UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 31: 217–221, 2012 *Original paper*
Originalni naučni rad

ADENOSINE DEAMINASE ISOENZYMES IN THE DIAGNOSIS AND MONITORING OF RHEUMATOID ARTHRITIS

IZOENZIMI ADENOZIN DEAMINAZE U DIJAGNOSTICI I PRAĆENJU REUMATOIDNOG ARTRITISA

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Summary: The aim of this study was determination of the catalytic activities of adenosine deaminase (ADA), ADA1 and ADA2 isoenzymes in the serum of patients suffering from rheumatoid arthritis (RA) who were and were not treated with methotrexate (MTX), and identification of the possibilities of using these biochemical parameters in diagnosing and monitoring the treatment effects in RA. Catalytic activities of total ADA (tADA) and ADA2 in serum were determined by a spectrophotometric method. A statistically significant correlation was found between the total ADA and ADA1 values, as well as between tADA and ADA2 in the serum of all patients suffering from RA. Determination of ADA1 and ADA2 isoenzyme catalytic activities in the serum of patients who might be suffering from RA improves the diagnostic value of total ADA catalytic activity determination. ADA2 catalytic activity in serum can be a useful biochemical marker in diagnosing and monitoring RA. Decrease in ADA1 isoenzyme catalytic activities in the serum of patients suffering from RA who were treated with MTX can help in the observation of MTX therapy effects.

Key words: adenosine deaminase isoenzymes, methotrexate, rheumatoid arthritis

Kratak sadržaj: Cilj rada bilo je određivanje katalitičkih aktivnosti izoenzima adenozin deaminaze (ADA), ADA1 i ADA2 u serumima obolelih od reumatoidnog artritisa (RA) lečenih uz pomoć ili bez terapije metotreksatom (MTX), kao i utvrđivanje mogućnosti uvođenja ovih biohemijskih parametara u dijagnostiku i praćenje terapijskih efekata u RA. Katalitičke aktivnosti ukupne ADA (tADA) i ADA2 u serumima određivane su spektrofotometrijskom metodom. Utvrđena je statistički značajna korelacija između katalitičkih aktivnosti tADA i ADA1, kao i tADA i ADA2 u serumima svih ispitanika sa RA. Određivanje katalitičkih aktivnosti izoenzima ADA1 i ADA2 u serumima pacijenata sa sumnjom na RA poboljšava dijagnostičku vrednost određivanja katalitičke aktivnosti tADA. Katalitička aktivnost ADA2 u serumu može da bude koristan biohemijski marker u dijagnostici i praćenju RA. Smanjenje katalitičkih aktivnosti izoenzima ADA1 u serumima pacijenata sa RA koji su dobijali MTX može da pomogne u praćenju terapijskih efekata MTX.

Ključne reči: izoenzimi adenozin deaminaze, metotreksat, reumatoidni artritis

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory autoimmune disease characterized by pain and swelling of the attacked joints, chronic proliferative synovitis that leads to progressive articular cartilage and subchondral bone destruction and the development of functional disability (1–3). Epidemiological studies show that RA is the most common connective tissue systemic disease, and 1–2% of the world's population is suffering from it (4). Although the most of epidemiological, pathophysiological,

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immunological and genetic aspects of RA are known today, it is still not clear what are the exact cause and factor that support the destructive inflammatory process in RA.

RA diagnosis is based on the determination of the basic clinical image and radiological and laboratory results respecting the revised criteria of the ACR (American College of Rheumatology) from 1987 (5). Six of the seven ACR criteria for RA are based on manifest clinical symptoms, and one is based on positive rheumatoid factor in serum. The fact that most of the clinical symptoms need to last for at least six weeks (6) limits the possibility of early RA diagnosis and points out the need for finding new sensitive and more specific markers in the RA diagnosis and monitoring (7). Hitoglou et al. (8) and Cordero et al. (9) determined the catalytic activities of total adenosine deaminase (tADA) and its isoenzymes (ADA1 and ADA2) in patients suffering from rheumatoid arthritis and systemic lupus erythematosus (SLE). They identified the correlation between RA activities and tADA and its isoenzyme ADA2 catalytic activities, whereby the progress of RA leads to increased activity of the mentioned enzyme and isoenzyme. Adenosine deaminase (ADA) is a key enzyme in the metabolism of purine derivatives which catalyses irreversible hydrolytic deamination of adenosine to inosine and 2'deoxyadenosine to 2'deoxyinosine with ammonium separation (10). It has an important role in acute and delayed inflammatory response (11–13) as well as in physiological functions including the differentiation and maturation of immunocompetent and hematopoietic cells (14). In their study, Sari et al. (15) identified a correlation between tADA catalytic activity in serum and its isoenzymes ADA1 and ADA2 and RA activity; therefore they can be used as biochemical markers that would give useful information about RA activity, together with the traditional inflammation indicators such as C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). Surekha et al. (16) confirmed these findings in their study. ADA is a marker of cellular immunity, so it is important to determine its catalytic activity not only when identifying the seriousness of an inflammatory process, but also for the improvement of treatment strategy and better monitoring of the therapy effects in RA (17).

