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Markers of apoptosis and oxidative stress in congestive heart failure

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Abstract

Background: The aim of this work is to study the markers of apoptosis (granzyme B) and oxidative stress (nitric oxide), as well as indicators of endothelial damage - cystatin C and lipid metabolism in patients with congestive heart failure, and to identify the relationship between these parameters.
Material and methods: The study included 114 patients (men and women) with congestive heart failure (CHF), out of which 41 patients with CHF, 39 patients with CHF and diabetes mellitus type 2, and 34 patients with CHF with metabolic syndrome. Biochemical parameters were measured with the help of reagent sets produced by "Human" company (Germany), the contents of granzyme B and cystatin C – with the help of commercial sets produced by "USCN Life Science Inc" company (China), while nitric oxide concentration was determined using a commercial kit by "R&D System"
Results: Analysis of indicators of apoptosis factors and oxidative stress in studied patients revealed a substantial increase in patients with heart failure in the presence of diabetes mellitus type 2, compared to two other groups of patients. The concentration of cystatin 3 in patients with diabetes mellitus type 2 increased significantly with *p <0,05.
Conclusions: The studied parameters allow us to suggest that complications in CHF are due to the intensity of oxidative stress, apoptosis and atherogenesis, and are interconnected with biochemical changes in lipid and carbohydrate metabolism.

Key words: apoptosis, metabolic syndrome, congestive heart failure, diabetes mellitus.

Introduction

Worldwide the prevalence and characteristics of the metabolic syndrome (MS) are linked to immobile lifestyle, consumption of high-calorie "fast food" products, as well as global unfavorable environment. According to recent research conducted by ARIC (Atherosclerosis Risk in Communities Study), the prevalence of metabolic syndrome among men and women constitutes 23% and 24%, respectively [1]. In the age group from 25 to 45, this pathological state makes up 35-53,1%. In patients with pathological syndrome the risk of cardiovascular complications increases, including myocardial infarction and stroke, which results in mortality, in 80% of cases MS leads to the development of diabetes mellitus [2].

Currently an increase in prevalence of diabetes mellitus type 2 (DM-2) is observed in economically developed countries. It is known that in patients with DM-2 the risk of development of vascular pathology, including coronary artery disease, increases by 2-4 times. In turn, combination of DM-2 and heart failure boosts the risk of fatal outcome by 4 times. In MS, as well as in DM, the risk of development of cardiovascular complications, including myocardial infarction and stroke, is very high [3].

Apoptosis plays an important role in the pathogenesis of cardiovascular diseases, such as atherosclerosis, cardiac ischemia and congestive heart failure [4]. Thus, the extent of damage to cardiomyocytes is determined by such pathological processes as apoptosis, oxidative stress, as well as metabolic changes. The aim of this work was to study the markers of apoptosis (granzyme B) and oxidative stress (nitric oxide), as well as indicators of endothelial damage – cystatin C and lipid metabolism in patients with CHF and to identify the relationship between these parameters.

Material and methods

The research included 114 patients (men and women) with congestive heart failure (CHF). Patients were divided into 3 groups: I group – 41 patients with CHF, II group – 39 patients with CHF and DM-2, and III group – 34 patients with CHF with metabolic syndrome. 10 healthy patients comprised the control group. During the stay in hospital laboratory and functional studies were conducted in all patients, antianginal and antiplatelet therapy was appointed.

The research was conducted in compliance with the Declaration of Helsinki (1975) which was revised in 1989 in Hong Kong.

From biochemical indicators in blood plasma the concentrations of total cholesterol (TC), α-lipoprotein cholesterol, β-lipoprotein cholesterol, glycosylated hemoglobin (HbAlc), glucose were measured. From apoptosis markers granzyme B was determined, from indicators of oxidative stress nitric oxide (NO) was measured, as well as cystatin C – as atherogenesis factor. Biochemical parameters were measured with the help of reagent sets produced by "Human" company (Germany), the contents of granzyme B and cystatin C – with the help of commercial sets produced by "USCN Life Science Inc" company (China), while nitric oxide concentration was determined using a commercial kit by

"R&D System". Statistical analysis was conducted with the help of Wilcoxon non-parametric criterion (Mann–Whitney test), differences were considered significant at *p <0,05; **p<0,01; *** p <0,001 compared to the control value.

