TIME COURSE OF THE NO REFLOW PHENOMENON DURING MYOCARDIAL REPERFUSION, DEPENDING ON DURATION OF PRECEDING ISCHEMIA

A. Kh. Kogan, A. Mieégombyn,
N. I. Losev, and A. N. Kudrin

UDC 616.127-005.4-092.9-036.8-07:
616.127-005-036.86

KEY WORDS: no reflow phenomenon, ischemia, myocardial reperfusion.

The "no reflow" phenomenon is the term given to incomplete restoration of the blood flow during postischemic reperfusion. The study of this phenomenon is interesting because it frequently accompanies angina, it lies at the basis of the development of coronary-occlusive cardiosclerosis and microinfraction, and it appears after surgical and thrombolytic revascularization. The question of the minimal duration of ischemia after which the no reflow phenomenon arises during reperfusion, and the character of its time course, remain topics for discussion. Different workers consider that the minimal duration of myocardial ischemia is 15 min [5], 30-45 min [10] or 90 min [9]. Some workers observed phasic changes in the no reflow phenomenon [5, 6], whereas others did not observe phasic changes [10].

The aim of this investigation was to study the time course of the no reflow phenomenon during myocardial reperfusion, depending on duration of the preceding ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 270 noninbred male albino rats weighing 180-220 g. Transient ischemia was produced in the rats by ligation of the left coronary artery, around a polyethylene tube 1.3 mm in diameter and 4 mm long, into the lumen of which a miniature rod (to give its walls sufficient rigidity), and on the edge of which two polyethylene rings were tightly mounted to prevent the ligature from slipping (Fig. 1). The level of ligation was 3-4 mm below the left angle of the base of the infundibulum. The topography of the left coronary artery, access to it, and the operative technique were described previously [1]. The area of the no reflow zone (NRZ) was determined by injection of the coronary vessels followed by morphometry. The vessels were injected with a suspension of blue latex microspheres (LM) in rats killed by division of the abdominal aorta and vena cava under ether anesthesia. The animal was given an intraperitoneal injection of 0.3-0.4 ml of undiluted heparin solution (Richter, Hungary) 1.5 h before sacrifice. The final concentration of LM was 2.6×10^6-3.2×10^6/ml. Their diameter as a rule (in 83% of cases) was 4.38-8.7 μ. To stain the microspheres, the resulting suspension was mixed with a 1% solution of trypan blue in the ratio of 6:1. The suspension of LM (total volume 0.8-1 ml) was injected from a syringe through a polyethylene cannula, introduced through the cartoid artery into a segment of the arch of the aorta, isolated between ligatures (duration about 1.5 min at an initial pressure of 100 mm Hg, about 1-1.5 min also at a final pressure of 150-165 mm Hg). During injection of the coronary arteries, the LM could pass directly from the aorta only into the vascular system of the normal myocardium, and potentially also through anastomoses, into the vessels of the ischemic myocardium. Direct entry of LM into the arteries of the ischemic region was ruled out, because the main artery of this region had been ligated. The zone with uninjected vessels (the unperfused zone UPZ) corresponded to the NRZ during reperfusion. Unlike normal myocardium, UPZ (NRZ) did not stain blue, because no LM had penetrated into it. After injection of the coronary vessels the heart was removed and subjected to segmental morphometry by the method described previously [2]. For better differentiation between the unstained and the stained zones, the segments of myocardium were wetted during morphometry or were immersed in distilled water. The total area of UPZ was calculated by adding together the areas of the unperfused (unstained) zones of all segments of the left ventricle subjected to morphometry. The absolute area of UPZ was converted to a percentage of the total area of the left ventricle (to the sum of the areas of all its segments). Morphometry was carried out with the aid of the MSB-1 stereoscopic microscope, equipped with ocular micrometer.

Department of Pathophysiology and Department of Pharmacology, I. M. Sechenov First Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 1, pp. 16-18, January, 1986. Original article submitted April 4, 1985.

