

KRZYSZTOF BORYSEWICZ¹, EWA SEWERYN², MAGDALENA SZMYRKA-KACZMAREK¹,
JACEK SZECHIŃSKI¹, TERESA BANAŚ²

Serum and Synovial Fluid Nitrite Levels in Patients With Rheumatoid Arthritis and Osteoarthritis

Stężenia azotynów w surowicach i płynach stawowych pacjentów chorych na reumatoidalne zapalenie stawów i osteoartrozę

¹ Department of Rheumatology and Internal Diseases Silesian Piasts University of Medicine in Wrocław, Poland

² Department of Biochemistry Silesian Piasts University of Medicine in Wrocław, Poland

Abstract

Objective. To assess serum and synovial fluid nitrite in patients with rheumatoid arthritis (RA) and osteoarthritis (OA). **Material and Methods.** Thirty-two patients with active RA, 30 with non-active RA, 22 with OA, and 20 healthy volunteers were entered into this study. Concentrations of nitrite in serum and synovial fluid were determined by a commercial kit. C-reactive protein and erythrocyte sedimentation rate levels were determined as markers of systemic activity of disease in the RA and OA groups.

Results. Serum nitrite levels were higher in the patients with active RA than in the non-active RA and OA groups ($p < 0.05$). In addition, serum nitrite levels were higher in all three disease groups than in the control group ($p < 0.05$). Synovial fluid nitrite levels were higher in the RA than in the OA group ($p < 0.05$). Nitrite levels in synovial fluid were higher than in the serum of the analyzed groups. There were no significant correlations between nitrite levels and CRP or ESR.

Conclusion. The findings suggest that nitrite production is enhanced in patients with rheumatoid arthritis compared with osteoarthritis. In addition, serum nitrite levels are enhanced in patients with joint diseases, both inflammatory and non-inflammatory, compared with healthy subjects. Furthermore, synovial fluid nitrite level is higher than in serum, which may suggest local NO synthesis in the synovium (*Adv Clin Exp Med* 2008, 17, 4, 395–398).

Key words: nitric oxide synthase, rheumatoid arthritis, osteoarthritis.

Streszczenie

Cel pracy. Ocena stężenia azotynów w surowicy i w płynie stawowym u pacjentów z reumatoidalnym zapaleniem stawów (r.z.s.) i chorobą zwyrodnieniową stawów – osteoartrozą (o.a.).

Materiał i metody. 32 pacjentów z aktywnym r.z.s., 30 z nieaktywnym r.z.s., 22 z osteoartrozą oraz 20 zdrowych ochotników wzięło udział w badaniu. Stężenie azotynów badano za pomocą komercyjnego zestawu diagnostycznego. Białko C-reaktywne (CRP) oraz OB służyły za wskaźniki oceny stanu zapalnego w grupie pacjentów z r.z.s. i o.a.

Wyniki. Największe stężenia azotynów w surowicy stwierdzono w grupie pacjentów z aktywnym r.z.s., następnie z nieaktywnym r.z.s. w porównaniu z o.a. ($p < 0,05$). Stężenia azotynów były statystycznie większe w grupach pacjentów z r.z.s. i o.a. w porównaniu ze zdrowymi ochotnikami ($p < 0,05$). Stężenie azotynów w płynach stawowych pacjentów z r.z.s. było większe niż w płynach stawowych pacjentów z o.a. ($p < 0,05$). Stężenia azotynów w płynach stawowych były większe niż w badanych surowicach. Nie stwierdzono wzajemnych znaczących korelacji między OB i CRP a stężeniami azotynów.

Wnioski. Uzyskane rezultaty wskazują na zwiększone wydzielanie azotynów u pacjentów z reumatoidalnym zapaleniem stawów w porównaniu z osteoartrozą. Stężenia azotynów są większe u pacjentów z chorobami stawów, zarówno zapalnymi, jak i niezapalnymi, w porównaniu ze zdrowymi ochotnikami. Stężenia azotynów w płynach stawowych są większe niż w surowicach, co może wskazywać na ich lokalną syntezę w błonie maziowej (*Adv Clin Exp Med* 2008, 17, 4, 395–398).

Słowa kluczowe: syntaza tlenu azotu, reumatoidalne zapalenie stawów, choroba zwyrodnieniowa stawów.

