TIME COURSE OF BLOOD POLYMORPH FUNCTION IN DOGS WITH REVERSIBLE MYOCARDIAL ISCHEMIA

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Acute or chronic ischemia caused by occlusion of the coronary arteries followed by reperfusion is one of the main causes of necrotic changes in the myocardium [12]. The onset and development of necrosis of the heart muscle are closely bound with activation of lipid peroxidation (LPO) [17]. Among the systems responsible for the sudden dramatic increase in the content of active forms of oxygen (AFO) [18], capable of initiating LPO during the formation of a zone of necrosis in the myocardium, a special place belongs to polymorphonuclear leukocytes (PML) of the peripheral blood [14].

Even in the early of development of myocardial infarction (MI) the inflammatory reaction of PML is expressed as an increase not only in the number of cells [2], but in their specific activity [3]. The increase in functional activity of PML, their chemotaxis, and infiltration into the zone of ischemia [9], bringing AFO and other prooxidants with them, against the background of a reduction of activity of endogenous antioxidants (superoxide dismutase, catalase, glutathione peroxidase, etc. [5]) — all these contribute to the initiation and acceleration of LPO and to injury and death of the cardiomyocytes [15]. Meanwhile it is not yet clear to what extent the activated phagocytes may take part in the formation of the necrotic zone in myocardial ischemia.

The aim of this investigation was to study the early manifestations of a change in polymorph function in experimental reversible myocardial ischemia in dogs.

EXPERIMENTAL METHOD

Eight mongrel dogs weighing 12-18 kg were used. A special device was implanted beforehand into the animal's chest so that, without repeating the operation, a loop could be tied tightly or relaxed around the circumflex branch of the left coronary artery, by alternate tension on two synthetic threads brought out on to the skin [6]. The chronic experiment on the prepared animal began not less than 15 days after the operation. The experiment was carried out after a single intramuscular injection of 2.5-5.0 mg droperidol and 0.05-0.1 mg fentanyl, followed (10-15 min later) by injection of 0.1 g/kg of 20% hexobarbital solution. Temporary occlusion of the coronary artery was then performed from 1 to 3 times, with intervals of not more than 5 min between them. The total duration of occlusion of the lumen of the coronary artery in each observation did not exceed 5 min. Transient disturbances and recovery of the coronary blood flow were confirmed by electrocardiographic monitoring of the state of the myocardium in standard leads I, II, and III, and they were accompanied by "ischemic" shifts (an increase in amplitude of the T wave in lead II by 0.05-0.85 mV). Blood was then taken from the femoral vein during the first 2 days after ischemia with an interval of 2-3 h. PML were isolated from peripheral blood by means of a Ficoll—Verografin gradient (ρ = 1.077 g/cm^3) [11]. The viability of the PML was not less than 97%. The level of polymorph function was determined by measuring the intensity of latex-stimulated luminol-dependent chemiluminescence of the leukocytes (LCL) [7]. TBA-active products in the blood plasma were determined by the method in [8].

EXPERIMENTAL RESULTS

It was shown previously that in both clinical [3] and experimental [9, 15] MI a marked increase in the level of polymorph function is observed, as shown by increased production of

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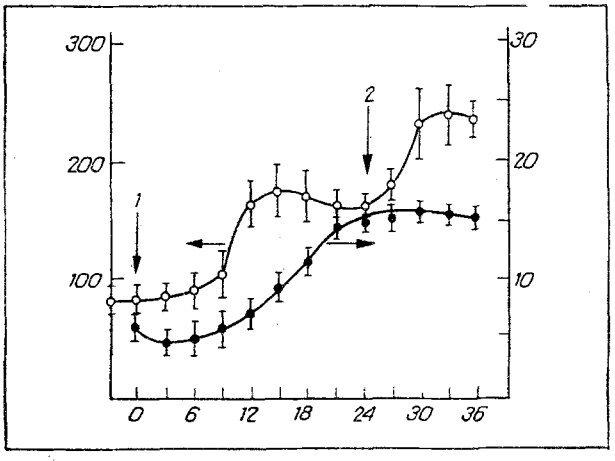

Fig. 1. Time course of change in LCL of PML and total number of peripheral blood leukocytes in dogs during primary (1) and repeated (2) single exposures to transient myocardial ischemia. Abscissa, time (in h); ordinate: on left — intensity of LCL (in relative units), on right — number of PML ($\cdot 10^6$/0.1 ml).

