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FORMATION OF A "PRO-OXIDANT" BOUNDARY ZONE AND ITS ROLE IN INTENSIFICATION OF LIPID PEROXIDATION IN AN AREA OF MYOCARDIAL ISCHEMIA AND INFARCTION

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A phenomenon known as the "free-radical peroxide paradox" (FRPP) during the development of myocardial ischemia and infarction was described in 1975 [6]. It is that, despite the oxygen deficiency present in the region of ischemia and the developing myocardial infarct, free-radical lipid peroxidation (LPO) is for a long time intensified. Its pathogenetic role has been proved, for inhibition of LPO by means of antioxidants reduces the severity and size of a myocardial infarct [6, 7, 9, 10]. Subsequent investigations confirmed these observations [9, 15]. The mechanism of FRPP has not yet been explained.

The writers postulate a definite connection between intensification of LPO in an area of ischemia and infarction with the formation of a "pro-oxidant" boundary zone (POBZ) at its periphery, which differs from other zones of the infarct in that, first, the blood flow (and, consequently, the oxygen supply) in it is partially restored and, second, the cardiomyocytes in this zone have largely lost their ability to utilize oxygen. This paper gives the results of experiments which confirmed this hypothesis.

EXPERIMENTAL METHODS

A model of myocardial ischemia and infarction was produced in noninbred male albino rats weighing 180-250 g by the method developed previously [3]. The left coronary artery was ligated by 3-5 mm below the left angle of the base of the infundibulum. The possible formation of a POBZ was studied by injecting the coronary vessels with latex microspheres (LM) and by the histochemical reaction for dehydrogenase activity (DA) using nitro-BT, followed by morphometry. By means of these methods, the area of the zone in which the vessels did not fill with LM, i.e., the unperfused (not supplied with blood) zone (UPZ) (one group of rats), the area of the zone not giving a positive reaction with nitro-BT, the so-called dehydrogenase-free zone (DFZ), corresponding to the zone of injury (ZI) (2nd group of rats), and also the difference between the area of the DFZ and UPZ, consisting of the zone without DA, but having patent vessels, communicating with the vascular bed of the normal myocardium, were determined. This last difference corresponds to the POBZ. The coronary vessels were injected with a suspension of blue LM into rats killed under anesthesia. The final concentration of LM was (2.6-3.2) $\times 10^6/mm^3$, and their diameter as a rule (in 83% of LM) was 4.38-8.7 μ. The suspension

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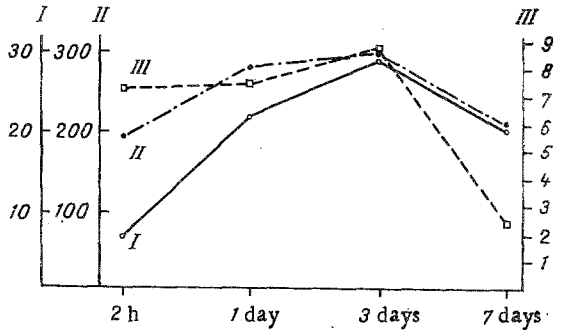

Fig. 1. Correlation between parameters of intensity of LPO and area of POBZ. Abscissa, times of investigation. I) POBZ (in % of area of DFZ), II) ICL (in cps/3 mg of lipids), III) MDA (in μmoles/0.6 mg of lipids). I, II) Coefficient of correlation between POBZ and LPO after 2 h and 1, 3, and 7 days of ischemia: $r = 0.97$ ($P < 0.01$), I, III) the same between POBZ and MDA after 1, 3, and 7 days: $r = 0.99$ ($P < 0.01$).

of LM was injected from a syringe through a polyethylene cannula, introduced via the carotid artery into a segment of the arch of the aorta isolated between ligatures. The UPZ did not stain blue, since the LM did not enter it. After injection of the coronary vessels the heart was removed and subjected to segmental morphometry, as described previously [5]. The DFZ, unlike normal myocardium, likewise did not stay blue as a result of the reaction with nitro-BT.

