Relationship between Apoptosis and Expression of Heat Shock Proteins in Peripheral Blood Lymphocytes of Patients with Myocardial Infarction

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We studied activity and dynamics of apoptosis of peripheral blood lymphocytes in patients with myocardial infarction and analyzed the relationship of these processes with expression of heat shock proteins with a molecular weight of 70 kDa playing an essential role in preventing cell death. Thus, we first demonstrated activation of apoptosis in peripheral blood cells of patients with myocardial infarction compared to the control (healthy individuals) and revealed the expected negative correlation between the expression of heat shock proteins with a molecular weight of 70 kDa by lymphocytes and intensity of their death. The observed dynamics of mononuclear cell apoptosis in the peripheral blood of patients with myocardial infarction can reflect activity of programmed cardiomyocyte death in the focus of ischemic injury.

Key Words: *heat shock proteins; apoptosis; myocardial infarction; peripheral blood; mononuclear cells*

Recent studies showed that ischemic injury during myocardial infarction is accompanied, apart from necrotic death of cardiomyocytes, by activation of their apoptosis [6]. A strict correlation was found between cardiomyocyte apoptosis in myocardial infarction and activity of Fas-receptor/Fas-ligand apoptosis-inducing system [5]. Fas-receptor is expressed on various cells, which suggests its universal function in cells of various tissues, including peripheral blood cells [1]. Another universal regulator of apoptosis, including apoptosis of cardiomyocytes and peripheral blood cells, is TNF-dependent apoptosis-inducing ligand (TRAIL) [9,11]. It is important from cardiologic point of view that the expression of TRAIL considerably increases in both the heart tissues and peripheral blood mononuclears [9]. These findings suggest that myocardial ischemia activates cascade reactions inducing programmed cell death not only in damaged tissues, but also in circulating immune cells. The relationship between the clinical picture of myocardial infarction and apoptosis of peripheral blood lymphocytes deserves more comprehensive investigation.

Apart from signals inducing apoptosis, there are various molecules preventing programmed cell death. The protective effects of antiapoptotic heat shock proteins (HSP) are now intensively studied. It was demonstrated that proteins of the HSP-70 family prevent cardiomyocyte death in ischemic and reperfusion injuries [3]. HSP-70 are expressed in both cardiomyocytes [7] and peripheral blood cells [4]. Taking into account the relationship between apoptotic processes in cells of the cardiac tissue and in leukocyte population we can hypothesize that myocardial ischemia affects HSP-70 expression in circulating blood cells. Clinical significance of changed functional state of peripheral blood cells during the development of myocardial infarction is analyzed [2,8].

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Here we studied activity and dynamics of spontaneous and induced apoptosis of peripheral blood lymphocytes in patients with acute myocardial infarction and analyzed the relationship between these processes and HSP expression.

MATERIALS AND METHODS

We examined 29 patients (16 men and 13 women; mean age 64.1±12.8 years) with acute Q-forming myocardial infarction hospitalized in intensive cardiologic care unit of N. N. Pirogov Municipal Hospital No. 1 (Moscow) within 24 h after disease onset. Standard complete examination and treatment were performed in all patients. The control group comprised 11 age- and sex-matched healthy donors.

The blood was taken from the cubital vein on days 1, 7, and 14 of the disease. For evaluation of subpopulation composition of lymphocytes and the intensity of their apoptosis we used fraction of mononuclear cells (MNC) of the peripheral blood isolated by standard centrifugation in Ficoll-verografin density gradient (1.077-1.078 g/cm^3). Isolated MNC were incubated in RPMI-1640 (Sigma Aldrich) supplemented with 10% FCS (PanEko) and 2 mM L-glutamine (Sigma Aldrich) for 4 h at 37°C and 5% CO_2 in the presence of 200 μM H_2O_2 (apoptosis induced by oxidative stress) or without stress factors (spontaneous apoptosis).