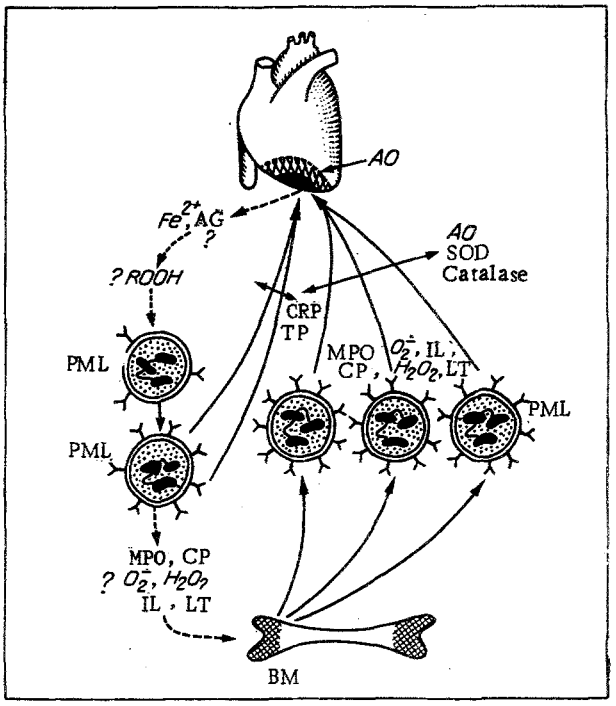

Fig. 2. Diagram showing possible mechanism of PML activation in ischemic myocardial damage. NZ) Necrotic zone; PZ) peri-infarct zone; AG) cardiac antigens; MPO) myeloperoxidase; CP) cationic proteins; IL) interleukins; LT) leukotrienes; CRP) ceruloplasmin; TP) tocopherol; SOD) superoxide dismutase; AO) antioxidants; BM) bone marrow.

AFO and of other prooxidants by these cells [15]. Activation of PML may be a delayed reflection of original ischemic injuries to cardiomyocytes or a rapid response to early changes taking place in the capillaries as a result of a previous ischemia—reperfusion cycle [12]. By the use of a model enabling myocardial ischemia of varied duration to be applied reversibly in dogs, it was found that a single exposure to transient myocardial ischemia (Fig. 1) with a total duration of not more than 5 min led to a significant increase in the intensity of LCL of PML toward the 12th-14th hour of the postischemic period. The level of LCL of PML reached by the end of the first day after ischemia was depressed by 10-12%. Repeated transient (5 min) myocardial ischemia, applied 24 h after the first exposure was accompanied by an even greater increase in the LCL-response of PML, which flattened out at a constant level twice as quickly as during the first ischemia, i.e., by the 6th-8th hour of the postischemic period. Thus acceleration of the LCL response of PML after repeated ischemia is evidence of the cumulative effect of repeated ischemia—reperfusion cycles.

The increase in polymorph functional activity during myocardial ischemia observed in the present and in other investigations [3, 9] was manifested only on the addition of stimulators to the cells [15]. This means that during ischemia the granulocytes were prepared for interaction with the stimulator, i.e., they were sensitized, due to a considerable increase in the number and affinity of various receptors on the cell surface [3]. The observed increase in blood polymorph activity of the dogs after the first and second ischemias may have been the result of an increase in the level of function of PML circulating in the blood stream, or the appearance of a population of more active PML in the blood stream.

