CONDITION OF THE PROCESS OF APOPTOSIS IN THE HUMAN POPULATION WITH ASYMPTOMATIC HYPERURICEMIA

59 patients at the age 46.4±3.49 years old have been observed, the first group consist of the patients with the normal level of uric acid in the blood (254.2 ± 16.01 mmol/l), the second group consist of the patients with the moderate level of uric acid in the blood (295.1 ± 15.83 mmol/l) and the third group consist of the patients with extremely high level of uric acid in the blood (until 350.0 ± 17.85 mmol/l). The performed investigations showed that the action balance of proapopotic and apoptic signals in the blood serum in asymptomatic hyperuricemia have been displaced from the initiation of metabolic processes to the accelerated apoptosis. This, possibly, is determined the development of cardio-vascular diseases, preparedness of cardiac histiocytes, system of central hemodynamic and heart in the development of cardio-vascular disorders in patients with asymptomatic hyperuricemia. The determined appropriateness of connection of the increasing level of uric acid in the blood serum with the soluble types of apoptosis sFasL and sFas with the degree disorders of the NOS balance, increasing level of TNFα in patients with asymptomatic hyperuricemia could allow us to assume its importance in the development mechanisms of cardio-vascular pathology. They have principal importance for the prognosis and elaboration of the preventive methods of prevention and therapeutic approaches of correction of the determined abnormalities in the purine metabolism from the generally accepted normal levels of uric acid.

Keywords: Asymptomatic hyperuricemia, apoptosis, nitric oxide


Introduction

Asymptomatic hyperuricemia (AH) according to the population investigations is found in 28% of people (Malyavskaya, Lebedev, Ternovskaya, 2007; Filippatos, Ahmed, Gladden et al., 2011) and in 54%-60% of patients not only with the reduced, but also with the saved left ventricular ejection fraction (Larina, Bart, Larin, Donskov, 2013). Moderate hyperuricemia (MH) is the most often considered as biologically inactive condition (Nasonov, 2008).

At the same time, some investigators MH, including AH refers to the independent forming factors of metabolic syndrome (MS) (Wasserman, Shnell, Boursi, Gurner-Gur, 2010; Larina, Bart, Brodskiy, 2011). In patients with MS the development risk of cardio-vascular diseases (CVD) and death reaches up to 75% (Fillipov, Khandjan, Solodukhin, 2008), development frequency of acute myocardial infarction (AMI) makes up to 10% and the development risk of heart failure (HF) makes up to 20-35% (Larbe, Torres, Torro et al., 2009). Among cases in the development of MH and AH as starting device of CVD it is considered the role of the endothelial dysfunction (ED) (Fillipov, Khandjan, Solodukhin, 2008). ED is conditioned by disorders of nitric oxide (NO) formation by the endothelial cells and disorders of vasodilation and other functions which performed by NO (Markov, 2005). The role of NO as a development factor of MH is actively discussed in the literature (Polovitkina, Oshepkova, Dmitriev, Titov, 2011). Its effect in supporting of the high level of uric acid (UA) NO is realized through the mechanisms of apoptosis.
In the physiological concentrations NO inhibits apoptosis which play a key role in the regulation of the activity of cardio-vascular system. The general ant apoptotic effect of NO could be mediated by the several mechanisms such as nitrolysis and inactivation of caspase, blocking delivery of prokaspase-9 to Apaf-I-apostome, activation of Bel-2 and Bel-XL with following inhibition of cytochrome C release from mitochondrion (Najafipur, Dolgov, Orlova, Cormer, Shevchenko, 2007).

The mechanisms of apoptosis could be realized through Fas (CD95)/Fas ligand system. Progression of HF and death of cardiac histiocytes have been connected with the activity of Fas-induced apoptosis. It is considered that the soluble types of Fas-mediated markers of apoptosis - sFas protein and its ligand sFasL - are the markers which could allow us to evaluate the significance of apoptosis process in patients with CF. It has been established that CD95 (Apo-I, Fas-antigen) represents superficial glycosytated protein of the cells membrane which belong to the group of tumor necrosis factor (TNF) / receptor of the nerve growth factor. Fas are expressed on the surface of B- and T-lymphocytes, cells of the different tumors and also on the surface of the some other cells of the human body.

The increasing expression of Fas protein on the cells’ surface induces interferon and TNF₂, and also activation of lymphocytes. Natural substance connected with Fas protein - its ligand (FasL) belongs to the TNF₂ group (Poradin, Salmsa, Cazimirski, 2006). Binding Fas with FasL or with antibodies against Fas lead to the trimerisation of Fas and the following interaction with proteins which forms DISC complex (death induced signal complex) which initiate processes of apoptosis (Najafipur, Dolgov, Orlova, Cormer, Shevchenko, 2007). Disorders of apoptosis could be reinforced by increasing of concentration NO during the activation of the biological systems. A number of authors consider that the most proapoptotic effects of NO belong to peroxynitrite (ONO₂) which forms in the reaction with superoxide (O₂). Concentration of NO in tissues increases due to activation of the pathological isoform of NOS - induced NOS (iNOS) which initiates suppression of macrophages starting up of apoptosis (Petrishev, Vasina, Lugovaya, 2008). Taking into consideration the importance of NOS and high level of UA in the realization of the process of apoptosis, development of MS and CVD, we should consider that in the human population with AH these processes are mediated by the mechanisms of changes in Fas (CD95) / Fas-ligand system.

