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Serum Markers of Apoptosis in Adults with Asymptomatic Hyperuricemia

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Abstract

The aim of our study was to evaluate the concentrations of soluble markers of Fas-related apoptosis (sFas and sFasL) and markers of endothelial function and their relationships with the blood uric acid (UA) level in patients with asymptomatic hyperuricemia. The study included 59 asymptomatic adults (mean age 46.4±3.49 years) divided into three *sex*- and age-*matched groups*. Group 1 consisted of 17(28.8%) adults with a normal level of blood UA (254.2±16.01 mmol/l); Group 2 consisted of 22(37.3%) adults with a moderate level of blood UA (295.1±15.83 mmol/l); and Group 3 consisted of 20(33.9%) adults with an extremely high level of blood UA (until 350.0±17.85 mmol/l). Parameters of endothelial function and serum concentrations of sFas and sFasL were assessed. The study showed that the balance between serum proapoptotic and apoptotic signals shifts toward initiation to the accelerated apoptosis in asymptomatic hyperuricemia. The determined associations between the increased blood level of uric acid and sFas, sFas, the NOS imbalance, the increased levels of TNFα in patients with asymptomatic hyperuricemia may indicate their importance in the development of cardiovascular pathology.

Keywords: *asymptomatic hyperuricemia; apoptosis; nitric oxide.*

Introduction

Asymptomatic hyperuricemia (AH), according to the population-based *cohort studies*, is found in 28% of people [1,2] and in 54%–60% of patients with left ventricular dysfunction [3]. Moderate hyperuricemia (MH) is often considered as a biologically inactive condition [4]. At the same time, some investigators consider the MH and AH as independent factors of metabolic syndrome (MS) [5,6]. In patients with MS, the risks of cardiovascular diseases, acute myocardial infarction, and heart failure rise to 75%, 10%, and 20%–35%, respectively [7,8]. Among the cases of hyperuricemia development, including AH, it has been found that endothelial dysfunction (ED) plays a role [7]. ED is primarily characterized by impaired regulation of vascular tone caused by loss of nitric oxide (NO) bioavailability. The role of NO in hyperuricemia development is actively discussed in the literature [9]. The effects of NO in supporting a high level of uric acid are realized through the mechanisms of apoptosis [10]. In the physiological concentrations, NO inhibits apoptosis. The general antiapoptotic effect of NO could be mediated by several mechanisms, such as nitrosylation and inactivation of kaspas, a block in delivery of procaspase-9 to Apaf-I-apostome, and an activation of Bel-2 and Bel-XL with following inhibition of cytochrome C release from mitochondrion [11]. The mechanisms of apoptosis could be realized through the Fas-mediated pathways. Progression of HF and death of cardiomyocytes are connected with the activity of Fas-mediated apoptosis. The Fas receptor – the first apoptosis signal (Fas) – binds the Fas ligand (FasL), a transmembrane protein part of the TNF family [12]. The interaction between Fas and FasL results in the formation of the death-inducing signaling complex (DISC). Some reports have suggested that the extrinsic Fas pathway is sufficient to induce complete apoptosis in certain cell types through DISC

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assembly and subsequent caspase-8 activation [13]. Fas is expressed on the surface of B- and T-lymphocytes, cells of different tumors, and on the surface of some other cells of the human body. The increased cell surface Fas protein expression induces the interferon-γ and TNF-α expression and activation of lymphocytes. Soluble forms of Fas(sFas), which have no transmembrane part, are circulated in the blood serum.

Disorders of apoptosis could be reinforced by an increase in NO concentration. A number of authors consider that the most proapoptotic effects of NO belong to peroxynitrite ($ONOO^-_2$) which are formed in the reaction with superoxide (O^-_2). The concentration of NO in tissues is increased due to activation of the inducible NOS (iNOS). A positive relation between apoptosis and iNOS expression has been described for several cell types [14].