Three isoenzymes of human ADA have been discovered in the existing studies: ADA1, ADA1+cp (consists of 2 ADA1 molecules interconnected by a connecting glycoprotein cp) and ADA2 (18, 19). ADA1 and ADA2 perform the deamination of 2 adenosine and 2'deoxyadenosine nucleotides which have immunosuppressive and antiinflammatory characteristics, and therefore the homeostasis of these substances and catalytic activities of ADA1 and ADA2 isoenzymes in human cells are very important for an organism (20). Many published studies (9, 15, 16) point out the importance of determining the catalytic activity of tADA and its isoenzymes in RA as possible biochemical markers for therapy effects monitoring in patients who were treated with methotrexate (MTX).

MTX is one of the most commonly used antirheumatic medications (21), as mono-therapy or in combination with the disease modifying drugs (LMB) because of its therapeutic efficiency and toxicity profile (6). The antiinflammatory effect of MTX is mediated by adenosine receptors stimulation (22, 23) so the determination of ADA catalytic activities is significant in monitoring the metabolism and therapy effects of MTX.

The aim of the study was the determination of catalytic activities of ADA1 and ADA2 isoenzymes in the serum of patients suffering from RA who were and were not treated with MTX, and the identification of the possibilities of using these biochemical parameters in diagnosing RA and monitoring the therapy effects.

Materials and Methods

Patients and controls

The study involved 120 subjects who were divided into three groups. The control group consisted of 60 healthy subjects (46 women and 14 men) with an average age of 52.78 years, who were from 28 to 74 years old; they also did not have family members suffering from rheumatoid arthritis and they were not medically treated. From the remaining 60 subjects, 20 were suspected to suffer from rheumatoid arthritis and 40 were diagnosed with rheumatoid arthritis. All the subjects were diagnosed with RA by specialized rheumatologists. Criteria for involving the patients in the study and for their exclusion were the revised ACR criteria from 1987 (5). The group A consisted of 30 subjects in the acute stage of RA (25 women and 5 men) with an average age of 56.67 years (their age ranged from 41 to 74 years). In this group, 20 subjects were suspected to have been suffering from RA for three to six months (they were not treated with MTX within the medical therapy for RA), and the diagnoses of RA were confirmed during our study. Ten subjects in group A were the new ones suffering from RA who were not treated by MTX within the medical therapy for RA. The group B consisted of 30 subjects (27 women and 3 men) with an average age of 56 years (the ages ranged from 29 to 74 years). All the subjects in group B were treated with a common therapeutic dose of MTX within the medical therapy for RA during more than two months.

Methods

tADA and ADA2 catalytic activities were determined by the modified Giusti's spectrophotometric method (24) combined with the method of Muraoka et al. (25), using adenosine (Sigma-Aldrich, USA) as

a substrate and 0.1 mmol/L of EHNA (Sigma-Aldrich, USA) as an ADA1 inhibitor. ADA1 catalytic activity was calculated from the found ADA2 (in the presence of 0.1 mmol/L of EHNA) and tADA activities. Reference interval of tADA catalytic activities for this method was 13.20–20.80 U/L (24).

The results of the study are expressed as arithmetic mean ± standard deviation ($\bar{x}$±SD). Testing of the significance of differences between the control group and the group A (subjects who did not get medical therapy) and between the control group and the group B (subjects who got medical therapy) was done by standard Student t-test based on results distribution. Values of $p<0.01$ were taken as statistically significant. Correlation coefficient (r) was determined by Pearson's correlation coefficient and the 95% confidence interval (CI) of examined parameters within the monitored groups.

Results

Table I shows tADA, ADA1 and ADA2 catalytic activities in the serum of subjects suffering from RA compared with their values in healthy subjects.

Correlation coefficient between the values of tADA and ADA2 catalytic activities in the group A was $r=0.95$ ($p<0.01$), and the correlation coefficient between the values of tADA and ADA1 isoenzyme catalytic activities was $r=0.90$ ($p<0.01$), so it was statistically significant. Correlation coefficient between the values of tADA and ADA2 catalytic activities in the group B was $r=1.00$ ($p<0.01$), and the correlation coefficient between the values of tADA and ADA1 isoenzyme catalytic activities was $r=0.90$ ($p<0.01$), suggesting a high degree of correlation.