The aim of investigation - to evaluate of the activity level of NOS and soluble forms Fas markers - mediated apoptosis (sFas and sFasL) in patients with AH and its relationships with concentration of UA in the blood.

**Materials and methods**

59 patients at the age 46.4±3.49 years old were included in the investigation, including 17 patients (28.8%) - 1 group with normal level of UA in the blood (254.2±16.01 mmol/l), 22 patients (37.3%) - 2 group with moderate level of UA in the blood (295.1±15.83 mmol/l) and 20 patients (33.9%) with extremely high level of UA in the blood (up to 350.0±17.85 mmol/l). Patients with the different level of UA were determined in the ambulatory level and fortuitousness during preventive investigations of people who joined to work.

The activity of the endothelial function was evaluated by the amount indexes of stable metabolites of NO (by the method of Golikov et al., 2004), activity of endothelial NOS (eNOS) - by the method of Sumbaev and Yasinskaya (2000), activity of iNOS and concentration of ONO₂ by the method of Komarin and Azimov (2005) in the blood serum.

At the same time the amount of factors which were able to regulate apoptosis of the cells - TNF₂ and soluble forms of Fas-ligands (sFasL) were determined in the blood serum with the use of immune-enzyme autoanalyzer with computer device AT-858 (LTD, China) with using of test-reagents of “Bender MedSystem” firm (Austria).
Statistical processing of the data was performed by the use of the SPSS 11.5.0 program. All values were presented as M±m form. The differences at P<0.05 values were considered as statistically significant.

**Results and discussion**

Expression on the membrane structures by the superficial CD95 molecules (sFas and sFasL) potentiates cells’ readiness to introduction to apoptosis as direct interaction of them with these ligands is one of the leading mechanisms of launching of apoptosis. Regulation mechanisms of apoptosis are diverse and can act in the different levels. Cytokines, growth factors and other cells incoming to the microenvironment of cardiac hystiocytes take part in regulation process of apoptosis. Interaction of Fas- FasL or TNFα molecules could be the signal to the development of apoptosis. In any case regulation of apoptosis has been carried out through genes’ activation either initiating apoptosis, such as p53 or inhibiting cells’ destruction, such as bcl-2.

The analyses of the received data have been showed that in the investigated groups the average level of sFas (inhibitor of apoptosis) with increasing concentration UA in the blood has been dynamically decreased. So, in comparison with the 1st group in patients of the 2nd group sFas was lower in 19.3% (P<0.05), and in patients of the 3rd group it was lower in 34.3% (P<0.01). In the 3rd group of patients the amount of sFas was lower than in the 2nd group in 18.6% (P<0.05). At the same time sFasL (inhibitor of apoptosis) and also cytokine TNFα in the blood with the increasing level of UA in patients with AH have been dynamically increased. In the comparison with the 1st group in the investigated group (the 2nd group) the level of sFasL was higher in 30% (P<0.01), and the level of TNFα was higher in 19.5% (P<0.05), and accordingly in the 3rd group of patients these levels were high in 50.0% (P<0.001) and in 46.2% (P<0.001). The ratio of the levels sFas and sFasL (sFas/sFasL) in patients of the 1st group were 14.0±0.68, and in the 2nd and 3rd groups of patients it was accordingly 8.7±0.38 and 6.1±0.33.

At the same time with the change of factors regulating apoptosis it has been revealed significant changes in the rate characterizing of ED. Thus, the dynamic reduction activities ofNO and eNOS occurred against a background of increasing the activity of inducible NOS and concentration of ONO⁻² (Table 1). In patients of the 2nd and 3rd groups level of NO was reduced in 17.6% (P<0.05) and 25.3% (P<0.01), eNOS - in 15.9% (P<0.05) and 22.5% (P<0.02), and activity of iNOS and content of ONO⁻² were high in 18.2 and 18.6% (P<0.05), and also accordingly in 40.0 and 44.2% (P<0.01) in the 3 group of patients.

