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DNA DAMAGE AND ITS PREVENTION IN THE NONISCHEMIZED AREA OF HEART MUSCLE IN RATS WITH EXPERIMENTAL MYOCARDIAL INFARCTION

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UDC 616.127-005.8-092.9-07:616.127-008.939.633.2-074

KEY WORDS: nonischemized zone; heart muscle; experimental myocardial infarction.

Marked disturbances of metabolism and function regularly develop in the nonischemized zones of the heart in infarction, and often they are explained by the presence of some degree of hypoxia in the zone surrounding the infarct. This explanation has recently been questioned because it has been shown that the demarcation line between the zone of ischemia and the nonischemized zone, based on parameters sensitive to hypoxia such as the ATP, acid phosphatase, glycogen, and lactate concentrations, is very sharp in experimental infarction: There is no gradual transition [9]. Similar results have been obtained by other workers [10] and it accordingly seems likely that it is not hypoxia or, at least, it is not only hypoxia that causes damage to the nonischemized zones of the heart in infarction. We have suggested that one factor damaging these zones is emotional-painful stress, which develops in myocardial infarction and exposes the myocardium to the action of an excess of catecholamines. It was shown previously that in animals with emotional-painful stress evoked by a certain environmental situation, lipid peroxidation (LPO) is activated in the myocardium and damage to DNA develops, simultaneously, in the form of a fall in its degree of polymerization [5, 4], but this can be prevented by administration of LPO inhibitors (antioxidants). In experimental myocardial infarction LPO activation has been proved and has been found to be well marked in nonischemized zones of the heart [2]. Accordingly the writers have postulated that damage and subsequent repair to DNA take place in nonischemized zones of the heart in infarction.

The aim of this investigation was to study injuries and subsequent repair to DNA in nonischemized zones of the heart in experimental myocardial infarction and to study the possibility of preventing these injuries by preliminary administration of the β-blocker inderal (propranolol) and the LPO inhibitor ionol.

EXPERIMENTAL METHOD

Male Wistar rats weighing 190-210 g were used. An experimental myocardial infarct was induced by ligation of the descending branch of the left coronary artery by Selye's method. A mock operation, thoracotomy under general anesthesia but without ligation of the coronary artery, was performed on control animals. Nuclei were isolated in the usual way [15], but magnesium was omitted from the medium and isolation carried out in the presence of EDTA [12] to inhibit intranuclear nucleases. Lysates of the nuclei were sedimented in an alkaline sucrose gradient by the method in [14] in the writers' own modification [4]. To evaluate reparative DNA synthesis, 2 h before sacrifice the animals were injected with hydroxyurea in 0.15 M NaCl solution, pH 7.4 (the remaining compounds also were injected in this solution), in a dose of 50 mg/100 g body weight, sufficient to inhibit replicative synthesis by 98%. Ten minutes after receiving hydroxyurea the animals were injected with thymidine-^{3}H (55 Ci/mmole, USSR) in a dose of 200 μCi/100 g body weight. Ionol [2,6-di(tert-butyl-4-methylphenol)], ground up beforehand with a small volume of Tween-60, was injected 96, 48, and 24 h before creation of the experimental infarct and immediately thereafter in a dose of 5 mg/100

Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 57-60, June, 1982. Original article submitted January 7, 1982.

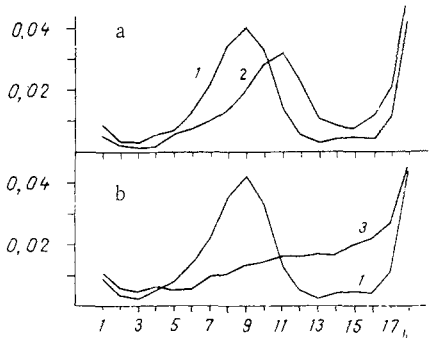

Fig. 1. Sedimentation profiles of alkaline lysates of nuclei from intact myocardium (control) and zone of infarction from rats with an experimental myocardial infarct. a) Character of damage observed in 7 of 10 cases; b) character of damage observed in remaining 3 cases. To isolate each nuclear preparation 2 or 3 animals were used. Direction of sedimentation from right to left. Significance of localization of peaks according to Student's t test: 1) control, 95%; 2) infarct, 7 parallels, 70%; 3) infarct, 3 parallels, 70%. Here and in Fig. 2: abscissa, nos. of fractions; ordinate, absorption at 260 nm.

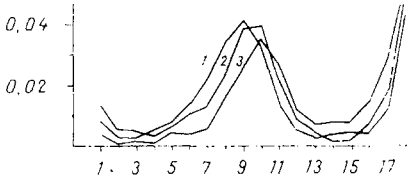

Fig. 2. Sedimentation profile of alkaline lysates of nuclei from nonischemized zone of heart from animals with experimental myocardial infarction after injection of ionol and inderal. 1) Control; 2) ionol + infarct; 3) inderal + infarct. Significance of localization of peaks of curves 1, 2, and 3 is 95, 70, and 70%, respectively.

