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Adenosine Deaminase at Septic Arthritis

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Adenosine deaminase (ADA, EC 3.5.4.4) is an important enzyme in purine metabolism [7]. The small isoform of the enzyme (SADA) is a catalytically active protein with a molecular mass of 36-40 kDa. [9].The large ADA isoform (LADA, Mm of 280-300 kDa) represents a non-covalent complex between the catalytic unit and an ADA-binding cell membrane enzyme dipeptidyl peptidase IV (DPPIV). The enhanced ADA activity was registered in different diseases, e.g. tuberculosis, rheumatoid arthritis, etc. (reviewed in [4]. The ADA substrate, adenosine, is known as anti-inflammatory agent. It suppresses the inflammation by decreasing of pro-inflammatory and increasing of anti-inflammatory cytokines, regulating the inflammatory function of endothelial cells [6].

Arthritis is one of the most common causes of people's disability in the world. There are 100 different forms of arthritis. Inflammatory joint diseases are heterogeneous group of disorders afflicting not only joint tissues but often multiple organs in the body. The group of the joint disorders includes septic arthritis (SA), rheumatoid arthritis (RA), osteoarthritis (OA), ankylosing spondylitis (AS), reactive arthritis (ReA), etc. [23]. RA is one of the most common types of arthritis. It affects 0.5–1% of the world population. It is an autoimmune disease accompanied with inflammation, causing chronic synovitis and progressive destruction of joints. The prevention or at least retardation of irreversible joint damage is a principal therapeutic aim in RA [20, 25].

Acute bacterial arthritis or "septic arthritis" is a rheumatologic emergency. Bacterial replication in the joint and the ensuing inflammatory process can lead to local joint destruction, and may be accompanied by systemic infection [22]. The predominant causative pathogens in septic arthritis are Staphylococcus aureus and Streptococcus, accounting for up to 91% of cases [11]. Staphylococci (S. aureus and coagulase-negative staphylococcus species) account for more than half of cases of prosthetic-hip and prosthetic-knee infection [24]. Typically septic arthritis presents with a short 1–2 week history of pain,

swelling, heat and restricted movement in the affected joint(s). There is a common misconception that septic arthritis affects one joint only, but evidence suggests that in up to 22% of cases the presentation is poly-articular [10].

Protein citrullination (deimination) catalyzed by enzymes of peptidyl arginine deiminase (PAD) family is a posttranslational modification, conversing arginine residues in proteins into citrulline residues [12]. PAD enzymes and the citrullinated proteins are implicated in numerous pathological disorders, so citrullination is considered as one of crucial process in the context of inflammation [16], particularly, linked to the pathogenesis of RA [14]. The citrullinated ADA was suggested as an ACPA antigen in RA synovitis [18]. Generally, the investigation of protein citrullination is an urgent, actual task in the medical biochemistry.

There are several works devoted to the ADA activity application for differential diagnosis of joint diseases [19]. Preliminary we demonstrated that in RA SFs the increasing of the ADA activity is accompanied with the increasing of SADA/LADA ratio. It was significantly higher as compared to the SFs from patients with ankylosing spondylitis (AS), gout (G), juvenile idiopathic arthritis (JIA) and reactive arthritis (ReA) [2]. SADA and LADA isoforms from the SFs of RA, G, AS, ReA and JIA were separated and their comparative amounts were evaluated. In the SFs from the most of studied arthritis types, the LADA isoform was prevailing over the SADA isoform. Actually, only in the SFs from patients with RA, the SADA isoform was presented in the substantial amount. The isolated ADA isoforms were purified and their citrullination states were analyzed. The LADA samples from the SFs of all the studied arthritis types were not citrullinated [13]. As to the SADA, only in the case of purified from RA SFs, SADA was citrullinated [3]. Considering, that one of 17 arginine residues in SADA [8], Arg-142, participates in SADA binding to DPPIV [21], we suggested the prevention of the SADA-DPPIV interaction because of Arg-142 citrullination. Occurring of these phenomena in RA SF, obviously, result in hindering of SADA transformation to LADA and to the registered accumulation of SADA especially in RA SF.

The present work demonstrates that from the 7 studied samples of septic arthritis (SA) SFs, only in one case the SADA was citrullinated similar to the SADA from RA SF.

Material and Methods

The synovial fluids from the patients diagnosed according to the accepted classification criteria and stored before use at -20^{o}C. L-citrulline, diacetylmonoxime (DAMO), thiosemicarbazide (TSC) and adenosine was purchased from Sigma (USA). All the used reagents were of the highest available purity.

The ADA activity was assayed by the phenol hypochlorite colorimetric method. The amount of ammonia, liberated in the catalyzed deamination of

adenosine was evaluated from the absorbance of the assay mixture at 630 nm [1, 17]. SADA and LADA isoforms were separated from SFs using gel-filtration chromatography procedures as described earlier [2].

