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The influence of the degree of activity of rheumatoid arthritis on the parameters of stable metabolites of nitric oxide

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Abstract. Rheumatoid arthritis (RA) is an immunoinflammatory disease characterized by progressive destruction of joints and damage to internal organs, which is based on disturbances in the humoral and cellular immune system [1,2]. In the absence of effective therapy, life expectancy in patients with RA is lower by 3 years in women and 7 years in men, primarily due to the high risk of developing comorbid diseases [5].

Keywords: Rheumatoid arthritis, nitric oxide metabolites, red blood cells

Rheumatoid arthritis (RA) is an immunoinflammatory disease characterized by progressive destruction of joints and damage to internal organs, which is based on disturbances in the humoral and cellular immune system [1,2]. In the absence of effective therapy, life expectancy in patients with RA is lower by 3 years in women and 7 years in men, primarily due to the high risk of developing comorbid diseases [5].

The heterogeneity of the pathogenetic mechanisms of RA allows us to consider it not as “one disease”, but as a clinical and immunological syndrome [3,4]. Despite the fact that the pathogenesis of RA remains incompletely understood, the role of oxidative stress in the pathogenesis of RA is beyond doubt, since the intensification of free radical reactions, activation of lipid peroxidation and stimulation of phospholipase A2 play a key role in triggering the inflammatory mechanism. Due to damage to the membrane structure and impaired vascular permeability, macrophages rush into the synovial fluid, the infiltration of which causes aseptic inflammation of the fibrous tissue [6]. Adhesion of macrophages to the vascular walls of joints in RA with the participation of LDL inhibits the activity of endothelial NO synthase and the formation of nitric oxide (NO) [7], which can cause endothelial dysfunction and vasospasm of the joints [8], which aggravates the inflammatory process.

Purpose of the study: to study the activity of the NO synthase mechanism in the membranes of erythrocytes and synovial fluid depending on the degree of RA activity.

Materials and methods. 35 patients with RA were under observation (24 women, or 68.6%, 11 men, or 31.4%). the average age of the patients was 39.4+1.1 years. The average duration of the disease is 9.8 + 2.1 years. The control group, comparable to the main group in age and gender, included 15 healthy volunteers. RA activity was assessed using the DAS28 test. All patients underwent a clinical examination, biochemical indicators of activity were determined (complete blood count, CRP), at the same time, in patients with RA in the membranes of erythrocytes, the Nox level was determined by the sum of stable metabolites NO2 and NO3 according to the method of P.P. Golikov et al. (2000), nitrate reductase activity according to T.P.Vavilov and Yu.A.Petrovich (1991), NADPH-diaphorase reaction rate according to V.T.Hope, S.R.Vinsent (1989), peroxynitrite level (ONOO-) as modified by R.K.Azimov , A.S. Komarina (2005). 28 (80%) patients were diagnosed with an articular form and 7 (20%) with an articular-visceral form. All subjects had a slowly progressive course of the disease. The activity of the I degree process was observed in 4 (11.4%), II - in 25 (71.4%), III - in 6 (11.2%) patients. X-ray stage I was noted in 16 (45.7%), stage II - in 17 (48.6%), stage III - in 2 (5.7%) patients. The obtained data were processed by the method of variation statistics using the standard application package Statatistica, Version 6. -Statsoft. Inc (2001). Significance of differences was accepted at $P < 0.05$.

Research results and discussion

As observations have shown, clinical signs depended on the degree of RA activity. In RA patients, the level of DAS28 increased due to the indicators of NPV, TSS and ESR (Table 1).

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Table 1
Main clinical signs and activity indicators in patients with RA, M±m

Assets. RA:	Pain, mm according to YOUR	Stiffness, min	ČBS	ČPS	OSZ, mm according to VASH	SOE, mm/h	DAS28
I degree	33,2+2,1	69,3+7,86	6,6+0,5	8,3+0,61	30,8+2,63	19,6+1,01	3,3+0,22
II degree	59,7+4,08*	168,3+15,4 *	14,1+0,96*	11,6+0,65*	48,5+3,21*	33,5+2,68*	5,9+0,31*
III degree	37,3+5,56*	189,6+11,3 *	21,3+2,22*	14,2+1,11*	55,61+4,84 *	38,3+3,12*	7,6+0,54*

Note. P<0.05 compared with minimal, radiographic stage I, slowly progressive, stage I activity and predominant joint involvement.

The state of the NO-ergic system was judged by the level of the main stable NOx metabolites (NO2- and NO3-), the activity of NO synthase, one of the markers of which is NADPH diaphorase (ND), and the activity of nitrate reductase (NR), a marker of inducible NO synthase (iNOS) and the content of peroxide nitrite - ONOO- in the membranes of erythrocytes, which are a convenient model for assessing the state of membrane-destructive processes at the systemic and organ level in pathological conditions of the body. At the same time, the same studies were carried out in the joint fluid.

