Apoptosis of Leukocytes as a Marker of Neutrophil-Endotheliocyte Interaction in Coronary Heart Disease

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We studied the mechanism of interaction of peripheral blood neutrophils with endothelial cells (expression of cell adhesion molecules and production of NO) and the role of neutrophil apoptosis in the development of endothelial dysfunction. The effects of mitochondrial dysfunction of neutrophils on the development of apoptosis of these cells after their interaction with endothelial cells were analyzed.

Key Words: *neutrophils; nitric oxide; aoptosis; endothelial dysfunction*

The role of neutrophil apoptosis in the pathogenesis of coronary heart disease (CHD) is an actual problem in modern cardiology, clinical biochemistry, and clinical pathophysiology [1,7]. Recent, studies of endothelial dysfunction are focused on the role of cell-cell interactions, in particular, on the mechanism of interaction of peripheral blood neutrophils (PBN) with endothelial cells (expression of cell adhesion molecules and production of NO) [3]. Adhesion of polymorphonuclear leukocytes to the endothelium requires expression of adhesion molecules by both endothelial cells and PBN. Under the effect of chemoattractant, neutrophils express adhesion molecules of the integrin family specifically interacting with intercellular adhesion molecules 1 and 2 (ICAM 1 and 2) belonging to the immunoglobulin family. For instance, leukocytic PECAM-1 (CD31) involves itegrin-associated protein (IAP) on the endothelium and stimulates Ca^{2+} entry into cells. This leads to endothelial retraction and loss of tight junctions, which enables leukocyte migration. There are experimental data that leukocytic IAP after binding of the corresponding ligand transmits the intracelluar signal inhibiting adhesion with the endothelial ligand PECAM-1, thus increasing cell mobility. Immunoglobulin molecules also play an important role in activation processes and cell-cell interactions. Three members of this superfamily (ICAM-1, ICAM-2, and VCAM-1) are involved into leukocyte-endothelium interaction. However, the role of regulation of leukocyte apoptosis after their interaction (under physiological and pathological conditions) with endothelial cells remains little studied [8].

It is interesting to study endothelial dysfunction in patients with different forms of CHD: stable angina and postinfarction cardiosclerosis (PCS). It is known that aortocoronary bypass (ACB) surgery in CHD patients often induces systemic inflammatory response accompanied by uncontrollable release of endogenous antiinflammatory mediators and systemic endothelial dysfunction [5,6]. In patients with PCS, endothelial dysfunction is associated with less pronounced chronic inflammation of the vascular wall than after ACB surgery. Thus, we can hypothesize that the mechanisms of regulation of leuko-

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cyte-endothelium interactions in these patients are seriously disturbed.

Here we evaluated some mechanisms underlying the development of endothelial dysfunction in CHD from the viewpoint of cell-cell interactions in the neutrophil—endotheliocyte system.

MATERIALS AND METHODS

The blood from patients with different forms of CHD was the object of the study. The patients were divided into 3 groups. Group 1 included 15 men with effort angina during the first 2 days after ACB surgery. The duration of artificial circulation during surgery was 60-90 min. Group 2 comprised 23 men with PCS. The control group consisted of 23 individuals without CHD. The mean age of the examinees was 57 years. Prolonged nitrates were withdrawn at least 24 h before blood sampling.

Neutrophils were isolated from the whole heparinized peripheral blood by centrifugation on multistep Ficoll-verografin density gradient. The densities of the upper and lower gradients were 1.142 and 1.062 g/cm^2, respectively. Neutrophils in a concentration of 1×10^6 cells/ml were used for detection of apoptosis using Annexin-V Apoptosis Detection Kit (Caltag). Four cell populations were thus identified: viable cells, cells with externalized phosphatidylserine, cells in the state of secondary necrosis, and necrotic cells.

Expression of CD31 on PBN was evaluated immunocytochemically by incubating fixed preparation containing 100 μl PBN suspension (1×10^6 cell/ml) with monoclonal FITC-labeled anti-CD31 antibodies (Caltag). The number of CD31$^+$ cells in the sample was counted under a fluorescent microscope.

Functional activity of mitochondria in PBN was evaluated by measuring their membrane potential [2]. PBN suspension (1×10^6/ml, 100 μl) was incubated with 1 μl 200 nM Rhodamine-123 (Sigma) for 40 min at 37°C. Fluorescence was detected using a CM2203 spectrofluorometer (Solar) at λ_{ex}=488 nm and λ_{em}=525 nm.

Activity of NO synthase was evaluated fluorometrically by plasma content of NO at λ_{ex}=375 nm and λ_{em}=415 nm using NO Assay Kit (Calbiochem).

The data were processed using Student *t* test and correlation analysis.