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>TNFα, ng/ml</th>
<th>sFas, ng/ml</th>
<th>sFasL, ng/ml</th>
<th>sFas/sFasL</th>
<th>NO, mcmol/l</th>
<th>eNOS, mcmol/min/l</th>
<th>iNOS, mcmol/min/l</th>
<th>ONO⁻², mcmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 group n=17</td>
<td>19.5±0.94</td>
<td>1.4±0.08</td>
<td>0.1±0.001</td>
<td>14.0±0.68</td>
<td>9.1±0.32</td>
<td>13.8±0.63</td>
<td>0.22±0.011</td>
<td>0.43±0.022</td>
</tr>
<tr>
<td>2 group n=22</td>
<td>23.3±1.03</td>
<td>1.13±0.05</td>
<td>0.13±0.007</td>
<td>8.7±0.38</td>
<td>7.5±0.29</td>
<td>11.6±0.47</td>
<td>0.26±0.010</td>
<td>0.51±0.018</td>
</tr>
<tr>
<td>3 group n=20</td>
<td>28.5±1.34</td>
<td>0.92±0.04</td>
<td>0.15±0.011</td>
<td>6.15±0.33</td>
<td>6.8±0.32</td>
<td>10.7±0.33</td>
<td>0.31±0.014</td>
<td>0.62±0.032</td>
</tr>
</tbody>
</table>

Note: * r = 0.55-0.63 (P <0.05), in the 2nd group of patients - r=0.69-0.71 (P<0.01) and in the 3rd group of patients - r=0.76-0.82 (P<0.01), and reverse relationship between levels of sFas: with indicators NO, eNOS and TNFα - r = -0.48-0.60 (P <0.05) during the assessment of relationship in...
the 1st group of patients, r=-0.59-0.68 (P<0.01) - in the 2nd group of patients and r=-0.67-0.78 (P<0.01) in the 3rd group of the investigated patients.

During the analyses of relationship of the levels of UA indexes in the blood with factors regulating apoptosis and activity of NOS it has been established that as concentration of UA is high, as high its correlation index. Thus in the 1st group of patients relationship of UA (r) with sFasL, iNOS, ONO₂, TNFα was within from 0.48 to 0.52 (P<0.05), in the 2nd group of patients it was within from 0.58 to 0.70 (P<0.01), in the 3rd group of patients it was within from 0.68 to 0.83 (P<0.001), and reverse relationship with the indexes of sFas, NO and eNOS - r= from 0.60 to 0.65 (P<0.05), from 0.73 to 0.81 and from 0.79 to 0.85 accordingly due to investigated groups.

So, the carried out investigations showed that the action balance of proapoptotic and apoptotic signals in the blood serum in AH have been shifted to the initiation of the metabolic processes and to the accelerated apoptosis. This, perhaps, has been determined in patients with AH development of CVD, readiness of cardiac hystiocytes, system of central hemodynamic and heart to the development of cardiovascular disorders, high development risk of HF and AMI. At the same time our investigation has demonstrated that in AH it is not only accelerated apoptotic processes, but also it is increased readiness to entry of the immune system to apoptosis. Perhaps, signal mechanisms from the beginning of forming apoptotic reactions in AH are universal and are not depended on the tissue localization. Perhaps in the peripheral blood the influence balance of the regulation factors such as expression of sFasL and reduction of sFas has shifted to the side of signal intensification in order to accelerated apoptosis by the factors of the immune system. This is also facilitated by ED, reducing NO, eNOS, iNOS activation and the formation of a cytotoxic compound ONO₂. Creating conditions are likely to be unfavorable factor for the normal functioning of cardiac hystiocytes that consequently increases their readiness to develop of CVD. However, high levels of UA over the long time period, presumably determine conditions for reducing the activities of eNOS and NO in the endothelium. Adequate in this process is the increased activity of iNOS to compensate the insufficient amount of NO and the excess of which goes to the formation of ONO₂. Apparently, the increased activity of iNOS and formation of ONO₂ create the conditions for amplification proapoptotic processes, favor to rise of the tension of the immune system that we have considered as a reaction of the body in patients with AH directed to the elimination of activated by apoptosis systems and prevent the development of systemic vascular lesions. Revealed increasing regularity due MUA in the blood serum with soluble forms of apoptosis sFasL and sFas with a degree of balance disorders NOS, increasing TNFα in patients with AH suggesting their importance in the development mechanisms of cardiovascular diseases. It has principal significance for prognosis and elaboration of the preventive measures of prevention and therapeutic approaches of correction of the determined abnormalities in the purine metabolism from accepted norms of UA.

**Conclusion**

On the basis of the received results we could formulate the following conclusion.

In patients with AH the increase UA level in the blood leads to increasing in the levels of sFasL, iNOS, ONO₂, TNFα, and decreasing in the levels of sFas, NO and eNOS. Values of the increased levels of UA in the blood serum have a clear direct correlation with sFasL - mediated apoptosis, iNOS, ONO₂ and TNFα and an inverse relationship with sFas, NO and eNOS. A direct and strong correlation of sFasL is found with iNOS, ONO₂ and TNFα and its reverse correlation with sFas, NO and eNOS. Meanwhile, sFas inverse correlation is established with iNOS, ONO₂ and TNFα and its direct strong correlation with NO and eNOS. The obtained data of the reduction integral index of sFas/sFasL in dynamic growth level of UA in the blood represent decreasing level of sFas apoptosis inhibitor and increasing level of apoptosis inducer such as sFasL, sFas/sFasL.
Revealed the presence of correlation between the dynamic increase of UA, indicators regulation of apoptosis, NOS and TNFα demonstrates the importance of AH in the reducing the adaptive protection of the body and increasing the risk of cardiovascular disorders.

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