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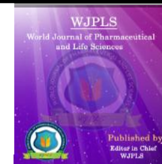

ADENOSINE DEAMINASE: A TARGET ENZYME IN DIFFERENT PATHOLOGIES – A REVIEW

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ABSTRACT

Adenosine is an anti-inflammatory agent, involved in the cellular energy and purine metabolism. Its derivatives of nucleotide and nucleoside nature have both the therapeutic significance in the treatment of immune, cardio and neurodegenerative disorders, and sometimes, can posses the toxic action on the cells and mammalian tissue. Adenosine deaminase is one of the enzymes participating in inactivation of the adenosine derivatives both in the intra- and intercellular medium. Therefore, the search for the regulators of activity of adenosine deaminase is a very important task of modern pharmacology. The solution of this task demands the knowledge on the catalytic peculiarities, active site structure, interaction with the substrates and with the probable inhibitors of the enzyme. This work reviews the information on the indicated fields available to date.

KEYWORDS: Adenosine deaminase, active site structure, substrates, inhibitors, therapeutic significance.

INTRODUCTION

Adenosine deaminase (Adenosine aminohydrolase, ADA, EC 3.5.4.4) is one of the key enzymes of purine metabolism. It catalyzes the conversion of (deoxy) adenosine to (deoxy) inosine releasing ammonia. ADA is widely distributed in all tissues of vertebrates, with the highest activity in the lymphoid tissues. Its amino acid sequence is highly conserved from bacteria to humans.[1] ADA plays a critical role in maturation and differentiation of lymphoid cells, maintaining the effective immune, neurological and vascular systems.[2,3]

There are two genetically different isoenzymes of ADA - ADA1 and ADA2, with different catalytic, biochemical and immunochemical characteristics, different distribution in mammalian, particularly in human, tissues. Catalytic sites of ADA1 and ADA2 - their substrate binding pockets differ significantly. ADA2 has 100-fold less affinity to adenosine and 2′-deoxyadenosine than ADA1. The optimum of its activity is at slightly acidic pHs (contrary to the neutral for ADA1). ADA2 is weakly inhibited by a strong inhibitor of ADA1 erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA).[4] ADA2 is responsible for a small part of total ADA activity in several tissues, but it is predominant circulating form in the normal human serum.[5] Human ADA2 may have become specialized during evolution to be specifically active in sites of high adenosine concentration and lower pH, as in tumor growth, hypoxia, inflammation, etc.[6]

The present review is considering the therapeutic significance of regulation of the activity of ADA1 isoenzyme.

ADA1: molecular forms and binding proteins

Two molecular isoforms of ADA1 (thereafter ADA) are known. In the mammalian tissues the enzyme is presented in the intracellular medium (cytosol) by a small monomer form (SADA) with molecular mass 35–40 kDa.[7] In the intercellular medium, a large ecto-enzyme is known (LADA) with molecular mass 280–300 kDa. This one represents a complex of SADA as a catalytic subunit, and a protein, called “ADA binding protein”, which has been identified as classification determinant 26 (CD26), identical to the dipeptidyl peptidases family enzyme, dipeptidyl peptidase IV (DPPIV).[8,9] The ecto-enzyme LADA as an ADA-DPPIV/CD26 complex is found in many mammalian tissues in both the circulating and localized on the cell membrane forms.

Binding of ADA on DPPIV/CD26

The main function of LADA consists in the reducing of the extracellular level of cytotoxic adenosine and 2´-deoxyadenosine and protecting the cells from apoptosis.[10] LADA has been demonstrated on the surfaces of fibroblasts, erythrocytes, lymphocytes, epithelial and dendrite cells.[11-13]

The point mutation study revealed the participation in ADA binding of L340, V341, A342 and R343 residues in the extracellular domain of DPPIV/CD26 [14]. On its turn, the P126–D143 segment (especially E139, R142 and D143 residues) in ADA appeared critical for binding to DPPIV/CD26.[15] The crystallized complex of bovine SADA and human DPPIV/CD26 showed that the dimer of the last binds two SADA.[16] The 3D-structure of their complex showed that the interaction of ADA and DPPIV results in the modulation neither of the catalytic sites,[17] nor the activities of the enzymes.[18] Moreover, the activities of CD26/DPPIV and ADA are not necessary for their binding.[19]