The area of DFZ, UPZ, and POBZ was calculated as a percentage of the total area of all segments of the ischemic left ventricle. In addition, the area of POBZ was calculated as a percentage of the area of DFZ. Parameters of LPO, namely the intensity of chemiluminescence of lipids (ICL) and the malonic dialdehyde (MDA) concentration in the lipids, were determined as described previously [7, 8]. Lipids were extracted by Folch's method. ICL, which reflects the concentration of peroxide radicals [1], was measured with a chemiluminometer in a constant weight of lipids (3 mg). The MDA concentration was determined in 0.6 mg of lipids by the reaction with 2-thiobarbituric acid [1]. All determinations were done on groups of five to eight animals 2 h and 1, 3, and 7 days after occlusion of the left coronary artery.

RESULTS

POBZ was discovered 2 h after coronary occlusion, and at that time it amounted to 6.9 ± 0.76% of the total area of DFZ (Fig. 1). ICL at this stage was 196.4 ± 4.0 cps compared with 61.7 ± 7.2 cps normally ($P < 0.05$). The MDA content was 7.53 ± 0.1 μmole compared with 1.15 ± 0.5 μmole normally ($P < 0.05$). The area of POBZ 1 day after coronary occlusion had increased to 21.6 ± 1.1% ($P < 0.01$ compared with the area after 2 h; everywhere from now on P relates to comparison with this time, and the area of POBZ is given as a percentage of the area of DFZ). In the region of the formed myocardial infarct ICL at this time increased to 265.7 ± 7.7 cps ($P < 0.001$). The MDA concentration at this time remained high (7.53-7.48 μmoles, $P < 0.05$).

The area of POBZ continued to increase and after 3 days it was 28.7 ± 2.4% ($P < 0.01$). There were corresponding increases in ICL to 282.7 ± 5.9 cps and the MDA concentration to 8.51 ± 0.26 μmole ($P < 0.01$). The area of POBZ 7 days after coronary occlusion was reduced, by contrast with previous times. At the same time ICL fell to 218 ± 5.9 cps and the MDA concentration to 22.51 ± 0.12 μmole (compared with after 3 days of ischemia). Analysis showed positive correlation between ICL and the area of POBZ at the zone of infarction (total for all four times of investigation) ($r = 0.97$, $P < 0.01$). Positive correlation also was found between the MDA concentration and area of POBZ in total for three of the four times of investigation (after 1, 3, and 7 days) ($r = 0.99$, $P < 0.01$). The presence of correlation between the values of LPO and POBZ suggests that POBZ makes a definite contribution to the mechanism of intensification of LPO throughout the region of the developed infarct and, consequently, to the mechanism of FRPP. In turn, the mechanism of intensification of LPO and POBZ arises from two of

its properties: First, there are vessels which communicate with the vascular bed of the normal myocardium, along which oxygen may be supplied, second, its cardiomyocytes contain no DA and their ability to utilize oxygen for tissue respiration is therefore disturbed. As a result, oxygen reaching this zone evidently creates relative hyperoxia in it and stimulates generation of active forms of oxygen (superoxide etc.) and LPO. The following phenomena, it can be tentatively suggested, directly intensify the formation of active forms of oxygen and activate LPO in POBZ, as indeed, to some extent, in other zones of the infarct also, where an adequate level of oxygen still remains: conversion of xanthine dehydrogenase D into xanthine oxidase O [14], auto-oxidation of accumulated reduced forms of NAD, NADP, and FAS [12], accumulation of free-radical forms of FAD and CoQ [12], accumulation of catecholamines, a decrease in activity of antioxidative enzymes [9], emigration of leukocytes into POBZ [4], and nonenzymic auto-oxidation of lipids [11]. The results are in agreement with data in the literature [13].

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