Discussion

Impossibility of early diagnosis and the problems in differential diagnosing that would permit to distinguish RA from other autoimmune diseases of connective tissues using only the ACR criteria are the reasons behind the search for some new markers which would make all this possible and at the same time be used for monitoring the disease course after therapy. It is still not completely clear what exactly causes the increase in ADA catalytic activities in the serum of patients suffering from RA, but it is supposed that ADA catalytic activity is increased because of its release from the damaged cells and the increased cell proliferation in RA (8). The results of this study are in accordance with the results of the mentioned authors (9, 15, 16). In the present study the values of ADA1 catalytic activities in the serum of healthy subjects were from 0.41 to 11.21 U/L. The reference interval (RI) for ADA1 was 0.71–10.69 U/L, and the 95% CI was 4.77–6.09 U/L. Average value of ADA1 catalytic activities in the serum of subjects suffering from RA who did not get medical therapy was higher and statistically significant ($p<0.001$) in comparison with the healthy subjects. However, the average value of the ADA1 catalytic activities in subjects suffering from RA who were treated with MTX was lower compared with the control group (*Table I*), and it is coherent with the MTX mechanism of action (26).

In this study, the RI for ADA2 was determined, and it was 6.32–22.68 U/L while the 95% CI was 13.47–15.53 U/L. Average value of ADA2 catalytic activities in the serum of subjects in the group A was higher and statistically significant ($p<0.001$) in comparison with the healthy subjects. In the group B, the average value of ADA2 catalytic activities was lower and statistically significant ($p<0.001$), compared with the control group (*Table I*), in accordance with the

Table I tADA, ADA1 and ADA2 catalytic activities in the serum of subjects suffering from RA compared with their values in healthy subjects.

	tADA (U/L) $\bar{x}$ ± SD	Statistical significance p	ADA1 (U/L) $\bar{x}$ ± SD	Statistical significance p	ADA2 (U/L) $\bar{x}$ ± SD	Statistical significance p
Control group (n=60)	19.65±3.56 (a)		5.43±2.36 (d)		14.50±4.09 (g)	
Group A (n=30)	23.37±6.64 (b)	p<0.001 (b ν a)	6.66±3.27 (e)	p<0.001 (e ν d)	16.70±4.62 (h)	p<0.001 (h ν g)
Group B (n=30)	10.22±2.67 (c)	p<0.001 (c ν a)	3.56±1.66 (f)	p<0.001 (f ν d)	6.64±2.12 (i)	p<0.001 (i ν g)

Control group – healthy subjects, Group A – subjects with RA without methotrexate treatment, Group B – subjects with RA with methotrexate treatment

Ricksen et al. (27) study. They proved that MTX causes inhibition of 5-aminoimidazole-4-carboxamide ribonucleotide-transformylase, leading to intracellular accumulation of the 5-aminoimidazole-4-carboxamide ribonucleotide that inhibits ADA and its isoenzymes.

