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SHORT COMMUNICATIONS

The Role of Endotoxin Aggression in Pathogenesis of Acute Myocardial Infarction

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Abstract—Endotoxin aggression of intestinal origin occurs in 89% of patients with acute myocardial infarction and may be a factor of the induction and/or progression of the disease. The most common sources of endotoxin aggression are *Escherichia coli, Bacteroides, Klebsiella, Proteus*, and *Pseudomonas.*

Keywords: acute myocardial infarction, endotoxin aggression, pathogenesis, ethiology
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It is well known that the primary pathogenic factor underlying acute myocardial infarction (AMI) is atherosclerosis of coronary arteries. In the 1980s, it was hypothesized that endotoxin (ET), which is a lipopolysaccharide (LPS), can induce atherosclerosis [1]. This hypothesis was based on the results of the studies that showed that the alteration of endothelial cells of rabbit coronary arteries was accompanied by proliferative and desmoplastic processes already in the first day of experiment [2]. This hypothesis was confirmed by clinical trials performed by Savel'ev et al. [3, 4], who showed a direct relationship between diffuse peritonitis and progression of diseases of atherosclerotic nature. This finding does not limit the possibility of involvement of excess ET in the general circulation, which was termed "endotoxin aggression" (ETA), and in the mechanisms of AMI development, since ETA may be the only cause of induction of both disseminated intravascular coagulation and local thrombosis at atherosclerotic plaque, because endotoxin plays a crucial role in the regulation of hemostasis [5]. This assumption has been indirectly confirmed by foreign authors who studied the pathogenesis of chronic heart failure [6]. This fact motivated us to perform this study.

We examined 72 patients in the intensive care unit of State Clinical Hospital no. 19 (Moscow), who were diagnosed with acute Q-wave myocardial infarction. The study was approved by the local ethics committee. In total, we examined 47 male and 25 female patients 39 to 93 years old (mean age, 65 years). Of them, 35 (48%) and 37 (52%) patients were diagnosed with anterior and posterior, respectively, myocardial infarction. The diagnosis of AMI was based on electrocardiographic, echocardiographic, physical, and anamnestic data. The biochemical diagnosis consisted in determining the level of the myocardial fraction of creatine kinase by a quantitative method using a Random A 25 biochemical analyzer and the troponin test using the Veda Lab diagnostic kit. In 56 patients, AMI had a favorable prognosis, and 16 patients died of its various complications (cardiogenic shock, pulmonary edema, and tamponade).

The total concentration of bacterial LPS was determined in the LAL test using the E-toxate reagent (Sigma, United States) in our modification adapted to clinical conditions (in EU/mL, with the use of the endotoxin reference standard (Sigma)), which is based on the ability of LPS to form fractals. The activity of the antiendotoxin immunity (AEI) was assessed by determining the concentration (in arbitrary optical density units (OD units)) of antibodies to the most common antigens of the LPS molecule—the hydrophobic moiety (Re-chemotype glycolipid, which is present in all endotoxins and is responsible for the whole range of general biological properties of endotoxins) and the hydrophilic part (common enterobacterial antigen (CEA)) by the screening assessment of the immune status by enzyme immunoassay. The results were compared with the values obtained in an age-matched control group of similar age (31 men and 27 women) without a verified chronic inflammatory pathology.

The ETA etiology was determined serologically by a procedure based on the determination of antibodies (in OD units) to *E. coli, Pseudomonas aeruginosa, Proteus, Bacteroides fragilis*, and *Klebsiella*. The source of ETA was considered verified if the antibody concentration was 3 times higher than the upper limit of nor-

Table 1. Mean LPS concentration and AEI activity in serum of patients with acute myocardial infarction

Group, number of patients	LPS Concentration, EU/mL	Concentration of antibodies, OD units	
		to glycolipid	to CAE
AMI patients, $n = 64$	1.76 [1.3; 1.9]	162 [134;186]	307 [256; 365]
Control, $n = 58$	0.70 [0.3; 0.9]	151 [123;197]	285 [234; 342]
Differences between groups	$p = 0.001$	$p = 0.05$	$p = 0.04$

For interpretation of abbreviations in tables, see "Methods."

Table 2. Comparison of LPS concentrations and antiendotoxin immunity (AEI) activity in patients with acute myocardial infarction (AMI) depending on the outcome of the disease

AMI patients, number of patients	LPS concentration, EU/mL	Concentration of antibodies, OD units	
		to glycolipid	to CAE
With favorable outcome, $n = 49$	1.79 [1.2; 2.5]	157 [132; 197]	300 [200; 346]
With lethal outcome, $n = 15$	1.65 [1.1; 2.0]	179 [120; 204]	409 [256; 454]
Significant differences between groups			$p = 0.02$

mal or 3 times smaller that the lower limit, which is accepted in clinical immunology. In addition, we used the serologic method for identification of general candidiasis. The results were compared with respective values detected in the control group, which comprised 127 donors (79 men and 48 women). If the concentration of an antibody to LPS of a given Gram-negative bacterium was more than 3 times greater than the upper limit of normal or, respectively, more than 3 times lower than the lower limit in the control group, the source of ETA was considered identified.