Results and discussion

Biochemical data obtained in all studied groups are provided in table 1, while markers of apoptosis and oxidative stress are given in table 2.

As it can be observed, the amounts of lipoprotein complexes, triglycerides and free cholesterol are significantly different from the control group. Thus, in the control group the level of cholesterol was 2,85 ± 0,21 mmol/l, while the level of triglycerides was 1,01 ± 0,14 mmol/l. An increase in both indicators was identified during comparison of average values in patients with CHF and CHF with metabolic syndrome and diabetes mellitus type 2. Thus, concentration of cholesterol increased up to 4,51 ± 0,23 mmol/l (p <0,001) in I group, while in II and III groups – up to 5,25 ± 0,33 mmol/l and 5,47 ± 0,27 mmol/l, respectively. At the same time, the concentration of α-lipoprotein cholesterol declined by 36% in patients in I group, by 45% in patients in II group and by 61% in patients in III group. Along with this, β-lipoprotein cholesterol increased by 45% in patients in I group, by 64% in patients in II group and in average by 94% on patients in III group. Hypercholesterolemia and hyper-β-lipoproteinemia observed during studies contribute to the damage of the vascular endothelium as a result of atherogenesis. It was found that high density lipoproteins, a major anti-atherogenic fraction of lipoproteins, protect endothelial cells from apoptosis, therefore providing an important and new dimension of its anti-atherogenic activity [5].

Concentration of triglycerides in I group increased by 3,7 times, in II group – by 4,6 times and in III group – by 4,1 times, which is consistent with changes in carbohydrate metabolism, namely glucose and glycosylated hemoglobin. In CHF with DM-2 the content of glucose reached 7,3 ± 1,1 mmol/l, while in II group this indicator stood at 5,1 ± 1,2 mmol/l, in III group – at 4,1 ± 0,1 mmol/l. The level of Hв A1 in CHF with DM-2 constituted 6,7 ± 0,07%, while in I and III groups this indicator was nearly the same and on average stood at 5,4 ± 0,01 %.

Thereby, quantitative imbalance between parameters of lipid metabolism and lipoprotein complexes, as well as dynamics of changes in levels of glucose and glycosylated hemoglobin show similar trend in all studied groups of patients with CHF. Similar results were obtained in the analysis of the average values of nitric oxide and granzyme B in patients with CHF (Table 2).

While the control value of nitric oxide was 10,2 ± 0,4 μmol/l, in all studied groups this parameter increased to the following values: 13,7 ± 0,4 μmol/l in I group, 23,4 ± 0,5 μmol/l in II group, 21,4 ± 0,5 μmol/l in III group.

NO is a short-living molecule which breaks down within 6-30 seconds. At the same time, NO is recovered by its involvement in dinitrosyl iron complexes with thiol ligands or in 8-nitrozols, which may later gradually release NO. Such NO-containing complexes are formed in the tissues of physiologically active depot. Deposition of NO in the arterial wall begins with any increase of NO levels in the body, regardless of its cause. Excess amount of NO at first performs a compensatory function, aimed at improving the tissue perfusion. Later on, there is a transformation of reaction into the pathological one with the induction of apoptosis, activation of oxidative stress, destructive processes, increasing myocardial dysfunction [6, 7].

Nitric oxide is irreversibly inactivated by the reaction with hemoglobin (in oxygenated and dioxygenated forms) in the lumen of the blood vessel, by superoxide radical in the blood vessel wall or by oxygen in free solution [8, 9]. Reaction of nitric oxide with oxygen is accompanied with the formation of stable end products – nitrite and nitrate, which are indirect markers of concentration of nitric oxide in the body [10].

Cathepsins are direct executors of apoptosis, which is based on proteolysis caused by cysteine proteases such as caspases, cathepsins, granzyme B [11].

Although the content of granzyme B is widely studied in immunological disorders, the role of granzyme B/perforin system in cardiovascular pathology is insufficiently studied [12, 13].

