GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVATION IN MOUSE PERITONEAL MACROPHAGES BY SERUM FROM PATIENTS WITH ISCHEMIC HEART DISEASE

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In many pathological states connected with the formation of destructive or inflammatory foci, signs of activation of the mononuclear phagocyte (MNP) system are observed in the body. For instance, in patients with acute myocardial infarction (AMI) the number of monocytes circulating in the blood is increased, activity of the serum lysozyme secreted by them is enhanced, the blood monocytes becomes more capable of adhesion, spreading, and phagocytosis [1]. The factors responsible for the increase in functional activity of the blood monocytes in AMI have not yet been explained. It can only be tentatively suggested that the activating signal is received by the cells through the blood serum.

It has been shown that activation of MNP is always accompanied by a metabolic burst, including activation of the hexose monophosphate shunt (HMPS) [6]. It is accordingly possible to assess activation of MNP by recording the increase in activity of the key enzymes of HMPS, namely glucose-6-phsophate dehydrogenase (G6PD) [7]. Because of the difficulty of obtaining sufficient numbers of human monocytes for biochemical investigation, the effect of human sera was studied in a model system of mouse peritoneal MNP.

The aim of this investigation was to study the effect of blood sera from patients with AMI on functional activity of MNP.

METHODS

Experiments were carried out on 48 patients with acute painful forms of ischemic heart disease (IHD) aged from 40 to 75 years. IHD with a major focus was diagnosed in 35 patients and an intermediate corconary syndrome manifested as an attack of acute coronary insufficiency (ACI) in 13 patients. The diagnosis of AMI was based on the characteristic clinical picture, ECG changes, and a study of activity of serium enzymes, including creatine phosphokinase (CPK). The diagnosis of ACI was based on an attack of angina lasting 30-60 min, not abolished by taking nitroglycerine, and accompanied by transient changes in the terminal part of the ventricular complex on the ECG without any diagnostically significant increase in serum enzyme activity. Blood sera were obtained from patients at different times (from a few hours until 7 days) after the beginning of the pain syndrome. The control group consisted of 37 healthy blood donors. The blood sera, obtained by centrifugation, were frozen at −70°C and kept in the frozen state not more than 3 days until required for investigation. Peritoneal resident MNP were obtained from noninbred mice by the method of flushing out the peritoneal cavity with standard Hanks's solution (pH 7.5) with heparin (5 U/ml), described previously [5]. The washings thus obtained contained $5 \cdot 10^6$ cells in 1 ml, of which 60-70% were MNP. The cell suspension was incubated with serum (experimental samples) in the ratio of 1:1 in siliconized test tubes for 30 min at 37°C with periodic shaking. In parallel experiments the same cells were incubated with Hanks's solution alone (control tests). Cells were incubated with each serum in 2 parallel tubes, the contents of one of which were frozen three times in a mixture of ice with salt (temperature about −12°C) and thawing after the end of incubation, whereas the contents of the second tube were subjected to the same pro-

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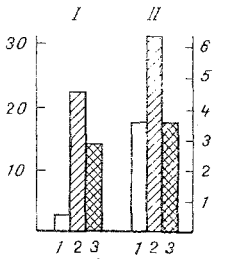

Fig. 1

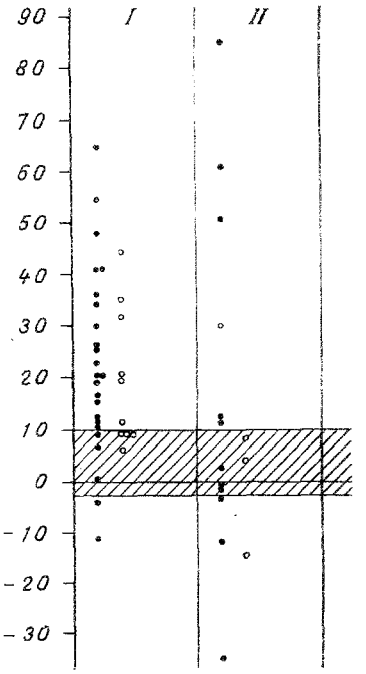

Fig. 2

Fig. 1. Parameters of activating action of sera from blood donors (1) and patients with IHD, obtained during first two days (2) and at later stages (3) after beginning of pain syndrome. I) Δ%, II) multiplicity of increase.

