 0.01$); under these circumstances the mean value of EPM of the erythrocyte was increased (Fig. 1: 10; $p < 0.01$). Incidentally, reduction of the erythrocyte mass in phase I was due mainly to reduction of the mean volume of the erythrocyte rather than to a change in the number of cells per unit volume of blood. The viscosity of blood flowing from the region was reduced by 8.3% of its initial level 1 h after ischemia ($p < 0.01$), the number of cells per unit volume of blood was reduced by 5.4% ($p < 0.01$), the volume of an erythrocyte remained reduced by 6.8% ($p < 0.01$), and the erythrocyte mass remained correspondingly reduced by 5.4% ($p < 0.01$): EPM of the erythrocyte continued to increase by up to 7.8% of the initial level ($p < 0.01$).

The study of correlation between the rheologic and morphometric parameters (Table 1) showed significant correlation both within and between the groups of parameters specified. In the control positive correlation was found between the total area of the vascular bed and the length of the vessels ($R = 0.843$), and to a lesser degree with diameter ($R_{1,5} = 0.021$), i.e., optimization of the total area in the control took place on account of variations in length of the bed, i.e., variability of the number of open vessels. Correlation

between the apparent viscosity of the outflowing blood and erythrocyte mass ($R_{6.9} = -0.399$) and EPM of the erythrocyte ($R_{6.10} = -0.236$) was not significant. Erythrocyte mass was more strongly dependent on the mean volume of an erythrocyte than on the cell concentration ($R_{9.8} = 0.932$; $R_{9.7} = 0.317$). The viscosity of blood flowing from the microcirculatory network did not correlate with the degree of vascularization, but it did exhibit correlation with the length of the microvessels ($R_{6.3} = -0.461$) and positive correlation with their mean diameter ($R_{6.5} = 0.779$: the higher the viscosity, the greater the diameter of the microvessels to viscosity was observed in [8], in which it was shown that vasoconstriction was observed when the viscosity of the blood in the mirovessels was reduced and vasodilatation when it was increased. This relationship between the parameters of the microvessels and blood is evidently autoregulatory in character. Immediately after ischemia dependence of viscosity of vascularization was weak, there was no correlation with length of the microvessels, but positive correlation with diameter still remained ($R_{6.5} = 0.466$). It can be postulated that as long as correlation between viscosity and diameter remains positive and sufficiently high, autoregulatory maintenance of the blood supply of the tissue will be observed. It is interesting to note that 1 h after ischemia correlation between viscosity and diameter was no longer significant, but by contrast, negative correlation with length (and, correspondingly, with vascularization) was strengthened: reduction of length of the vascular bed may be accompanied by increased viscosity, but viscosity in turn may bring about the dilatation of the microvessels observed in this case.

The approach to examination of correlation between rheologic and morphologic parameters of the microvascular bed is useful in order to analyze the pattern of regulation of pathological processes in one tissue and also to analyze morphological and functional differences between different tissues.

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