The intensity of programmed cell death was evaluated by flow cytofluorometry using DNA-specific fluorochrome propidium iodide (Sigma Aldrich). Intracellular content of HSP-70 was evaluated after preliminary 30-min fixation of cells with 2% paraformaldehyde in the presence of 0.5% Triton X-100 at room temperature followed by staining with antibodies in phosphate buffered saline containing 1% FCS and 0.1% Triton X-100. The relative intracellular concentration of HSP-70 was measured by flow cytofluorometry by the mean fluorescence intensity of cells in samples treated with BRM22 antibodies (Sigma Aldrich) and second FITC-labeled antibodies (Sigma). Cytofluorometry was performed on FACSscan flow cytofluorometer (Becton Dickinson). Not less than 10,000 cells were analyzed in each sample. The data were processed using Cell Quest and WinMDI software.

RESULTS

In patients with myocardial infarction, the level of spontaneous apoptosis of peripheral blood MNC on day 1 of the disease 3.7-fold surpassed the corresponding parameter in the control group (Fig. 1). The intensity of MNC apoptosis induced by oxidative stress also surpassed the control level (by about 2.2 times) at this term. By day 7, the intensity of spontaneous and induced apoptosis of MNC increased: the percentage of apoptotic cells at this term was maximum over the whole observation period and surpassed the control levels for spontaneous and induced apoptosis by 5.7 and 2.7 times, respectively. By day 14, the recorded levels of spontaneous and induced apoptosis in MNC from patients with myocardial infarction significantly decreased, but still remained above the control by 2.8 and 1.7 times, respectively (Fig. 1).

The observed increase in apoptosis intensity of peripheral blood MNC in patients with myocardial infarction supplements published data on the effect of ischemic damage to the cardiac tissues on the functional state of circulating lymphocytes [2,8,9]. We also first characterized the dynamics of the percentage of apoptotic cells in MNC samples from patients with myocardial infarction (Fig. 1). Taking into account

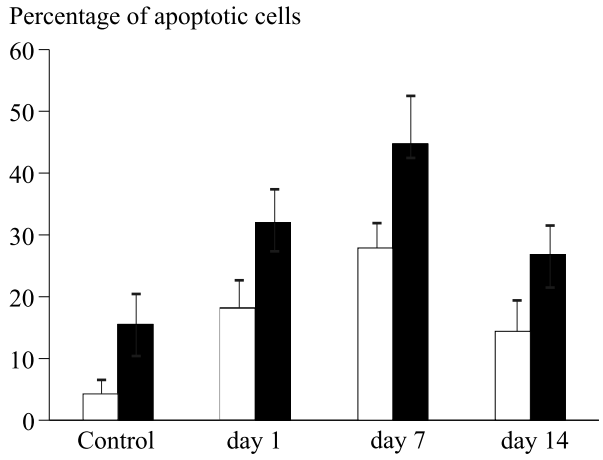

Fig. 1. Percentage of apoptotic cells in *in vitro* cultured MNC samples isolated from the peripheral blood of patients with myocardial infarction at different terms of the disease (cytofluorometry data). Here and on Fig. 2: open bars: spontaneous apoptosis; dark bars: stress-induced apoptosis.

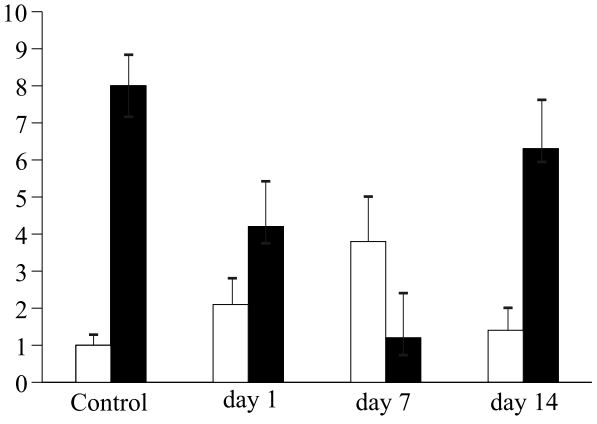

Fig. 2. Intracellular content of HSP-70 in *in vitro* cultured MNC samples isolated from the peripheral blood of patients with myocardial infarction at different terms of the disease. Ordinate: mean relative fluorescence of cells stained with monoclonal antibodies to HSP-70 and FITC-labeled antibodies.

the universal nature of signaling systems, *e.g.* Fas-receptor/Fas-ligand and TRAIL regulating the processes of programmed cell death in the organism [1,5,11] we can hypothesize that the observed changes in the level of peripheral blood MNC apoptosis in patients with myocardial infarction reflect the intensity of cardiomyocyte apoptosis in the focus of damage.