Nitric oxide (NO) is an inorganic gaseous free radical produced by the enzyme nitric oxide synthase (NOS). Three distinct isoforms of NOS have been identified: inducible (iNOS), endothelial (eNOS), and neuronal (nNOS). Nitric oxide synthase is a remarkably complex enzyme which acts on molecular oxygen and arginine in neurons, endothelial cells, platelets, neutrophils, and other cells to produce nitric oxide. NO is a unique second-messenger molecule that readily diffuses through cell membranes to exert a variety of biological actions in mammalian cells. Previous studies have suggested that it has several physiological roles in immune regulation, inflammation, autoimmunity, and arthritis [1, 2]. Increased levels of NO in serum and synovial fluid have been reported in patients with rheumatoid arthritis (RA) and osteoarthritis (OA) [3, 4], in animals with induced arthritis [5], and in autoimmune arthritis [6]. Several cell types present in the joint, including macrophages, endothelial cells, chondrocytes, and synoviocytes, can be induced by proinflammatory cytokines to produce NO [1]. Administration of NOS inhibitors in experimental arthritis reduces disease activity. In humans with inflammatory joints diseases, beneficial effects of inducible NOS inhibition is observed with glucocorticoid, methotrexate, or non-steroidal anti-inflammatory drug administration. These drugs reduce enhanced NO synthesis and disease activity in different ways [2, 3]. This may be a new experimental therapeutic approach in the treatment of joint diseases, not only of inflammatory origin.

NO itself is difficult to measure directly because of its very short half-life in biological fluids (6–10 seconds). Under aqueous aerobic conditions, NO spontaneously oxidizes to its inactive stable end-products nitrite and nitrate [2, 3]. In this study the levels of nitrate in the serum and synovial fluid of patients with active and non-active RA or with osteoarthritis (OA) as well as controls were compared and correlations with systemic activity variables were tested.

Material and Methods

Patients with rheumatoid arthritis (RA) were divided into groups according to C-reactive protein (CRP) concentration. The active RA group were patients with CRP > 20 mg/l and non-active RA with CRP < 20 mg/l. Thirty-two patients with active RA (22 female, 10 male, mean $\pm$ SD age: 48.6 ± 16.7 years), 30 patients with non-active RA (23 female, 7 male, mean age: 42.3 ± 11.8 years), and 22 patients with OA (16 female, 6 male, mean age: 56.1 ± 9.4 years) who attended

the Department of Rheumatology, Silesian Piasts University of Medicine in Wrocław, were studied. Twenty healthy volunteers (13 female, 7 male, mean age: 41.2 ± 7.8 years) served as a control group. Informed consent was obtained from everyone who participated in this study. Exclusion criteria were other inflammatory diseases, post-traumatic joint effusion, and treatment with prednisolone > 10 mg/day.

To determine the serum nitrate levels, 5 ml of blood was required. Blood samples were taken at the same time for the determination of CRP and erythrocyte sedimentation rate (ESR). In patients with active RA and OA with joint effusion, arthrocentesis was done at the same time and synovial fluid was taken for nitrate level determination. Blood samples and synovial fluids for nitrate were stored at -70°C until use. A commercial colorimetric assay for the determination of NOS activity was used in this study (Bioxytech Nitric Oxide Synthase Assay Kit, Oxis International Research, no. 22113). This kit is intended for the quantitative determination of total nitrite as an indicator of nitric oxide synthase activity in biological samples. Blood sampling time was standardized and done two to four hours after intake of a low-nitrite/nitrate breakfast. Under these conditions, the effect of dietary nitrite/nitrate intake should be minimal. ESR was determined according to the method of Westergren and serum CRP was measured by the nephelometric method. These parameters were determined in the serum of patients with RA and OA.

Statistical analysis was undertaken using the Statistical Package for Sciences (Statistica, version 6.0). Data in tables are expressed as the mean (SD). The independent sample *t* test, Mann-Whitney *U* test, and Spearman's non-parametric correlation test were used as appropriate statistical methods for analyses. Differences were considered significant if *p* values were < 0.05.