The aim of our study was to evaluate the concentrations of soluble markers of Fas-related apoptosis (sFas and sFasL) and markers of endothelial function and their relationships with the blood uric acid (UA) level in patients with AH.

Materials and Methods

The study included 59 asymptomatic adults (mean age 46.4±3.49 years) divided into three *sex*- and age-*matched groups*. Group 1 consisted of 17(28.8%) adults with a normal level of blood UA (254.2±16.01 mmol/l); Group 2 consisted of 22(37.3%) adults with a moderate level of blood UA (295.1±15.83 mmol/l); and Group 3 consisted of 20(33.9%) adults with an extremely high level of blood UA (until 350.0±17.85 mmol/l). Written informed consent was obtained from patients and their parents.

Patients with different blood UA levels have been determined at random in the ambulatories during preventive investigations before applying for a job.

Endothelial function has been evaluated by the levels of stable NO metabolites (by the method of P.P Golikov et al., 2004), endothelial NOS (eNOS) activity (by the method of V.V. Sumbaeva and I.M. Yasinskiy, 2001) and iNOS activity (by the method of M.O. Kamotoe et al. 2000), as well as serum ONO^-_2 concentration (by the method of R.K. Azimov, A.S. Komarin, 2005).

Serum concentrations of sFas and sFasL were assessed by ELISA using an AT-858 analyzer (LTD, China) and «Bender MedSystem» kits (Austria).

Results were statistically processed using the *software* package "SPSS" 11.5.0. The mean (M) and standard error of the mean (SEM) were deduced. For data with normal distribution, inter-group comparisons were performed using Student's t-test and F-test. The mean (M) and standard error of the mean (m) were calculated. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups (for nonparametric data). A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The system of membrane receptor Fas and its ligand FasL compose one of the main pathways triggering apoptosis.

Our analyses revealed that average serum concentration of sFas (an inhibitor of apoptosis) is dynamically decreased with an increase in the blood UA concentration. In the Group 2 patients, the blood sFas level was less by 19.3% ($P<0.05$) compared to Group 1 patients; in the Group 3 patients, it was less by 34.3% ($P<0.01$) compared to Group I patients and by 18.6% compared to Group 2 patients ($P<0.05$). At the same time, average serum concentrations of sFasL (an inductor of apoptosis) and TNF-α were dynamically increased with an increase in the blood UA concentration. In the Group 2 patients, the blood concentrations of sFasL and TNF-α were more by 30.0% ($P<0.01$) and 19.5% ($P<0.05$), respectively, compared to Group 1 patients; in the Group 3 patients, these concentrations were more by 50% ($P<0.001$) and 46.2% (P<0.001), respectively, compared to Group I patients.

The sFas/sFasL ratio was 14.0±0.68, 8.7±0.38, and 6.1±0.33 in Groups 1, 2, and 3, respectively. Simultaneously, the endothelial function changes were identified. In particular, the level of the stable NO metabolites and eNOS activity decreased against a background of increases in iNOS activity and concentration of ONO^-_2 (Table 1).

In patients of Groups 2 and 3, the level of the stable NO metabolites was reduced by 17.6% ($P<0.05$) and 25.3% ($P<0.01$), respectively, compared to Group 1; and eNOS

Table 1

Parameters of apoptosis and ED in patients with AH according to blood UA levels

Groups of patients	TNF_a ng/mL	sFas ng/mL	sFasL ng/mL	sFas/sFasL	NO µmol/L	cNOS µmol/min/L	iNOS µmol/min/L	ONO^-_2 µmol/L
Group 1 n=17	19.5±0.94	1.4±0.08	0.10±0.001	14.0±0.68	9.1±0.32	13.8±0.63	0.22±0.011	0.43±0.022
Group 2 n=22	*23.3±1.03	*1.13±0.05	*0.13±0.007	*8.7±0.38	*7.5±0.29	*11.6±0.47	*0.26±0.010	*0.51±0.018
Group 3 n=20	*Δ28.5±1.34	*Δ0.92±0.04	*Δ0.15±0.011	*Δ6.15±0.33	*Δ6.8±0.32	*Δ10.7±0.33	*Δ0.31±0.014	*Δ0.62±0.032

** – P < 0.05 versus Group 1; Δ – P < 0.05 versus Group 2.*

activity was reduced by 15.9% (*P<0.05*) and 22.5% (*P<0.02*), respectively.

