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# ADENOSINE DEAMINASE ISOFORMS IN SYNOVIAL FLUID AT RHEUMATOID ARTHRITIS

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The level of adenosine deaminase (ADA) activity in synovial fluids of patients with inflammatory arthritis (IA) and non-inflammatory joint diseases differ significantly. In this work from synovial fluids with different levels of the ADA activity, low- and high-molecular isoforms were separated. The increasing of low-/high- molecular ratio upon increasing the total ADA activity was manifested. It was supposed that the increase of total ADA activity at inflammation takes place, because of the release of intracellular, low-molecular ADA. To prevent the anti-inflammatory agent, adenosine, against metabolizing by the enzyme, its inhibition might be considered as a new approach to the IA treatment.

***Keywords*:** adenosine deaminase isoforms, inflammatory arthritis, synovial fluid.

**Introduction.** Rheumatoid arthritis (RA) is a chronic disease, characterized by extracellular matrix degradation, destruction of joint cartilage and loss of function. It is a multisystem autoimmune disease of unknown cause. The characteristic feature of RA is persistent inflammatory synovitis, usually involving peripheral joints. It is characterized by infiltration of T-cells and monocytes into the synovium, proliferation of synoviocytes and presentation of secreted products of activated lymphocytes, macrophages, fibroblasts and leucocytes [1, 2]. Currently, along with the quest of biomarkers, which can help in differentiating the IA from other types of arthritis, intensive investigations are in process of finding the indices for evaluating the severity of the disease [3].

A purine nucleoside, adenosine, is known as an effective anti-inflammatiory mediator, which decreases pro-inflammatory and increases anti-inflammatory cytokines, modulates macrophages and monocytes, and regulates the inflammatory functions of endothelial cells [4]. Adenosine deaminase (ADA, E.C. 3.5.4.4) is an enzyme metabolizing adenosine [5]. During inflammation ADA is released to the extra cellular medium and to pathological effusions, synovial fluid (SF), etc., resulting in the considerable increase of activity in these fluids. Hence, actually,

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ADA can be considered as an index for developing inflammation [6]. The evaluation of ADA activity can be used as an appropriate approach for diagnosis of some diseases, among them, pleural tuberculosis [7, 8]. Several works are devoted to the valuation of application of ADA activity for differentiating the etiology of joint diseases [9, 10].

In our previous work we have found that the levels of ADA activity in joint effusions of patients with RA and osteoarthritis differ significantly. The cutoff value of the enzyme activity in SF was evaluated to differentiate the RA from the arthritis of other etiologies [11]. These results allowed us to offer their usage in clinics of Armenia the ADA activity in SF as a sensitive, specific, low-cost and technically convenient test for quick diagnosis of rheumatoid arthritis.

The goal of the present study was to analyze the level of ADA activity in SF of patients with inflammatory arthritis (IA) and to compare the levels of two isoforms of the enzyme: low-molecular (catalytic unit of ADA) and high-molecular (catalytic unit in complex with ADA-binding protein), aiming to find the reason of the enzyme level increasing in inflammatory SF.

**Experimental Part.** The samples of synovial fluid from 33 RA patients (diagnosed according to the criteria of ACR for RA, 1987) were obtained in Erebouni Medical Center. The samples were stored at –20º*C*. Before processing, they were refrozen and centrifuged. The ADA activity was determined by evaluation of the amount of ammonia, liberated in the catalysis by enzyme reaction of adenosine deamination. Earlier described [11] phenol-hypochlorite colorimetric method was used: the assay mixture in 0.5 *mL* of 40 *mM* K-Na-phosphate buffer, pH 7.4, contained 0.4 *mM* adenosine. The reaction was started by addition of SF, and the mixture was incubated for 30 *min* at 37º*C*. Then phenol-nitroprusside and hypochlorite reagents, 1 *mL* each, were added, and the absorbance at 630 *nm* was registered. Ammonium sulfate solution was used as a standard. The enzyme activity was expressed in International Units (IU/*L*).

To compare the levels of low-molecular and high-molecular isoforms of ADA, the SF samples were subjected to gel-filtration on the column with G-200 Sephadex. Elution diagrams regarding the protein and the ADA activity in the obtained fractions were constructed.

Statistical analysis was performed with GraphPad software, version 3 for Windows (USA). Unpaired two-tailed *t*-test with Welch correction was applied. The data are expressed as mean ± standard error.

**Results and Discussion.** The mean value of ADA activity in the studied 33 RA patients was 34 ± 23.2 (mean ± SD) IU/*L*. The samples were divided into two groups according to the demonstrated ADA activity: 1$^{st}$ group ($n = 24$) – with the enzyme activity near or less of the mean value, and the 2$^{nd}$ group ($n = 9$) – with the activity significantly higher of the mean value. Per 3 SF samples from each group were subjected to gel-filtration on the column with Sephadex G-200.