Knowing that tADA catalytic activity represents a marker of cellular immunity, ADA2 gives information about monocyte/macrophage activities (related with RA). Further, as ADA1 is thought to be of lymphocytic/neutrophil origin (28), from the results of this study it can be concluded that the diagnostic value of tADA may be increased by measuring the ADA2 isoenzyme catalytic activity in the serum of patients suffering from RA. Decreased ADA1 and ADA2 catalytic activities in the serum of patients suffering from RA who were treated with MTX are in accordance with the MTX mechanism of action. Therefore, the determination of their catalytic activities in serum might help to monitor the MTX therapeutic effects in RA.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Müller-Ladner U, Pap T, Gay RE, Neidhart M, Gay S. Mechanisms of disease: the molecular and cellular basis of joint destruction in rheumatoid arthritis. Nat Clin Pract Rheumatol 2005; 1: 102–10.
2. Cojocaru MI, Cojocaru M, Silosi I, Vrabie DC. Central nervous system manifestations in rheumatic diseases. Journal of Medical Biochemistry 2011; 30: 1–4.
3. Tešija A. Novi biomarkeri u dijagnostici i praćenju bolesnika s reumatoidnim artritisom. Biochem Med 2003; 13 (1–2): 141–2.
4. Vallbracht I, Rieber J, Oppermann M, Förger F, Siebert U, at al. Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. Ann Rheum Dis 2004; 63: 1079–84.
5. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987. Revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31: 315–24.
6. Popović M, Stefanović D, Mitrović D. Reumatične i srodne bolesti, dijagnoza i terapija. Beograd, 2000: 76–38.
7. Härle P, Bongartz T, Schölmerich J, Müller-Ladner U, Straub RH. Predictive and potentially predictive factors in early arthritis: a multidisciplinary approach. Rheumatology 2005; 44: 426–33.
8. Hitoglou S, Hatzistilianou M, Gougoustamou D, Athanassiadou F, Kotis A. Adenosine deaminase activity and its isoenzyme pattern in patients with juvenile rheumatoid arthritis and systemic lupus erythematosus. Clin Rheumatol 2001; 20(6): 411–16.
9. Cordero OJ, Salgado FJ, Mera-Varela A, Nogueira M. Serum interleukin-12, interleukin-15, soluble CD 26 and adenosine deaminase in patients with rheumatoid arthritis. Reumatol Int 2001; 21: 69–74.
10. Nikolić J, Kapetanović R. Biohemija. Medicinski fakultet u Banjaluci, 2007: 246.
11. Adanin S, Yalovetsky IV, Narduli BA, Sam ADII, Jonjev ZS, Law WR. Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis. Am J Physiol 2002; 282: 1324–32.
12. Cohen ES, Law WR, Easington CR, et al. Adenosine deaminase inhibition attenuates microvascular dysfunction and improves survival in sepsis. Am J Resp Crit Care Med 2002; 166: 16–20
13. Law WR, Conlon BA, Valli VE. Therapeutic potential for transient inhibition of adenosine deaminase in systemic inflammatory response syndrome. Crit Care Med 2003; 31: 1475–81.
14. Lee G, Lee SS, Kwang YK, Dong WK, Sangdun C, Hong KJ. Isolation and characterization of a novel adenosine deaminase inhibitor, IADA-7, from Bacillus sp. J-89. J of Enzym Inhib and Med Chem 2009; 24 (1): 59–64.
15. Sari RA, Taysi S, Yilmaz Ö, Bakan N. Correlation of serum levels of adenosine deaminase activity and its isoenzymes with disease activity in rheumatoid arthritis. Clin Exp Rheumatol 2003; 21: 87–90.
16. Surekha RH, Madhavi G, Srikhant BMV, Jharna P, Rao URK. Serum ADA and C – reactive protein in Rheumatoid Arthritis. Int J Hum Genet 2006; 6 (3): 195–8.
17. Mehanović-Nikolić J, Laloš-Miljuš J, Stajčić-Nalesnik M, Lakić Lj, Bobić Ž, et al. The diagnostic value of anticyclic citrullinated peptide antibodies, adenosine deaminase activity and other potential biomarkers for predicting and monitoring rheumatoid arthritis. Journal of Medical Biochemistry 2008; 27 (3): 383–8.
18. Koraćević D, Bjelaković G, Đorđević V, Nikolić J, Pavlović D, i sar. Biohemija. Savremena administracija Beograd, 2000: 152–600.
19. Chiba S, Saitoh M, Kashiwagi M, Kobayashi N, Matsumoto H. Isoenzyme Analysis of the High Serum Adenosine Deaminase Activity in Patients with Myasthenia gravis. Inter Med 1995; 34 (2): 81–4.
20. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. Eur Respir J 1996; 9: 632–3.
21. Stamp LK, O'Donnell JL, Chapman PT, Zhang M, Frampton C, James J, Barclay ML. Determinants of red

blood cell Methotrexate polyglutamate concentrations in rheumatoid arthritis patients receiving long-term Methotrexate treatment. Arthritis & Rheumatism 2009; 60(8): 2248–56.

22. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev 2005; 57: 163–72.
23. Wessels JAM, Huizinga TWJ, Guchelaar HJ. Recent insight in the pharmacological actions of methotrexate in the treatment of rheumatoid arthritis. Rheumatol 2008; 47: 249–55.
24. Giusti G. Adenosine deaminase. In: Bergmeyer HU, ed. Methods in enzymatic analysis. New York: Academic Press, 1974; 1092–9.
25. Muraoka T, Katsuramaki T, Shiraishi H, Yokoyama MM. Automated Enzymatic Measurement of Adenosine Deaminase Isoenzyme Activities in Serum. Anal Biochem 1990; 187: 268–72.
26. Bansard C, Lequerre T, Daveau M, Boyer O, Tron F, Salier JP, Vittecoq O, Le-Loët X. Can rheumatoid arthritis responsiveness to methotrexate and biologics be predicted? Rheumatol 2009; 48: 1021–8.
27. Riksen NP, Barrera P, Van den Broek PHH, Van Riel PLCM, Smits P. Methotrexate modulates the kinetics of adenosine in humans in vivo. Ann Rheum Dis 2006; 65: 465–70.
28. Ungerer JPJ, Oosthuizen HM, Retief JH, Bissbort SH. Significance of Adenosine Deaminase Activity and Isoenzymes in Tuberculous Effusions. Chest 1994; 106: 33–7.

Received: January 13, 2012

Accepted: February 25, 2012