In studied patients concentration of granzyme B significantly increased in I group by 1,5 times (p<0,001), in II group by 2,5 times (p<0,001), in III group – by nearly 2 times (p<0,001) in comparison with the control group.

Granzymes enter the target cell through pores formed by perforin and trigger apoptosis by activating caspases. Granzymes belong to the family of exogenous serine proteases. To date, five classes of granzymes are identified, such as A, B, H, K and M. These granzymes are produced in an inactive form and are activated by cathepsin C-mediated removal of the propeptide.

Granzymes A and B are the most studied ones. Granzyme B cleaves Asp or Glu residue of pro-caspases, resulting in activated caspase cascade, which ultimately leads to the death of the target cell. Activated caspases induce DNA fragmentation and cell apoptosis [14, 15]. Under normal conditions, the system granzyme B/perforin plays an important role in liquidation of abnormal cells, having antiviral activity and participating in the elimination of tumor cells. Thus, granzyme plays an important role in CTL-mediated immune response.

Table 1

Biochemical parameters in patients with CHF, M ± m

Parameters	Control group (N=10)	I group (N=41)	II group (N=39)	III group (N=34)
Cholesterol, mmol/l	2,85 ± 0,21	4,51 ± 0,23***	5,25 ± 0,33***	5,47 ± 0,27***
Triglycerides, mmol/l	1,01 ± 0,14	3,74 ± 0,37***	4,66 ± 0,24***	4,19 ± 0,29***
α-LP, mmol/l	1,29 ± 0,16	0,83 ± 0,11*	0,72 ± 0,08**	0,50 ± 0,03***
β-LP, mmol/l	2,49 ± 0,21	3,60 ± 0,26**	4,06 ± 0,35**	4,83 ± 0,18***
Glucose, mmol/l	4,2 ± 0,2	5,1 ± 1,28*	7,3 ± 1,1**	4,1 ± 0,1
Нв A1, %	5,5 ± 0,08	5,3 ± 0,01	6,7 ± 0,07*	5,4 ± 0,01
Cystatin C, mg/l	0,8 ± 0,01	1,1 ± 0,04	1,4 ± 0,07*	1,2 ± 0,06

*p <0,05; **p<0,01; ***p <0,001 compared to the control value

Table 2

Markers of apoptosis in patients with CHF, M ± m

Parameters	Control group (N=10)	I group (N=41)	II group (N=39)	III group (N=34)
NO, μmol/l	10,2 ± 0,4	13,7 ± 0,4***	23,4 ± 0,5***	21,4 ± 0,5***
Granzyme B, ng/ml	14,5 ± 0,9	22,0 ± 1,4***	36,4 ± 0,8***	28,8 ± 1,1***

*p <0,05; **p<0,01; *** p <0,001 compared to the control value

A number of researchers have suggested that granzyme B can cleave extracellular matrix [16]. It is assumed that granzyme B is involved in chronic, as well as acute inflammation in the atherosclerotic processes in coronary artery. It can be assumed that the inhibition of granzyme B can introduce a new therapeutic approach to the treatment of cardiovascular diseases and such conditions as prevention of progression of atherosclerosis, plaque rupture.

To characterize CHF complications we investigated the levels of cystatin C and found that the largest increase was observed in II group (by 1,8 times), while in I and III groups this indicator increased by 1,4 and 1,5 times, respectively. The literature shows that increased levels of cystatin C, regardless of other factors, are associated with the severity of induced ischemia [17, 18]. Normally cystatin C, as an inhibitor of cysteine proteinases, prevents the development of atherosclerotic lesions. However, increased level of cystatin C in atherosclerosis may serve as an evidence of large atherosclerotic plaques [19, 20]. Cystatin C in patients with DM-2 can serve as a reliable predictor of development of cardiovascular complications. For instance, it allows to predict the occurrence of arterial hypertension in patients with DM-2 and to some extent to measure the degree of progression of coronary atherosclerosis in these patients [21, 22].

Thus, it can be concluded that the studied parameters of oxidative stress and apoptosis, either individually or in combination, determine the degree of progression of CHF, can serve as markers of complications, and are correlated with biochemical changes in parameters of lipid and carbohydrate metabolism.

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