Results

There were no significance differences in age between the groups (*p* > 0.05). As expected, there was a significance difference in the proportions of women and men in the RA groups of patients and healthy subjects (*p* < 0.05). Serum nitrite levels were higher in patients with active RA (48.62 ± 13.42 $\mu\text{mol/l}$) and non-active RA (39.41 ± 17.49 $\mu\text{mol/l}$) than in the OA group (28.06 ± 12.26 $\mu\text{mol/l}$). All differences among the analyzed groups were statistically significant (*p* < 0.05). In addition, serum nitrite levels in the healthy volunteers (18.13 ± 8.45 $\mu\text{mol/l}$) were lower than in all the patient

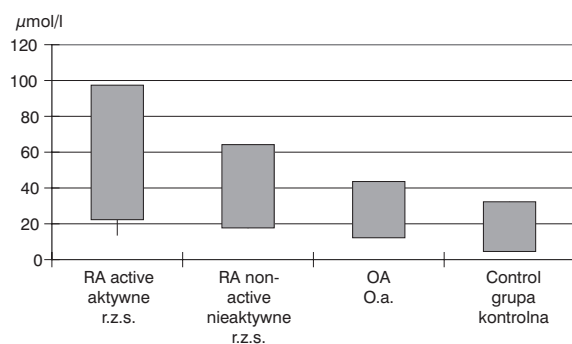


Fig. 1. Serum nitrite levels

Ryc. 1. Stężenia azotynów w surowicy

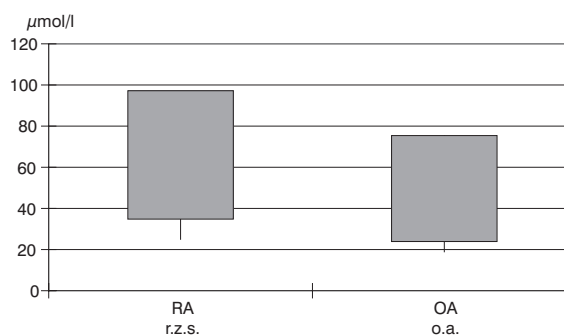


Fig. 2. Synovial fluid nitrite levels

Ryc. 2. Stężenia azotynów w płynie stawowym

groups ($p < 0.05$) (Fig. 1, Tab. 1). Synovial fluid nitrite levels were higher in patients with active RA ($65.21 \pm 24.75 \mu\text{mol/l}$) than in the OA group ($40.73 \pm 18.68 \mu\text{mol/l}$) (Fig. 2, Tab. 1). Difference between these results were also statistically significant. Nitrite levels in synovial fluid were higher than in serum in the analyzed groups (active RA and OA). There were no significant correlations between nitrite levels and CRP or ESR.

Discussion

In this study the most important finding was that serum nitrate levels were increased not only in inflammatory joint disease, i.e. RA, but also in osteoarthritis compared with healthy volunteers. The next intriguing finding was that, at the same time, the nitrate concentration in the synovial fluid was statistically significantly higher than in serum. There were no correlations between nitrate level and protein concentration (in serum or synovial

fluid); however, despite the fact that serum protein concentration was higher than that in synovial fluid, this correlation should be much stronger. These data may suggest local NO synthesis in the synovium. This accords with the nitrate levels in synovial tissue and serum in a previous study [4, 7]. The origin of these increased levels of nitrate is not clear. Synovial inflammation increases synovial fluid levels of NO; when SF is cleared by the lymphatic system and enters the systemic circulation, the serum levels of nitrate might increase. A possible source of increased nitrate is the systemic vasculature or other inflammatory cells in which the induction of NO has been shown [1, 4]. Although OA is not considered an inflammatory disease, some studies have shown evidence of mild inflammatory changes in the OA synovium, consistent with the fact that proinflammatory cytokines (IL-1, TNF) have also been detected in OA synovial fluid [8].

Increased serum and synovial nitrite concentrations have been found in patients with RA and OA

Table 1. Systemic activity variables and serum and synovial fluid nitrite concentrations in the analyzed groups. Results are shown as mean (SD)

Tabela 1. Wskaźniki stanu zapalnego, stężenie azotynów w surowicy i w płynie stawowym w badanych grupach; wartości średnie (w nawiasach SD – odchylenie standardowe)

	Serum (Surowica)				Synovial fluid (Płyn stawowy)	
	Active RA (Aktywne r.z.s.) (n = 32)	Non-active RA (Nieaktywne r.z.s.) (n = 30)	OA (O.a.) (n = 22)	Control (Grupa kontrolna) (n = 20)	RA (R.z.s.) (n = 32)	OA (O.a.) (n = 22)
ESR (mm/1 st -h)	52.08 (18.76)	24.55 (13.87)	13.35 (5.75)	14.74 (4.98)		
CRP (mg/l)	42.2 (17.54)	10.8 (4.2)	5.2 (1.9)	3.6 (2.2)		
Nitrite (μmol/l)	48.62 (13.42)	39.41 (17.49)	28.06 (12.26)	18.13 (8.45)	65.21 (24.75)	40.73 (18.68)