0007-4888/86/0001-0019$12.50

TABLE 1. Changes (in % of area of ischemic left ventricle) in Area of Zone with Uninjected Vessels (UPZ) during Transient Ischemia ("no reflow" phenomenon) and Continuous Myocardial Ischemia (M ± m)

Duration of reperfusion	No. of column						
	1	2	3	4	5	6	7
	Duration of ischemia						
	10 min	30 min	1 h	2 h	4 h	1 day	3 days
0 min	32±1,81 ($n = 6$)	31,7±1,06 ($n = 10$)	31,6±1,1 ($n = 10$)	31,9±1,25 ($n = 10$)	32±1,02 ($n = 8$)	27,8±1,1 ($n = 10$)	19,6±1,35[a] ($n = 10$)
5 min	0 ($n = 6$)	11,2±0,97[b] ($n = 10$)	19,2±1,19[b,d] ($n = 10$)	30,2±1,08[b] ($n = 10$)	—	—	—
30 min	0 ($n = 6$)	17,3±1,15[c,d] ($n = 10$)	19,6±1,13 ($n = 10$)	30,1±1,26[b] ($n = 10$)	—	—	—
2 h in column No. 1	0 ($n = 6$)	—	—	—	—	—	—
$1^1/_2$ h in column	—	24,7±1,3[c,d] ($n = 10$)	—	—	—	—	—
1 h in column No. 3	—	—	28,5±1,23[c] ($n = 10$)	—	—	—	—
4 h in column No. 1	0 ($n = 6$)	—	—	—	—	—	—
$3^1/_2$ h in column No. 2	—	27,4±1,1 ($n = 10$)	—	—	—	—	—
3 h in column No. 3	—	—	30,0±1,06 ($n = 10$)	—	—	—	—
1 day	0 ($n = 6$)	21,9±1,03[c,d] ($n = 10$)	25±1,39[c,d] ($n = 10$)	26,5±1,04[c,d] ($n = 10$)	27,1±1,2[d] ($n = 8$)	—	—
3 days	0 ($n = 6$)	14±1,38[c,d] ($n = 8$)	19,3±1,32[c,d] ($n = 8$)	19,1±1,03[c,d] ($n = 8$)	20,2±1,06[d] ($n = 8$)	—	—

Legend. $P < 0.05$: a) compared with ischemia for 30 min, b) compared with previous period of ischemia, with same duration of reperfusion, c) compared with previous period of reperfusion, with same period of ischemia, d) compared with initial ischemia in the same column (1, 2 or 4 h), n) number of animals.

EXPERIMENTAL METHOD

The course of development of the no reflow phenomenon in the myocardium was studied 5 and 30 min, 1-1.5 and 3-3.5 h, and at the end of the 1st and 3rd days after the beginning of reperfusion, preceded by ischemia for a duration of 10 or 30 min or 1, 2, or 4 h. The results showed (Table 1) no reflow reperfusion after ischemia for 10 min. After ischemia for the periods mentioned above, the phenomenon appeared for the first time during reperfusion after ischemia for 30 min and 1 h, three phases could be distinguished: The first phase of primary maximal limitation of the no reflow area, corresponds to 5 min after the beginning of reperfusion ($P < 0.001$), the second phase consisted of an increase in the no reflow area: a tendency toward an increase first appeared at the 30th minute of reperfusion, and a marked increase was observed 1-1.5 and 3-3.5 h after the beginning of reperfusion (as a rule, $P < 0.05$); the third phase consisted of a second period of limitation of the no reflow area, and corresponded to the 1st-3rd days (after 1-3 days $P < 0.05$).

During reperfusion after ischemia for 2 h the phase of maximal limitation of the area of no reflow was absent. Instead, during reperfusion the area of UPZ was almost unchanged compared with the preceding ischemia. Later, at the end of the 1st-3rd day, the phase of unchanged area of UPZ was replaced by a phase of a relative decrease in its area (after 1 day $P > 0.05$, after 3 days $P < 0.05$).