Analysis of the results showed that in patients with RA in the membranes of erythrocytes and in the synovial fluid of inflamed joints, the level of NO, ONOO-, ND and NR activity depends on the functional activity of the process (Table 2). Thus, with the first degree of functional deficiency in the membranes of erythrocytes, the level of NO statistically significantly decreases by 21.0% (P <0.05) against the background of an increase in ND activity by 24.1% (P <0.01). The ONOO- content increases by 50% (P <0.001), and HP- activity by 29% (P <0.001). In the synovial fluid, the level of stable NO products decreases by 16.3% (P <0.05), ND activity decreases by 18.1% (P <0.05). At the same time, overexpression of ONOO- by 26.2% (P <0.01) and a decrease in the activity of the HP enzyme by 55.3% (P <0.001) were recorded.

Table 2
Level of NO-, ONOO-, ND and NR in erythrocyte membranes (numerator) and synovial fluid (denominator) in RA patients before treatment

Group	NOx, нмоль/мл	$ONOO^-$, нмоль/мл	НД, нмоль/мин/мл	НР, нмоль/мин/мл
Control	14,05+0,76	3,0+0,19	21,25+1,87	8,49+0,66
	3,37+0,18	0,61+0,05	10,5+0,85	0,56+0,05
RA patients	11,10+0,92*	3,15+0,22	26,88+1,9*	8,74+0,72
I degree	2,82+0,20*	0,77+0,61*	8,65+0,70*	1,18+0,09*
II degree	19,39+1,17* #	3,67+0,46* #	17,86+1,23* #	10,26+0,78* #
	2.35+0.19* #	1,02+0,83* #	7,25+0,84* #	1,58+0,08* #

Note. * P <0.05 compared to control # P <0.05 compared to grade I, ^P <0.05 compared to grade II.

In RA patients with stage II functional impairment, a 38.01% (P <0.02) increase in the level of NO in erythrocyte membranes is associated with inhibition of ND activity by 16.0% (P <0.05). In the synovial fluid, the level of NO decreases by 30.3% (P <0.001), at the same time there is a depression of ND activity by 31.5% (P <0.001) against the background of an increase in the level of ONOO- by 67.2% (P <0.001) and activity NR by 64.6% (P < 0.001).

It should be emphasized that as the disease progresses, the severity of disturbances in NO-, ONOO-, ND and NR increases both in the membranes of erythrocytes and in synovial fluid.

Thus, the studies have shown that disturbances of the NO-ergic system in the joint fluid play an important role

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in the pathogenesis of RA. Changes in the level of activity of the NO-ergic system in erythrocytes apparently reflect the adaptive process that develops in this disease. To substantiate the role of the disturbed NO-ergic system in the pathogenesis of inflammatory processes in the joints, we conducted a correlation analysis between stiffness indicators, DAS28 and PVA levels in the synovial fluid of NOx, ONOO-, ND and HP in patients with RA. Patients with I and II degrees of activity were selected, since there were not enough patients with III degrees of RA activity to conduct an objective assessment and statistical analysis.

Analysis of the results showed that the level of NOx and ND activity in the synovial fluid did not correlate at all with the clinical parameters of the process activity in patients with I and II functional joint insufficiency. When assessing ONOO- and NR, a strong positive correlation of indicators of the NO-ergic system was revealed with clinical signs of the disease. Moreover, their connection increases with increasing degree of activity.

Thus, one of the important factors in the development of the articular form of RA is the increase in ONOO- in the synovial fluid of the joints, due to the activity of HP.

Consequently, changes in the activity of the NO-ergic system in erythrocytes apparently reflect the adaptation process that develops in RA. As a result of exposure to free radicals, the balance in both the innate and acquired immune systems is disrupted, resulting in the development of synovitis and joint erosions [10]. Adhesion molecules are expressed on the walls of blood vessels, which cause the migration of T-lymphocytes and monocytes to the site of inflammation. After penetrating the tissue, macrophages cause an increase in the concentration of TNF-alpha and interleukin 1, which activates T lymphocytes. TNF-alpha is considered a key cytokine in RA, as it stimulates the expression of adhesion molecules and the proliferation of blood vessels feeding the lesion; activates osteoclasts and causes proliferation of synovial fibroblasts with the formation of pannus. Synovial fibroblasts, together with interleukin-1 activated chondrocytes, express matrix metalloproteinases [12]. In the inflamed synovium, interaction occurs between a variety of cells, in particular macrophages, T and B lymphocytes, synovial fibroblasts, as well as mast, dendritic and plasma cells with the release of pro-inflammatory mediators (TNF-alpha, IL-1beta, IL-6) [eleven]. An increased level of NOS2 gene transcripts in RA patients at the onset of the disease indicates the involvement of inducible NO synthase in the hyperproduction of NO and in the pathogenesis of this disease [13].

Conclusions: thus, an imbalance of NO in endothelial cells is one of the factors in the pathogenesis of RA. In patients with RA, the severity of clinical symptoms is combined with a reduced content of the main NO metabolites in the joint fluid, NADPH-diaphorase activity, and nitrate reductase hyperactivity.

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