The hypothesis of "priming" of the phagocytes, essentially that preincubation of PML with a small quantity of different stimulators, of which the most interesting are LPO products, is accompanied by activation of the cell and corresponding expression of receptors [13], is currently under extensive discussion in the literature. It is a noteworthy fact that however priming of the granulocytes took place in vitro, the increase in activity of the cells was not more than two-threefold [13], whereas in clinical and experimental cases of MI, PML activity was increased tenfold or more [3, 9, 15], and the time of switching of PML activity to a higher level during the priming process was 5-7 min, but not several hours. This suggests that the increase in the LCL response of PML after the first ischemia was due to the appearance of a more active cell population in the blood stream. The fact that PML constitutes a heterogeneous cell population, and that their life span in the blood stream does not exceed 5-6 h, i.e., shorter than the latent period after the first ischemia, and also the leukocytosis observed in MI, characterized by an increase in the proportion of neutrophilic leukocytes [2, 16] — all these facts also are evidence in support of our hypotheses. Incidentally, the time course of the rise of the blood leukocyte count after the first exposure to ischemia coincides with that of the increase in the LCL response of PML (Fig. 1). As a result of the ischemia–reperfusion cycle the increase in functional activity of the phagocytes is accompanied by increased production of AFO by these cells, which are capable of initiating LPO and giving rise to peroxide degradation products of phospholipids and proteins [1, 2].

In primary reversible short-term myocardial ischemia the increase in functional activity of PML may thus take place in two stages (Fig. 2). As a result of the formation of LPO products [1], degradation of the cardiomyocytes, and activation of the complement system, PML circulating in the blood stream undergo priming (first stage), which switches them to a higher level of readiness for interaction with those stimulators which are beginning to appear, and which may include myocardial antigens, circulating immune complexes [4], and so on. The power of the "respiratory burst" is thereby increased: production of AFO, interleukins, leukotrienes, and interferons and other biologically active substances is increased; some of these, such as interleukin I [10], can initiate granulocytopoiesis, as a result of which a more active population of PML appears (second stage). Activated PML are then attracted by chemotaxis into the zone of inflammation, where they infiltrate into the necrotic zone of the myocardium, into which AFO and other prooxidants have been locally introduced, and against the background of weakened activity of the endogenous antioxidant protection system [5, 14], these initiate structural and functional lesions of the cardiomyocytes in the zone of necrosis and the peri-infarct region, thereby widening the zone of myocardial damage. Consequently PML and the AFO and various prooxidants produced by them, together with other systems producing AFO, may therefore participate in ischemic damage to the myocardium.

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CORRELATION BETWEEN CHANGES IN Na^+/H^+-EXCHANGE AND CYTOPLASMIC Ca CONCENTRATION DURING PLATELET ACTIVATION

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Stimulation of platelets by various agonists (physiological and artificial) isknown to induce the passage of Na^+ ions into the cytoplasm in exchange for intracellular H^+ ions, an indication of activation of Na^+/H^+-exchange [3, 4, 11-13]. At the same time there is an increase in the concentration of intracellular free Ca (Ca_i^{++}), which plays a key role in the triggering of most intracellular processes [5]. The writers showed previously that, on the one hand, an increase in the Na^+ ion concentration in the platelets (for example, by the aid of monensin) leads to a rise of Ca_i^{++} level, but on the other hand, replacement of Na^+ by a nonpenetrating organic cation (choline) does not cause any significant changes in the Ca_i^{++} concentration in response to inducers such as PAF [3]. This fact, together with data in the literature [10], have led to the suggestion that correlation exists between Na^+/H^+-exchange and the Ca_i^{++} level during platelet activation.

This paper describes the further study of these relationships by analyzing data on changes in the intracellular pH (a marker of Na^+/H^+-exchange activity) and [Ca_i^{++}] during the action of agonists on platelets.

EXPERIMENTAL METHOD

Venous blood from healthy blood donors or from Wistar rats, anesthetized with ether, mixed in the ratio of 6:1 with an anticoagulant of the following composition: sodium citrate 93 mM, citric acid 7.7 mM, glucose 140 mM (pH 6.5) — was used in the experiments. Platelet-enriched plasma (PEP) was obtained by centrifuging the blood for 15 min at 100g. Platelets were isolated by centrifuging the mixture of anticoagulants and PEP (1:1) for 10 min at 350g and resuspension in buffer solution containing 138 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 0.5 mM Na_2HPO_4, 0.2% bovine serum albumin, 0.2 U/ml apyrase, and 10 mM HEPES, pH 6.5 (buffer A) up to a con-

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