The area of "no reflow" during reperfusion preceded by ischemia for 30 min and 1 h was usually smaller than the area of UPZ before reperfusion (as a rule, $P < 0.05$). During reperfusion preceded by ischemia for 2 h the area of no reflow became less than the area of UPZ during ischemia before reperfusion, only after the end of the 1st-3rd days. The results are in agreement with data published previously [5, 6].

The mechanism of development of the no reflow phenomenon is linked with secondary disturbances of the microcirculation. In turn, factors directly causing disturbances of the microcirculation, it can be tentatively suggested, are: 1) a decrease in prostacycline formation by the endothelial cells under the influence of lipid peroxidase [11, 8], the quantity of which increases during reperfusion on account of intensification of free-radical lipid

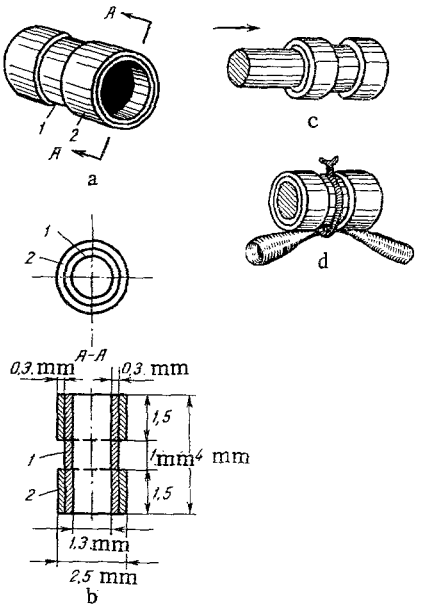

Fig. 1. Coronary occluding device made from polyethylene tube to produce transient ischemia. a) General view: 1) body, 2) polyethylene rings to prevent coronary-occluding ligature from slipping; b) dimensions; c) introduction of matchstick into lumen of device to make it rigid; d) ligation of artery around body of device.

peroxidation [3], and this leads to adhesion and aggregation of the platelets occluding the microvessels [7, 8]; 2) injury to endothelial cells, partly perhaps under the influence of free-radical lipid peroxidation products with the development of edema of the endothelium and the formation of cytoplasmic outgrowths, which occlude the lumen of the microvessels [5, 9]; 3) edema of the cardiomyocytes and interstitial tissue [5, 9], which leads to compression of the microvessels; 4) contracture of the cardiomyocytes [5], evidently under the influence of accumulating Ca^{++} and catecholamines [4]; 5) and increase in viscosity of the blood, a decrease in flexibility of the erythrocyte membranes, and aggregation of erythrocytes [5, 9]; 6) true thrombosis, although the role of the latter is very doubtful [5, 9].

The no reflow phenomenon thus arises during reperfusion after ischemia for 30 min but not after ischemia for 10 min, and it developed in phases. The results confirm that no reflow may lead to the appearance of an infarct, and that treatment of transient ischemia (including angina) should be aimed not only toward the ischemia itself, but also toward the no reflow phenomenon which accompanies postischemic reperfusion.

LITERATURE CITED

1. A. Kh. Kogan, Modeling of Myocardial Infarction [in Russian], Moscow (1979).
2. A. Kh. Kogan, N. I. Losev, A. N. Kudrin, and A. Mieégombyn, Byull. Éksp. Biol. Med., No. 3, 271 (1984).
3. P. F. Litvitskii, A. Kh. Kogan, A. N. Kudrin, and L. O. Luk'yanova, Byull. Éksp. Biol. Med., No. 3, 271 (1981).
4. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
5. J. P. Camilleri, D. Joseph, J. N. Fabinai, et al., Arch. Path. Anat. Abt. A, 369, 315 (1976).
6. A. Deloche, J. N. Fabiani, J. P. Camilleri, et al., Am. Heart J., 93, 358 (1977).
7. N. V. Demopoulos, E. S. Flamm, M. L. Seligmann, et al., in: Neural Trauma, New York (1979), pp. 63-78.
8. R. J. Gryglewski, S. Bunting, S, Monacada, et al., Prostaglandins, 12, 685 (1976).