RA – rheumatoid arthritis, OA – osteoarthritis.

r.z.s. – reumatoidalne zapalenie stawów, o.a. – osteoartroza.

and in the spondyloarthropathies [2, 3, 7]. In the spondyloarthropathies, correlations between nitrite, ESR, and CRP were found. ESR decreased with non-steroidal anti-inflammatory drug treatment, although serum nitrate and CRP concentrations remained unchanged [2]. In other clinical studies, positive correlations between serum nitrate levels and parameters of clinical presentation and severity of disease have been shown in patients with RA [7, 9].

The present study confirms that serum NO levels are increased in patients with active and non-active rheumatoid arthritis in comparison with controls. However, no correlations between nitrate

level, ESR, and CRP in patients with RA were found. In other studies there were also no correlations between NO production and laboratory parameters of disease activity [10, 11].

The findings of the present study suggest that nitrate level provides a measure of endogenous NO synthesis and show that this level may be measured in humans without complex preparatory steps. In this regard, the likely benefit of some therapeutic interventions, including antioxidants, that potentiate the antioxidative defense mechanism and reduce peroxidation in the management of rheumatoid arthritis and osteoarthritis is underscored.

References

- [1] Clancy RM, Amin AR, Abramson SB: The role of nitric oxide in inflammation and immunity. *Arthritis Rheum* 1998, 41, 1141–1151.
- [2] Stichtenoth DO, Frolich JC: Nitric oxide and inflammatory joint diseases. *Br J Rheumatol* 1998, 37, 246–257.
- [3] Stefanovic-Racic M, Stadler J, Evans CH: Nitric oxide and arthritis. *Arthritis Rheum* 1993, 36, 1036–1044.
- [4] Farrell AJ, Blake DR, Palmer RM: Increased concentrations of nitrate in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. *Ann Rheum Dis* 1992, 51, 1219–1222.
- [5] Stefanovic-Racic M, Meyers K, Meschter C: N-Monomethyl arginine an inhibitor of nitric oxide synthase, suppresses the development of adjuvant arthritis in rats. *Arthritis Rheum* 1994, 37, 1062–1069.
- [6] Weinberg JB, Granger DL, Pisetsky DS: The role of nitric oxide in the pathogenesis of spontaneous murine autoimmune disease: increased nitric oxide production and nitric oxide synthase expression in MRL-Ipr /Ipr mice, and reduction of spontaneous glomerulonephritis and arthritis by orally administered NG-monomethyl-L-arginine. *J Exp Med* 1994, 179, 651–660.
- [7] Ersoy Y, Özerol E, Baysal Ö: Serum nitrate and nitrite levels in patients with rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. *Ann Rheum Dis* 2002, 61, 76–78.
- [8] Grabowski PS, Wright PK, van Hof RJ: Immunolocalisation of inducible nitric oxide synthase in synovium and cartilage in rheumatoid arthritis and osteoarthritis. *Br J Rheumat* 1997, 36, 651–655.
- [9] Onur Ö, Akinci AS, Akbiyik F: Elevated levels of nitrate in rheumatoid arthritis. *Rheumat Int* 2001, 20, 154–158.
- [10] Choi JW: Nitric oxide production is increased in patients with rheumatoid arthritis but does not correlate with laboratory parameters of disease activity. *Clin Chim Acta* 2003, 336, 83–87.
- [11] Özgöçmen S, Sogut S, Ardicoglu O: Serum nitric oxide, catalase, superoxide dismutase, and malondialdehyde status in patients with ankylosing spondylitis. *Rheumat Int* 2004, 24, 80–93.

Address for correspondence:

Krzysztof Borysewicz
Silesian Piasts University of Medicine
Department of Rheumatology and Internal Diseases
Borowska 213
50-556 Wrocław
Poland
Tel.: +48 71 734 33 30
E-mail: krzysztof1964@poczta.onet.pl

Conflict of interests: None declared

Received: 29.04.2008

Revised: 4.07.2008

Accepted: 5.07.2008