CHANGES IN SUPEROXIDE DISMUTASE ACTIVITY DURING STIMULATION OF PERIPHERAL BLOOD POLYMORPHONUCLEAR LEUKOCYTES

G. I. Klebanov, M. V. Kreinina, I. I. Chukaeva, O. L. Barbarash, I. M. Korochkin, and Yu. A. Vladimirov

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The "respiratory burst" of phagocytic cells, including polymorphonuclear leukocytes (polymorphs), is accompanied by a marked increase in the production of active forms of oxygen (AFO) and other pro-oxidants [5], which can realize the bactericidal and cytotoxic effects of activated phagocytes [9], especially through the initiation of lipid peroxidation (LPO).

Many diseases with a course typical of specific and also nonspecific inflammation are accompanied by activation of phagocytes. For example, it has been shown that production of AFO and other pro-oxidants is increased tenfold in clinical [2] or experimental [3] myocardial infarction. Chemotaxis and subsequent infiltration of these activated phagocytes into the zone of ischemia can lead to involvement not only of cardiomyocytes [13], but also of the polymorphs themselves. Activation of endogenous antioxidative enzyme systems (superoxide dismutase, catalase, glutathione peroxidase, etc.) is one way in which cells are protected against the cytotoxic action of generated AFO [2]. We know from the literature that in some diseases accompanied by activation of polymorphs, for example in rheumatoid arthritis, increased superoxide dismutase (SOD) activity is observed in the blood plasma, erythrocytes, and platelets [6]. However, it is not yet clear how SOD activity changes during stimulation of polymorphs under normal and pathological conditions.

The aim of this investigation was to study the character of the change in SOD activity of circulating polymorphs from normal individuals and patients with myocardial infarction.

EXPERIMENTAL METHOD

Blood from 19 healthy individuals and 26 patients with myocardial infarction (MI) was studied. The patients' ages ranged from 26 to 70 years (average 48 years). Polymorphs were isolated from the peripheral blood of these subjects [7]. The viability of the cells, determined by the trypan blue test, was not below 98%. The state of polymorph function was determined by measuring latex-stimulated luminol-dependent chemiluminescence (ChL) [4]. To measure SOD activity in the polymorphs, 1 ml of a suspension of $5 \cdot 10^6$ cells in phosphate buffer (2.68 mM KCl, 137.74 mM NaCl, 1.5 mM KH_2PO_4, 8.09 mM Na_2HPO_4 pH 7.4) was poured into centrifuge tubes, after which 0.2 ml of the stimulator (polystyrene latex in the proportion of 80 latex particles to one cell) was added. The mixture was incubated at 37°C. Samples were taken every 20 sec for 20 min. Lysis of the cells was induced by addition of 1 ml of chloroform to the sample. After vigorous shaking the samples were centrifuged for 15 min at 400*g* to separate the phases. The aqueous phase (the SOD-containing cell lysate) was used for further analysis. SOD activity was determined as in [14]. The system for generating superoxide radicals consisted of the reaction of oxidation of β-NAD (the reduced disodium salt of NADH, from "Reanal," Hungary), 5-methylphenazine metasulfate (PMS, from "Ferak Lab. GMBH," West Berlin). Nitroblue tetrazolium (nitro-BT, from "Chemapol," Czechoslovakia) was used as the radical carrier-indicator. To assay activity of the enzyme 1 ml of SOD-containing cell lysate was treated with 0.1 ml of a 0.1% solution of nitro-BT, 0.5 ml of

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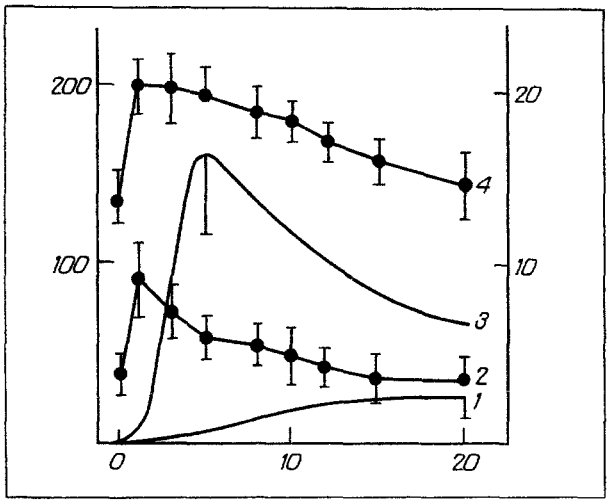