The presence of citrulline in the proteins was shown using the colorimetric assay based on the specific reaction of DAMO with ureido group under highly acidic conditions [5]. The protein sample was subjected to acidic hydrolysis by incubating in a water bath for 1.5 hours in the presence of 3 N HCl. To 0.5 ml of hydrolyzed protein, 1.5 ml of freshly prepared chromogenic solution was added. The chromogenic solution in 30 ml contained 1 mg TSC, 10 ml 0.5 % DAMO in water and 20 ml 0.025% $FeCl_3$ solution in 15 % H_2SO_4. The mixture of hydrolyzed protein and chromogenic solution in a capped test tube was incubated for 15 min in a water bath. After cooling, the quantity of citrulline was measured as optical absorbance at 530 nm. In the work it was expressed as A_{530}/A_{280} ratio.

The spectral measurements were carried out on the spectrophotometer Cary 60 (USA).

Results and Discussion

The present work characterizes one SF sample from 7 studied septic arthritis (SA) samples, in which the SADA isoform was citrullinated. The patient was diagnosed by SA of shoulder joints, infected with Staphylococcus aureus. He took Metformin as he had type II diabetes mellitus. The physical examination showed obvious swelling of shoulder and limitation of movement (both actively and passively) cause of severe pain. Initial laboratory investigations (C reactive protein, 35 mg/L; white blood cell count $12x10^3$/μl; erythrocyte sedimentation rate 50 mm/hour) evidenced acute inflammation process. Because the rheumatoid factor and anti-CCP tests were negative, the patient was not diagnosed as RA.

The total ADA activity in the SF from shoulder joints of this patient was equal to 66.5U/L, the highest among 7 of the SA SF samples and being an additional indication of inflammation. We studied this SF sample more rigorously. After dialysis against 7mM phosphate buffer (pH 7.4), it was concentrated on the ion-exchange DEAE-cellulose. An aliquot from the central protein fraction, eluted from this column was subjected to gel-filtration through Sephadex G-200sf. In all the eluted fractions, the protein (A_{280}), the ADA activity (A_{630}), and the level of protein citrullination (A_{530}/A_{280}) were evaluated. Figure 1 shows the results of this gel-filtration. The diagram for the ADA activity (-●-) evidences the significant level in the region of LADA molecular mass (250 kDa) and the lower level in the region of SADA molecular mass (35-40 kDa). The diagram for the level of citrullination (-**Δ**-) evidences the absence of citrullination in the high-molecular region fractions, and noticeable citrullination in the small-molecular fractions with low ADA activity. This

result evidences the protein citrullination in SADA fractions separated from shoulder SA SF.

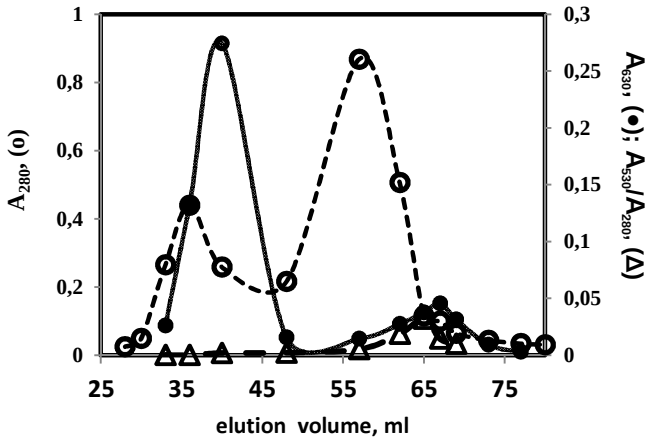

Fig. 1. The elution diagram of gel filtration of SA SF

As it was noted in the Introduction, earlier, we registered that SADA in RA SF is usually citrullinated. For comparison with the SADA from shoulder joints SA SF, Figure 2 shows the result of identical gel filtration on Sephadex G-200sf of RA SF with separation of small and large isoforms of ADA. The obtained picture evidences some protein citrullination in the LADA preceding fractions (Mm > 300kDa), and significant citrullination in protein fractions in the region of SADA activity (Mm ~ 35-40 kDa).