No significant correlations between the intensity of spontaneous and induced MNC apoptosis and the age and sex of the examinees and the presence of concomitant diabetes mellitus were found in patients with myocardial infarction and in the control group. The intensity of apoptosis in the main group also did not depend on the previous history of myocardial infarction or angina pectoris attacks. We revealed no relationships between the levels of spontaneous and induced apoptosis and parameters of contractile functions of the myocardium (ejection fraction and size of the left ventricle). However, it was found that activity of programmed death of peripheral blood MNC is affected by concomitant arterial hypertension in patients with myocardial infarction (data are not shown).

In a special experimental series in parallel with evaluation of the intensity of spontaneous and induced apoptosis of MNC we analyzed the expression of intracellular HSP-70 in peripheral blood lymphocytes of healthy donors and patients with myocardial infarction (Fig. 2). Cytofluorometry revealed the maximum intracellular content of HSP-70 in lymphocytes from healthy donors in oxidative stress. In patients with myocardial infarction, synthesis of intracellular HSP-70 in MNC during spontaneous and induced apoptosis also increased on day 1 compared to the control, but on day 7 increased expression of HSP-70 was observed only in MNC incubated in the absence of stress factors (spontaneous apoptosis). In contrast, oxidative stress (induced apoptosis) considerably reduced HSP-70 content in lymphocytes at this term (this reaction of lymphocytes to stress requires additional analysis and can be related to the release of cytoplasmic HSP-70 into the extracellular space). On day 14 of the disease, a tendency to recovery of the intracellular concentration and production of HSP-70 to the control values was observed under the effect of oxidative stress (Fig. 2).

The results of two experimental series (Figs. 1 and 2) agree with the concept of antiapoptotic functions of HSP-70. These findings attest to a negative correlation between the intensity of stress-induced MNC apoptosis and the expression of HSP-70 by these cells, because the decrease in the production of HSP-70 under conditions of oxidative stress was accompanied by an increase in apoptosis intensity. This correlation was not observed on the model of spontaneous apoptosis; the dynamics of apoptosis intensity in myocardial infarction was similar to the dynamics of intracellular content of HSP-70 in lymphocytes (Fig. 1, 2). The observed difference in the results of measurement of HSP-70 expression and MNC apoptosis intensity on the models of spontaneous and induced apoptosis can be explained by the existence of two forms of HSP-70 molecule: constitutive and stress-induced. According to current notions, induced HSP-70 exhibit the most pronounced protective and antiapoptotic properties, while constitutive form acts primarily as chaperones [7,10]. Specific antibodies used in our experiments detected both forms of HSP-70, but the expression of induced antiapoptotic form of this protein predominated over the synthesis of constitutive HSP-70 under conditions of oxidative stress, which most likely determines the observed decrease in apoptosis intensity with increasing the intracellular content of HSP-70. Under normal physiological conditions in the absence of stress (spontaneous apoptosis), primarily constitutive HSP-70 not exhibiting pronounced protective potential are synthesized, which determines the absence of the negative correlation between apoptosis activity and HSP-70 expression in the experiment.

Thus, our experiments demonstrated a relationship between apoptosis of peripheral blood MNC, expression of HSP-70 by these cells, and dynamics of myocardial infarction. The observed dynamics of spontaneous and induced programmed death of peripheral blood MNC in patients with myocardial infarction can reflect activity of programmed cardiomyocyte death in the focus of ischemic injury.

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