Blood samples were taken directly in the intensive care unit at different time intervals from the first clinical manifestations of AMI (in the range from 2 to 24 h).

The results were processed by the nonparametric statistical analysis using the StatSoft software ver. 8.0 (StatSoft Inc., United States). Data were represented as the median and interquartile ranges: *Me* [*X*1/4; *X*3/4]. The significance of differences between the groups was estimated using the Mann–Whitney test; the critical level of significance of differences was taken to be 0.05.

In the study, we found the signs of ETA in 64 (89%) patients. The data summarized in Table 1 indicate a 2.5-fold increase in the concentration of endotoxin and antibodies to CEA, which is the first response of the immune system to the endotoxin entering the bloodstream; only several days later, a widely varying increase in the concentration of antibodies to the glycolipid (from 22 to 468 OD units) was observed, which may indicate that patients with AMI were in different phases of ETA development. In this regard, it was interesting to find out if there is a correlation between the studied parameters and the outcome of the disease (Table 2).

The analysis of results showed significant differences only for the antibodies to CEA, whose concentration at a lethal outcome was 25% higher. Since high concentrations of ABs to GLP are typical for the ETA-induced systemic inflammatory response syndrome [7], we decided to divide the group of AMI patients into two subgroups with low (113 OD units [98, 134]) and high (339 ± 23 OD units [267, 378]) content of antibodies to the glycolipid in serum. The first and second subgroups included 78 and 22% of patients, respectively.

The mortality in the second subgroup of patients was significantly greater than in the first (20 and 36%, respectively). These results allowed us to postulate the involvement of ETA in the AMI pathogenesis in the vast majority of patients, which prompted us to establish its sources (Table 3). The latter included *Bacteroides* (in 66.7% of cases), *Klebsiella* and *Pseudomonas aeruginosa* (30%), *Proteus* (22.2%), and *Escherichia coli* (20.6%). These bacteria are the etiological factors in the ETA development, whose selective elimination from the intestine can improve the effectiveness of

Table 3. Frequency of involvement of Gram-negative bacteria in the development of endotoxin aggression in patients with acute myocardial infarction

Endotoxin aggression source	Patients with acute myocardial infarction			
	favorable outcome	lethal outcome	total number	percentage of involvement
Bacteroides	32	10	42	66.7%
Escherichia coli	9	4	13	20.6%
Klebsiella	12	7	19	30.0%
Pseudomonas aeruginosa	10	3	13	30.0%
Proteus	10	4	14	22.2%

Table 4. Number of endotoxin aggression sources in patients with acute myocardial infarction depending on the outcome of the disease

Acute myocardial infarction, its outcome	Number of identified etiological factors of endotoxic aggression						Total
	1	2	3	4	5	0	
Favorable	20	14	9	3	2	1	49
Lethal	4	6	2	2	–	–	14
All patients	24	20	11	5	2	1	63
Percentage	38.0%	31.7%	17.5%	8.0%	3.2%	1.6%	100%

treatment. It should be noted that antibodies to LPSs of all five Gram-negative bacteria were always present in the serum of both AMI patients and healthy subjects from the control group. This fact indicates that the sources of systemic endotoxemia (as a physiological phenomenon) and ETA (as a "pre-disease" and/or a universal factor in the pathogenesis of diseases) are most diverse Gram-negative bacteria of the intestinal microflora, including those unidentified in this study, because the etiology of ETA was established in 62 of 63 patients (i.e., in 98.4% of cases; Table 4). The source of ETA was usually represented by one or two Gram-negative bacteria (in 38% and 31.7% of patients with AMI, respectively); more rarely, three (17.5%), four (8.0%), and five (1.6%). The number of ETA sources in AMI patients was not associated with the outcome of the disease. This fact indirectly indicates that an important factor of ETA development is an increase in intestinal permeability.

Another important aspect of the AMI pathogenesis may be the presence of systemic candidiasis in every third patient, because the conserved structures of *Candida*, similarly to LPS, are able to interact with Toll-receptors and activate the innate immunity and, as a consequence, increase the activity of the adaptive immune system, which functions stochastically, i.e., indiscriminately marks both own and foreign antigens for subsequent destruction [8, 9]. Systemic candidiasis can potentiate the LPS-induced inflammation, a manifestation of which is thrombosis and progression of coronary artery occlusion. This is confirmed by the following fact established in this study: the laboratory signs of systemic candidiasis in the case of a lethal outcome were detected 2.25 times more frequently than in the patients with a favorable prognosis of AMI.

CONCLUSIONS

(1) The vast majority of patients with acute myocardial infarction suffer from endotoxin aggression, which can be the cause of initiation and progression of atherosclerosis and thrombosis and the pathogenic effect of which can be potentiated by systemic candidiasis.

(2) The most common etiological factors in the development of endotoxin aggression in the patients with acute myocardial infarction are *Bacteroides*; more rarely, *Klebsiella, Proteus, Pseudomonas aeruginosa*, and *Escherichia coli*.

(3) The contribution of endotoxin aggression to the pathogenesis of acute myocardial infarction requires further investigation.

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