RESULTS

PBN are the first cell responding to endothelial damage. Their is preceded by participation in inflammation cell activation, respiratory burst, production of inflammation mediators and oxygen radicals by NADPH oxidase. These changes then lead to leukocyte apoptosis. In our study, the number of viable PBN was higher in the control group (78%) and was reliably decreased in the ACB (68%) and PCS groups (70%; $p<0.001$). The number of cells with externalized phosphatidylserine was higher in ACB and PCS group (23 and 21%) compared to the control group (13%, $p<0.001$). The numbers of necrotic cells and cells at the stage of secondary necrosis were similar in all three groups (Fig. 1). When evaluating functional activity of mitochondria we revealed a significant decrease in membrane potential in ACB and PCS groups (0.41± 0.16 and 0.37±0.18 arb. units) compared to the control group (0.62±0.16 arb. units). It is known that CD31 expression by neutrophils is important for their adhesion to the endothelium, transendothelial migration, and production of reactive oxygen species. The number of cells expressing CD31 was higher in the ACB and PCS groups (27 and 23%) compared to the control group (17%, $p<0.001$, Fig. 2). Stimulated adhesion and transendothelial migration of PBN lead to dysregulation of endothelial NO synthase. Plasma NO concentration was considerably lower in ACB and

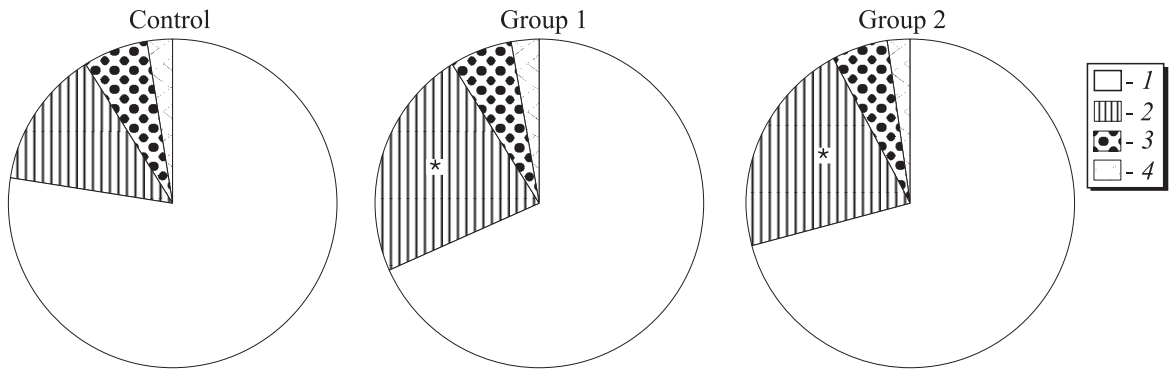

Fig. 1. Relative content of different PBN populations. *1*) viable cells; *2*) apoptotic cells; *3*) necrotized cells; *4*) cells in a state of secondary necrosis and terminal apoptosis. *$p<0.001$ compared to the control.

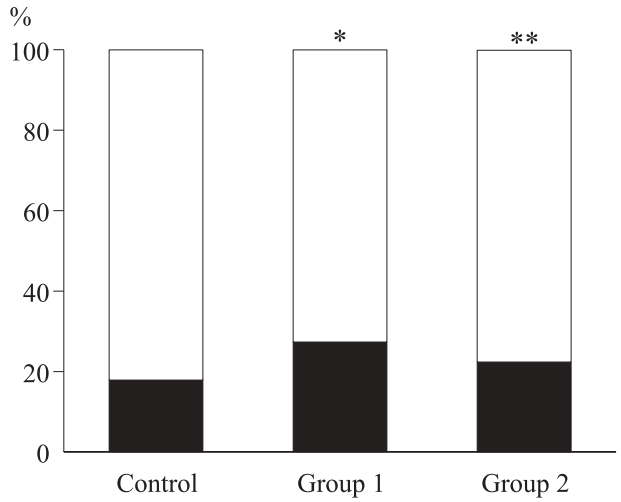

Fig. 2. Relative content of $CD31^+$(light bars) and $CD31^-$ (dark bars) cells. *$p<0.0001$, **$p<0.01$ compared to the control.

PCS group (12.2 and 6.1 nM) compared to the control group (26.9 nM, $p<0.001$).

Correlation analysis revealed a close relationship between PBN apoptosis and CD31 expression ($r=0.67$) and a positive medium-strength relationship between CD31 expression and NO concentration ($r=0.53$) in group 1. A weak positive correlations were found between apoptosis and number of $CD31^+$ cells in the control group ($r=0.4$), apoptosis and NO concentration in groups 1 ($r=0.42$) and 2 ($r=0.34$), CD31 expression and NO concentration in group 2 ($r=0.35$).

It can be hypothesized that the development of systemic inflammatory reaction in the postoperation period in patients with stable angina after ACB or in patients with PCS is the main cause of enhanced expression of cell adhesion molecules on PBN (probably, due to stimulating effect of proinflammatory cytokines). This, in turn, provokes intensification of cell-cell interactions in the neutrophil—endotheliocyte system, which leads to the development (or aggravation), of endothelial dysfunction manifested in decreased production of some vasodilator factors, including NO. It is known that activation of phagocytizing cells initiates their apoptosis [4]. Thus, the intensity of apoptosis in PBN population reflects the degree of endothelial disfunction.

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