In Fig. a and b per one presentable elution diagrams for the samples with total ADA activity below of the mean value 27.5 IU/*L* (1$^{st}$ group), and that with above mean value 58.9 IU/*L* (2$^{nd}$ group) respectively are presented. The obtained elution diagrams showed the presence of both isoforms of ADA in the SF of two groups. The elution volumes of maximal points of activity of high-molecular isoform in a and b diagrams are 37 and 36 *mL* respectively. The elution volumes of

maximal activity of low-molecular isoform in these diagrams are 57 and 52 *mL* respectively. The diagrams demonstrate the dependence of the ratio of two molecular forms from the initial level of enzyme activity. In the diagrams of samples from the 1st group (with the activity near or less of the mean value), the diagram in Fig. a shows the prevalence of high-molecular ADA toward the low-molecular: the ratio of activity at maximal point of low-molecular isoform, 0.112 IU/*L*, to that of high-molecular isoform, 0.151 IU/*L*, is 0.74. In the case of the 2nd group (with the ADA activity higher of the mean value), the diagram in Fig. b shows nearly equal levels of two forms of the enzyme: the ratio of activity at maximal point of low-molecular isoform, 0.206 IU/*L* (nearly twice of that in diagram a), to that of high-molecular isoform, 0.185 IU/*L* (only a little higher of that in diagram a), is 1.1.

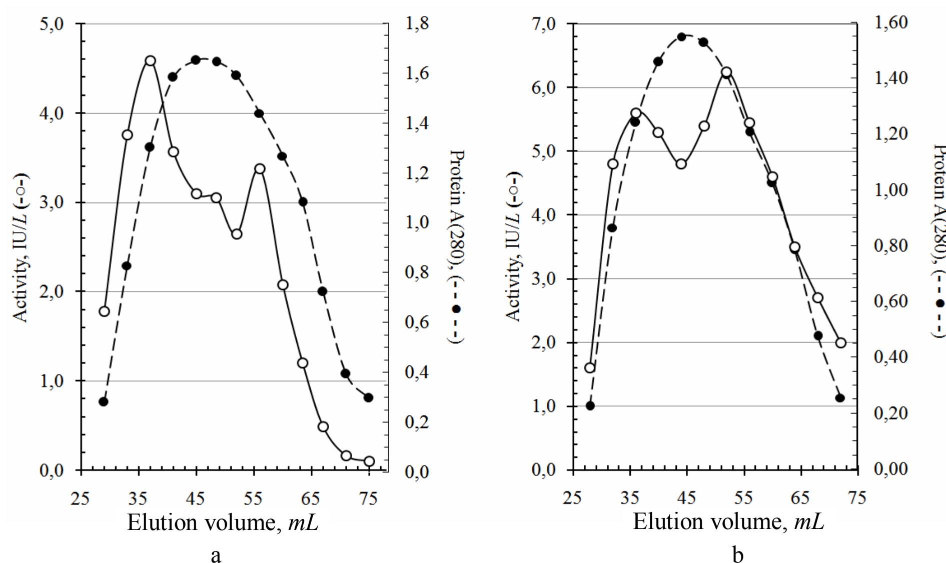


Fig. 1. The presentable elution diagrams, obtained at gel-filtration of SF through the column with Sephadex G-200$_{sf}$:
a – 1st group, the initial total activity of ADA was 27.5 IU/*L* (below the statistical mean value, 34±23.2 IU/*L*);
b – 2nd group, the initial activity of the enzyme was 58.9 IU/*L* (higher of the mean value).

Seemingly, the general ADA activity in SF samples from 1st group (low activity of the enzyme) like any other biological fluids (blood plasma, pleural fluid, etc.) is presented in high molecular ectoform, in form of a complex of ADA catalytic unit with the ADA-binding protein [12]. In the mammalian tissues, the ADA-binding protein is identical with the protein, provided with the dipeptidyl peptidase activity known as the dipeptidyl peptidase IV, which is found both freely circulating and localized on lymphocyte membranes.

The results of presented gel-filtration study allow us to suggest that in the inflammatory SF the increase of total ADA activity takes place mainly, because of the release of intracellular, low-molecular ADA. This phenomenon has a non-beneficial consequence: the released ADA metabolizes adenosine, an anti-inflammatory mediator, the high level of which is required to regulate anti-inflammatory

processes. Hence, in the SF of the 2nd group, with the ADA activity significantly above the statistical mean value, the development of severe inflammation leads to the ADA release, because of tissue destruction and cell death. But the ADA release by itself might be the cause of severity increase of inflammation, as it metabolizes and deactivates a very important anti-inflammatory mediator adenosine.

In this investigation as an inflammatory arthritis was used the rheumatoid arthritis. To involve other inflammatory joint disorders in the study would allow us to assert the importance of ADA level as an index of IA development.

Altogether, the current investigations evidence that the inhibition of ADA activity can be considered as a new approach to IA treatment.

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