Fig. 2. Dependence of activating action of sera from patients with AMI (filled circles) and ACI (empty circles) from time elapsing after beginning of pain syndrome. Ordinate, Δ%. I) first two days, II) later times. Shaded strip corresponds to confidence interval of median Δ% for group of blood donors.

cedure of freezing at −70°C in liquid nitrogen with alternate thawing. Completeness of destruction of the cells in both tubes was verified microscopically. G6PD activity was determined spectrophotometrically by recording absorption at 340 nm as the result of the formation of NADHP during the reaction [8]. Two parameters were used as criteria of the activating action of the sera of G6PD: the multiplicity of increase and Δ%. The first parameter was calculated as the ratio of G6PD activity in the experiment to its activity in the control, in the case of freezing to −12°C. The second parameter was calculated as follows: first, G6PD activity in the case of freezing in a mixture in liquid nitrogen, for both control and experimental samples; the difference between the values obtained (Δ%) in experiment and control was then found. Serum CPK activity was determined by the method in [2].

RESULTS

Sera from blood donors, on incubation with cells, in most cells had a distinct activating action on G6PD of MNP, as reflected in a multiplicity of increase of 3.47 ± 0.30, although it was hardly reflected at all in the value of Δ%, which averaged 1.98 ± 1.78.

The study of the effect of sera of patients with IHD on G6PD activity of mouse peritoneal MNP revealed significant differences in their activating action depending on the form of IHD, the severity of complications, and the time elapsing after the beginning of the pain syndrome. Blood serum from patients with AMI and ACI, obtained during the first two days after the beginning of the pain syndrome, had the strongest activating action on G6PD of MNP. In this group the value of Δ% averaged 22.17 ± 2.98 and the mean multiplicity of increase was 6.21 ± 0.52 ($P < 0.05$). Blood sera obtained from patients with AMI and ACI at the later stages (3-7 days) from the beginning of the disease, had a weaker activating action, characterized by a mean value of Δ% of 13.94 ± 7.91 and a mean multiplicity of increase of 3.50 ± 1.32 ($P < 0.05$; Fig. 1).

When individual differences in the activating action of the blood sera from patients with AMI and ACI on G6PD of MNP were assessed, confidence intervals of the medians of these parameters for the group of healthy subjects were used as the basis, with the adoption of 95 and 99% levels of significance of the differences. Among 35 sera from patients with AMI, 26 showed an increase in their activating action on G6PD, to judge by the fact that at least one parameter differed with a significance of not less than 95%. With respect to Δ%, 25 of the 35 sera differed with a significance of 95% from the donors' sera, and 17 of the 35 sera differed with respect to this parameter but with a significnace of 99%.

Of the 13 sera from patients with ACI 9 had an increased activating action on G6PD, with a significance of not less than 95% with respect to at least one parameter.

Patients' sera obtained in the early stages of the acute period of the disease (the first two days) had an increased activating action on G6PD of MNP in 87.9% of cases. Meanwhile, sera obtained in the later stages of the pain syndrome had an increased activating action on G6PD of MNP in only half of the cases (Fig. 2). The components activating MNP were evidently present mainly in sera of patients with AMI and ACI obtained during the first 48 h after the beginning of the disease.

As was shown previously, the early stages of the acute period of AMI are characterized by a significant increase in serum CPK activity [3]. CPK levels and the intensity of the activating action on G6PD of MNP were therefore compared in the same sera obtained from patients in the early stages of AMI. CPK activity was raised in 17 of 23 patients of this group; in 15 cases, moreover, increased CPK activity was combined with an activating effect of the same sera on G6PD of MNP, in 5 cases the action of the sera on G6PD of MNP was not accompanied by any increased CPK activity, and only in one patient were no changes found in either parameter.

Among 35 patients with AMI there were 14 with a complicated or severe course of the disease. A distinguishing feature of these sera compared with those of patients with uncomplicated AMI was the fact that in 12 of 14 cases they differed from sera of normal blood donors in the value of Δ% with a 99% level of significance.

Using an increase in G6PD activity of mouse peritoneal MNP as the model, an activating effect on sera from patients with acute painful forms of IHD, obtained during the first 48 h of the disease, on MNP was thus discovered. The nature of the serum warning factors requires further investigation. Participation of components of the serum from patients with acute forms of IHD such as C-reactive protein, regulatory peptides, and proteolytic enzymes, in the processes of MNP activation can only be postulated [4, 9].

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