At the same time, the iNOS activity and ONO^-_2 concentration were high by 18.2% (*P<0.05*) and 18.6%, respectively, in Group 2 compared to Group 1, and by 40.0% and 44.2% (*P<0.01*), respectively, in Group 3 compared to Group 1.

We found that the serum sFasL level directly correlated with iNOS activity, serum concentrations of ONO^-_2 and TNF-α (*r*=0.55-0.63, *P<0.05* in Group 1; *r*=0.69-0.71, *P<0.01* in Group 2; *r*=0.76-0.82, *P<0.01* in Group 3); the reverse correlations between serum sFas and the concentrations of the stable NO metabolities and eNOS activity (r =-0.48-0.60, *P<0.05* in Group 1; *r*=-0.59-0.68, *P<0.01* in Group 2; *r*=-0.67-0.78, *P<0.01* in Group 3) were found.

The analysis of correlation between blood UA levels and serum sFas, sFasL and NOS activity revealed the relationship between the blood UA concentration and *r* levels. Thus, *r* between the blood UA concentration and sFasL, ONO^-_2, iNOS, TNF-α ranged from 0.48 to 0.52 (*P<0.05*) in Group 1, from 0.58 to 0.70 (*P<0.01*) in Group 2, and from 0.68 to 0.83 (*P<0.001*) in Group 3; *r* between the blood UA concentration and sFas, NO and eNOS ranged from -0.60 to -0.65 (*P<0.05*) in Group 1, from -0.73 to -0.81 (*P<0.05*) in Group 2, and from -0.79 to -0.85 (*P<0.05*) in Group 3.

The study showed that the balance between serum proapoptotic and apoptotic signals shifts toward initiation to the accelerated apoptosis in asymptomatic hyperuricemia. The determined associations between the increased blood level of uric acid and sFas, sFas, the NOS imbalance, the increased levels of TNFα in patients with asymptomatic hyperuricemia may indicate their importance in the development of cardiovascular pathology. The TNFα-pathway has a broad range of inflammatory and apoptotic properties. Dysregulation of these processes may contribute to injury of the cardiomyocytes.

The mechanism of action of the soluble Fas receptor has not been well known but may be similar to that of TNF receptors in that it leads to an enhanced Fas-mediated response in the vessels. The Fas-related system is involved mainly in regulation of apoptosis [15], whereas the TNF-system regulates apoptotic and inflammatory responses. Some evidence also suggests that TNFα may induce Fas-mediated apoptosis [16,17].

However, high levels of blood uric acid that last a long time presumably determine conditions for a decrease in eNOS activity and NO formation. Adequate upregulation of iNOS leads to NO replenishment, and its excess is used for ONO^-_2 formation. Apparently, the increase in iNOS level and formation of ONO^-_2 creates conditions for strengthening proapoptotic processes associated with enhancement of the immune response aimed to constrain the development of systemic vascular lesions.

In sum, our study provides data that asymptomatic hyperuricemia is associated with markers of the TNF- and Fas-mediated apoptosis pathway and endothelial dysfunction. These findings support the hypothesis that uric acid is involved in the risk development of cardiovascular diseases, as are inflammation, endothelial dysfunction, and apoptosis. This knowledge may facilitate the development of new diagnostic tools for identifying patients with a high risk of cardiovascular diseases and help the search for preventive methods and therapeutic approaches for correction of abnormalities in the purine metabolism.

Competing interests

The authors declare that they have no competing interests.

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