Fig. 1. Kinetics of development of ChL-response of polymorphs (1, 3) and changes in activity of intracellular SOD (2, 4) during latex stimulation of polymorphs of healthy individuals (1, 2) and patients with acute MI (3, 4). Abscissa, time (in min); ordinate: on left — intensity of ChL (in relative units), on right — SOD activity (in activity units).

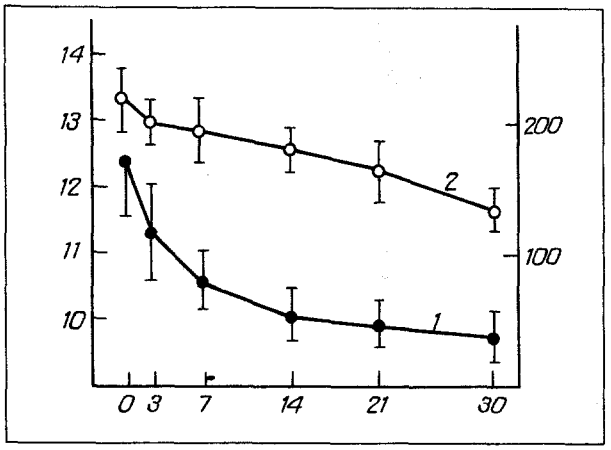

Fig. 2. Changes in latex-stimulated ChL-response (1) and SOD activity of polymorphs (2) during development of MI. Abscissa, time of disease (in days); ordinate: on left — SOD activity (in activity units), right — intensity of ChL (in relative units).

0.01% PMS, and 0.2 ml of a 0.1% solution of NADH. All the solutions were made up before the experiment in potassium-sodium phosphate buffer, pH 7.8, containing 10^{-4} M EDTA. The samples were incubated for 1 h at 37°C with constant mixing. The reaction was stopped by the addition of 1 ml of 1 M HCl. Next, the hydrophobic formazan particles thus formed were sedimented by centrifugation for 15 min at 400*g*. The residue was treated with 3 ml of dimethyl sulfoxide (DMSO). To dissolve the formazan particles more completely in DMSO the samples were kept for 20 min in a boiling waterbath. The sensitivity of the method was increased by using a modification [15] consisting of subsequent addition of 3 ml of 10 M KOH to the samples. After rapid mixing the samples were centrifuged for 15 min at 400*g* to separate the phases. The optical density of the DMSO-containing fraction was measured at λ = 720 nm on a "Spektromom-410" spectrophotometer (Hungary). Control samples contained 0.1 ml of a 0.1% solution of nitro-BT, 0.5 ml of a 0.01% solution of PMS, 0.2 ml of a 0.1% solution of NADH, 1 ml of phosphate buffer, pH 7.8, and also 0.2 ml of a suspension of latex particles ($4 \cdot 10^8$ particles) or phosphate buffer for determining SOD activity after or before stimulation of the polymorphs respectively. Activity of the enzyme was calculated as the

degree of inhibition of the reaction, using a commercial preparation of SOD ("Sigma," USA, 400 activity units/mg protein). The protein concentration in the SOD-containing cell lysate was estimated by the method in [12].