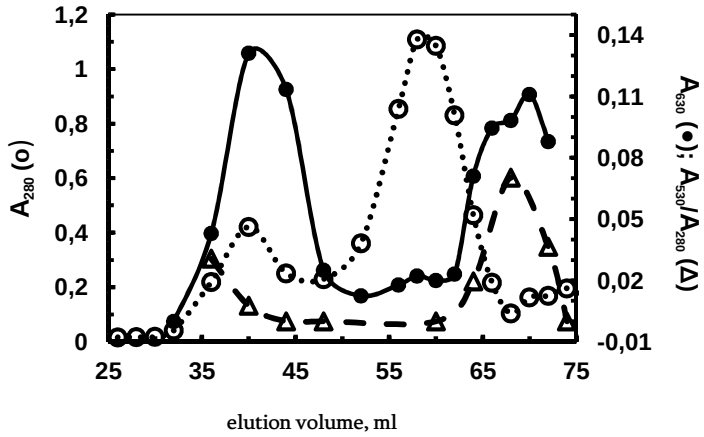

Fig. 2. The elution diagram of gel filtration of RA SF

Figure 3 compares the results from the diagrams in Figures 1 and 2. This picture evidences: a) in RA SF the specific activity of SADA fraction, A_{630}/A_{280}

ratio, averaged through all the activity possessing fractions, is 2.7 times higher than at SA SF; b) the averaged SADA/LADA ratio at RA is 3.2 times as higher as compared with SA; c) the averaged citrullination state of all the SADA fractions expressed as A_{530}/A_{280} ratio, is nearly the same (1.1) at two, SA and RA samples. Hence, in the single case of SF from SA with an acute inflammation, even being presented in the lower level compared to the RA SF, the citrullination state of SADA is close to the citrullination of SADA in RA SF.

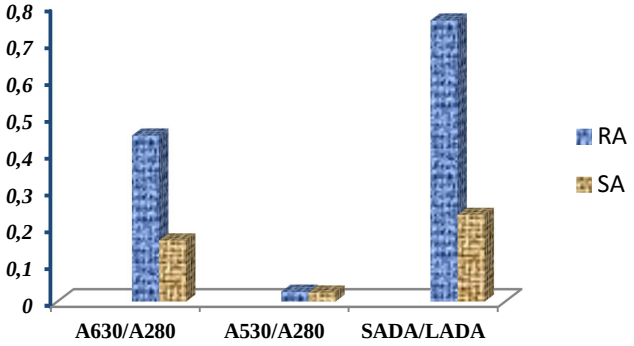

Fig.3. The comparison of the data from diagrams in Fig. 1 and Fig.2

Conclusion

Septic arthritis is a medical emergency which can lead to serious complications and mortality. For this reason, the prompt accurate diagnosis and the correct treatment are critical to ensuring a good result.

Therefore, along with the bacteriological analysis, the biochemical investigations of SFs, as protein citrullination can be very informative and valuable.

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Ադենոզինդեամինազան սեպտիկ արթրիտի դեպքում

Լ.Գ. Կարապետյան

Պեպտիդիլարգինինի դեիմինացմամբ (ՊԱԴ) հետտրանսլացիոն ձևափոխումը սպիտակուցներում արգինինի մնացորդները վերածվում են ցիտրուլինի: Ցիտրուլինացված սպիտակուցներ են գրանցվում մար-

դու մի շարք լուրջ հիվանդությունների ժամանակ՝ Ալցհայմերի և Պարկինսոնի հիվանդություններ, ռևմատոիդ արթրիտ (ՌԱ) և այլն: ՌԱ-ի սինյովալ հեղուկում (ՍՀ) ադենոզին դեամինազի (ԱԴԱ) ակտիվության զգալի աճ է գրանցվել, որն ուղեկցվում էր ցածր մոլեկուլային ձևի աճով (ՑԱԴԱ): Ավելին, հինգ տարբեր արթրիտների ՍՀ-ից անջատված ՑԱԴԱ և ԲԱԴԱ (բարձրամոլեկուլային ԱԴԱ) իզոձևերից ցիտրուլինացված էր միայն ՌԱ ՍՀ-ից անջատված ՑԱԴԱ-ն: Այս աշխատանքում ներկայացված տվյալները ցույց են տալիս սեպտիկ արթրիտի ՍՀ-ում ՑԱԴԱ-ի ցիտրուլինացված լինելը:

Аденозиндезаминаза при септическом артрите

Л.Г. Карапетян

Посттрансляционная модификация пептидиларгининдеиминазами (ПАД) превращает остатки аргинина в белках в цитруллин. Цитруллинированные белки регистрируются при некоторых тяжелых заболеваниях человека, таких как болезни Альцгеймера и Паркинсона, ревматоидный артрит (РА) и др. Было показано значительное повышение активности аденозиндезаминазы (АДА) в синовиальной жидкости (СЖ) ревматоидного артрита (РА), сопровождающееся увеличением его низкомолекулярной формы (НАДА). Более того, среди НАДА и ВАДА (высокомолекулярная АДА) изоформ из СЖ пяти типов артрита была цитруллинирована только НАДА из СЖ РА. Данные, представленные в настоящей работе, свидетельствуют о цитруллинировании НАДА в СЖ септического артрита.

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