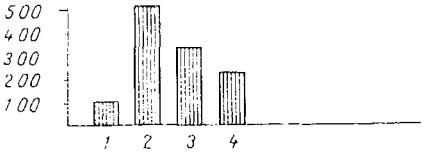

Fig. 3. Reparative DNA synthesis in intact myocardium (control) and in nonischemized zone of heart in rats within infarction (effect of antioxidant protection and β-receptor blockade). 1) Control (7 parallels); 2) infarct (7); 3) infarct + inderal (5); 4) infarct + ionol (5). Scatter of data reflected as standard deviation about 3%, not exceeding 5%. Ordinate, radioactivity in CPM/mg DNA.

g body weight. Inderal was injected in a dose of 10 μg/100 g body weight immediately before and 6 h after creation of the experimental infarct. The animals were killed 24 h after creation of the infarct to isolate the nuclei and 2 h after injection of thymidine-^{3}H, i.e., 26 h after creation of the infarct, to assess reparative DNA synthesis. DNA was then isolated from the heart muscle [13], determined quantitatively with diphenylamine [8], and its radioactivity measured by counting its fallout on membrane filters in toluene scintillator.

EXPERIMENTAL RESULTS

The results of sedimentation analysis (Fig. 1) showed heterogeneity of DNA injuries after infarction in the nonischemized zone of the myocardium. In 7 of 10 cases, for instance, the pattern of DNA damage observed was typical of the action of most DNA-damaging agents (Fig. 1a) and similar to that found in stress [4]. Displacement of the sedimentation peak into the region of lower molecular weights and the appearance of low-molecular-weight fragments of DNA (fractions Nos. 13-17) on individual sedimentation profiles compared with the control are evidence of fragmentation of DNA on account of single and, possibly, double breaks.

In 3 of 10 cases a different picture was observed. As Fig. 1b shows, no marked peak was present on the experimental sedimentation profiles. The reason was probably the more intensive fragmentation of DNA due to the high concentration of free radicals, produced by breakdown of lipid hydroperoxides; nuclease degradation of DNA at this period (the first day after infarction) can evidently be ruled out [7]. However, another explanation of this fact is possible. Free-radical DNA damage, the classical example of which is radiation injury, can be expressed not only as the appearance of breaks and, as a result, a decrease in the molecular weight of DNA, but also as the appearance of DNA–protein and DNA–DNA cross-linkages [1, 6]. DNA–protein cross-linkages formed by alkali-stable protein fragments in the composition of the DNA chain can persist even after lysis in an alkaline medium [11]. Under these circumstances a network of DNA molecules may be formed, sedimenting abnormally slowly, especially under conditions of overloading of the gradient, which we were compelled to use for technical considerations [4].

As was recently established [7], in experimental infarction 1 h after the operation an increased DNA concentration is found in the blood, and it continues to rise until 24 h. At this same time a decrease in the DNA concentration in the zone of ischemia was found compared with the intact zone, but not on account of activity of nucleases, which does not increase until after 3 days. The facts described above are evidence that primary DNA damage in the zone of ischemia in infarction takes place through a free-radical mechanism on account of reactable products of peroxidation, which is intensified in the early postinfarction period both in the zone of the infarct and in the nonischemized zone [5].

This conclusion received further experimental verification from experiments in which peroxidation was blocked by the antioxidant ionol. In a parallel study the effect of the β-blocker inderal, which inhibits the action of catecholamines which in the modern view trigger LPO activation in the heart [3], was tested on the degree of damage to DNA in infarction. The results showed (Fig. 2) that injection of inderal, and to an even greater degree that of ionol, reduced postinfarction DNA degradation in the noninfarcted zone of the myocardium. Incomplete protection of myocardial DNA in both cases can evidently be explained by the impossibility of neutralizing the effect of the focus of ischemia, a constant source of toxic products, including hydroperoxides, the action of which can be reduced but not completely suppressed so long as the focus of injury itself remains.

DNA damage through myocardial infarction is accompanied by activation of reparative DNA synthesis. The results showed (Fig. 3) that reparative DNA synthesis in the zone around the infarct in the heart of animals undergoing infarction took place several times more actively than in the control. After injection of inderal the high level of postinfarction reparative DNA synthesis noted above was reduced by one-third, but after injection of ionol it was reduced by half; this result evidently is in agreement with the data given above on blocking DNA damage in the noninfarcted zone of the myocardium under these experimental conditions described above.

The results of this investigation and earlier facts are unambiguous evidence that in experimental infarction damage and subsequent repair to DNA arise in the nonischemized zones of the heart, and can largely be prevented by β-receptor blockade and inhibition of LPO, and they are thus the result of LPO activation induced by catecholamines.

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