9. R. A. Kloner, C. E. Ganote, and R. B. Jennings, J. Clin. Invest., 54, 1496 (1974).
10. A. Krug, J. Thorac. Cardiovasc. Surg., 60, 242 (1970).
11. S. Moncada, R. J. Gryglewski, S. Bunting, et al., Prostaglandins, 12, 715 (1976).

ENZYME ACTIVITY OF PERIPHERAL BLOOD CELLS IN EXPERIMENTAL CHRONIC MYOCARDITIS LINKED WITH PERSISTENCE OF COXSACKIE A VIRUS

V. V. Bogach, N. A. Braude, R. K. Katosova,
I. A. Komissarova, T. T. Kondrashova,
L. S. Lozovskaya, and R. P. Nartsissov

UDC 616.127-002.2-022:578.835.
17/- 092.9-07:616.155.2/.3-
008.931-074

KEY WORDS: lymphocytes; cell metabolism; enzyme systems; cytochemical analysis

Cytochemical parameters of peripheral blood cells reflect the characteristics of metabolism in the tissues of the internal organs [2, 5, 7, 9] and they can accordingly be used to assess the severity of a pathological process. The enzyme profile of the blood leukocytes has been studied experimentally only in acute virus diseases [3, 6]. It has been shown that a chronic virus infection develops in animals infected with strains of Coxsackie viruses A13 and A18, isolated from patients with rheumatic carditis and myocarditis [1, 4, 11].

In human cardiopathies associated with chronic virus infection, intravital determination of the character of the pathomorphological changes in the myocardium may be very difficult, and accordingly the aim of the present investigation was to look for informative criteria for assessing activity of the process and the degree of damage to the internal organs in experimental chronic virus myocarditis.

EXPERIMENTAL METHOD

Experiments were carried out on 18 noninbred male albino rats weighing 200-260 g. The experimental animals (n = 12) each received an intraperitoneal injection of 1.0 ml of culture fluid containing $10^{5.6}$ TCD_{50} of Coxsackie A13 virus (strain 4523) isolated from a child with rheumatic carditis [4]. The virologic methods included investigation of the thymus and blood clots from all animals for presence of the virus 60 days after infection. The virus was reisolated by infection of primary trypsinized tissue cultures of human embryonic fibroblasts (HEF) with the test material. The titer of antibodies against Coxsackie A13 virus was determined in the blood sera by the neutralization of cytopathic activity of the virus test. Blood was taken for cytochemical investigation from the caudal vein 8 days and 2 months after infection, and succinate dehydrogenase (SDH) activity of the lymphocytes and platelets, α-glycerophosphate dehydrogenase (α-GPDH) activity of the lymphocytes [8], and alkaline phosphatase (AlP) activity of the neutrophils were determined by the azo-coupling method [10]. For the histopathological investigation sections from preparations of the heart, fixed in Carnoy's fluid, were stained with hematoxylin and eosin. Statistical analysis of the results was carried out by the Student and Kolmogorov–Smirnov tests. Correlation analysis was done by Nairi-2 computer.

EXPERIMENTAL RESULTS

During virologic investigation Coxsackie A13 virus was reisolated from the thymus or blood of eight of the 12 animals 60 days after infection. Antibodies of the appropriate specificity were present at this time in the blood in titers of 4 to 6 $\log_2$ in all infected animals. On histologic investigation the degree of heart damage was estimated from the presence of signs of virus myocarditis such as the formation of perivasuclar and subendocardial granulomas, infiltration of the myocardium with lymphocytes, destructive changes in the cardiomyocytes, cardiosclerosis, and petrification. The intensity of myocardial damage was pronounced in animals in whose thymus no virus was present (Table 1). Significantly strong correlation was found

Research Institute of Pediatrics, Academy of Medical Sciences of the USSR, Moscow. Research Institute for Biological Testing of Chemical Compounds, Ministry of the Medical Industry of the USSR, Staraya Kupavna. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 1, pp. 18-20, January, 1986. Original article submitted January 30, 1985.

 0007-4888/86/0001-0022$12.50