EXPERIMENTAL RESULTS

Investigation of polymorph function showed that the intensity of latex-stimulated luminol-dependent ChL of peripheral blood granulocytes of patients with MI was significantly higher than that in the healthy controls (Fig. 1). Many diseases with a course typical of aseptic inflammation, including MI, are known to be accompanied by leukocytosis. However, the raised level of polymorph function in patients with MI reflects qualitative changes taking place in the cells in this disease, for the same number ($5 \cdot 10^5$), was always used for measurements of ChL. Analysis of curves of ChL versus time of development of luminescence showed that not only the magnitude of the ChL response changes in patients with MI, but also the shape of the curves: the time taken to reach a maximum of the ChL-responses and its latent period are reduced [1]. This type of change in the parameters of the ChL-response of polymorphs may be due to several causes: an increase in the number and (or) affinity of specific receptors and nonspecific binding sites on the surface of the phagocytes [1]; changes in activity of enzyme systems involved in the "respiratory burst" and reduction of oxygen; changes in the physical state of the cell membranes; changes in activity of endogenous enzymic antioxidative systems (SOD, catalase, etc.).

The results of measurement of SOD activity during stimulation of the polymorphs by latex particles are given in Fig. 1. It was found, first, that the initial SOD activity of the blood phagocytes of patients with MI is higher than that in normal individuals, and second, only 30 sec after addition of the stimulator to the polymorphs there was an appreciable increase in SOD activity, which was followed by a decrease. For example, by the 20th minute of measurement activity of SOD in the blood polymorphs of healthy individuals had fallen virtually to its initial level. In other words, during stimulation of polymorphs there was a simultaneous increase both in endogenous SOD activity and in production of AFO by them. Comparison of the kinetics of development of the ChL response and activation of SOD during stimulation of the polymorphs indicates that the maximal increase in activity of the enzyme coincides with the latent period of development of the ChL-response.

Assuming that activation of polymorphs is the result of interaction between stimulator and cell, it must be recalled that the act of this interaction itself takes place very quickly, and the presence of a latent period in the development of the ChL-response to stimulation of granulocytes is evidently due to the property of polymorphs of transforming the signal for interaction between stimulator and receptor into AFO production. In this connection, one cause of the presence and duration of the latent period of the ChL response of polymorphs may be the taking up of that small quantity of AFO produced by polymorphs in the early stage of their stimulation of maximally activated SOD. The subsequent development of the events of the "respiratory burst" of the polymorphs leads to an avalanchelike increase in the amount of AFO produced, with which the SOD is unable to cope, and as a result, the intensity of the ChL response increases.

The fact that such rapid activation of SOD occurs during stimulation of polymorphs evidently rules out the possibility of de novo synthesis of enzyme molecules, more especially because the intensity of synthesis in the granulocytes is significantly lower than in other phagocytes. It can be concluded from an analysis of data in the literature that during contact between stimulator and cell, only the change in permeability for calcium ions is comparable in time with the course of activation of SOD of the polymorphs. For instance, it has been shown that the maximum of the increase in intracellular calcium concentration in polymorphs is observed during the first 10 sec after addition of the stimulator to the cells [11]. This suggested that changes in SOD activity during stimulation of polymorphs are the result of partial proteolysis of the enzyme in the cell. Support for this hypothesis is given by the rapid accumulation of Ca^{2+} ions in the cytosol of the cells during their stimulation [11]; the presence of Ca^{2+}-dependent proteolytic enzymes, such as of calpain-like protease [10], the presence of AFO, increasing the sensitivity of the proteins (possibly of SOD also) to the action of proteolytic enzymes [8].

In other words, the increase in the intracellular calcium concentration in response to stimulation of polymorphs, leading to activation of enzymes of partial proteolysis, and an increase in local amounts of AFO, increasing the sensitivity of the substrate proteins to the action of proteolytic enzymes, creates favorable conditions for partial proteolysis to take place, one possible result of which may be increased SOD activity.

It follows from the results in Fig. 1 that completely satisfactory agreement was found between the initial level of SOD activity and the intensity of the ChL-response of the polymorphs. We next studied correlation between these parameters during treatment of MI. We found that when the course of the disease was favorable, gradual normalization of the ChL-response of the polymorphs, accompanied by a very small decrease in intracellular SOD activity took place (Fig. 2). This constancy of increased

SOD activity in the polymorphs may quite well be one of the causes of depression of polymorph function during the course of this disease.

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