It is worth noting, that our experiments revealed some differences in the catalytic peculiarities of monomer SADA and its complex with DPPIV/CD26 (LADA).[20] The Michaelis constant, *K*m, evidenced a higher affinity of two substrates (in particular of more toxic 2´-deoxyadenosine) for LADA, proving the increase of this toxic nucleoside neutralization in the extracellular medium due to formation of ADA-DPPIV/CD26 complex. Some of the studied derivatives of EHNA demonstrated stronger inhibiting activity towards DPPIV/CD26-bound ADA. Then, the works[21,22] reported the synthesis of a series of compounds: 1-(4-substituted phenyl)-3-[4-substituted piperazino(piperidino)]propiophenones, α-Phenyl-β-amino-4-substitute propiophenones, 1-(4-Ethoxyphenyl)-1-alkyl-2-phenyl-3-piperidinopropanols and 1-(4- substituted phenyl)-3-[4-substituted piperazino(piperidino)]propan-1-ols. The synthesized compounds were identified by IR and NMR^{1}H spectrometry, characterized with some of their biological activities. A rather effective *in vitro* structure-dependent inhibition of ADA by several of these compounds was revealed. Most interesting was that for the first time the compounds were synthesized, which inhibited SADA more effectively than LADA. The observed differences in the catalytic properties of SADA and LADA are the results of complex formation between SADA and DPPIV.

Some influence of ADA binding on the enzymatic activity of DPPIV was observed also: the pH optimum of DPPIV activity in the ADA-DPPIV/CD26 complex appeared in a more alkaline region (>9) than the known optimum for the free enzyme (in the region 7.5-8.5).[23]

The independent from the enzymatic activity of ADA, the costimulatory effect, promoted by the ADA-DPPIV/CD26 complex, consists in inducing of IL-6 production, increasing INF-*γ* and TNF-*α* secretion, and enhancing the pro-inflammatory and Th1 response.[13] ADA enhanced the CD3-mediated proliferation of T cells, Th1 and pro-inflammatory cytokines secretion in HIV-1-infected individuals. Since ADA tightly enhanced specific T-cell responses against HIV *in vitro*, it has been suggested that this enzyme satisfy all the requirements to be assayed as an anti-HIV vaccine adjuvant.[24,25]

Binding of ADA on DPPII

Using a resonant mirror biosensor, fluorescence polarization, and differential spectroscopy methods, we revealed for the first time the *in vitro* binding of ADA to purified DPPII, another enzyme of dipeptidyl peptidases family. While DPPII is localized in lysosomes and lysosome membranes, but ADA in the cell cytoplasm and surface, the *in vivo* interaction of the enzymes cannot be excluded.[26] The similarity (at least, by molecular mass, ~200 kD) of ADA-DPPII complex to the *"high molecular ADA"* isolated by Lindley and Pisoni from human fibroblast lysosomes[27] seems rather interesting. These authors suggested the probable importance of lysosomal compartmentalization of ADA for maintenance of cellular energy requirements under conditions of nutritional deprivation. Their suggestion might be considered as one of the working hypotheses for the unknown physiological significance of ADA binding to DPPII.

Binding of ADA to adenosine receptors

The adenosine receptors (ARs) are the other type of proteins, anchoring ADA on the cell surface. Three subtypes of ARs: A1R, A2AR and A2BR have been reported to anchor ADA to the cell surface.[28-30] A1R is the ADA anchoring protein in the brain.[31] The selective A1R agonists and antagonists have many potential therapeutic applications. The A1R agonists have been identified as antilipolytic, neuroprotection and pain management agents, which can be applied in type 2 diabetes treatment also. A1R antagonists are studied as effective agents for the treatment of acute heart failure, asthma, Alzheimer's disease, chronic heart diseases and as diuretics for kidney protection.[32,33] It was suggested that human brain ecto-ADA has dual functions: reducing the extracellular adenosine concentration and serving as an allosteric regulator of A1R. ADA modulates receptor function enhancing agonist and antagonist binding and, independent from its enzymatic activity, increases receptor signaling.[34] So far as ARs are widespread, ADA modulators could be promising therapeutic targets in a wide variety of pathological conditions associated with these receptors, including neurological, cardiovascular, immune and inflammatory diseases and cancer.[35]

Hence, ADA is a multifunctional protein that can degrade adenosine, act as an allosteric modulator of ARs and regulate the hormonal effects of adenosine.[36]

Physiological significance of ADA substrates

A substrate of ADA, adenosine (Ado) is an endogenous purine nucleoside, acting as a regulator in living systems.[37] Ado is involved in the cellular energy and purine metabolism, released to or produced in the extracellular medium, where it binds to the cell membrane ARs.[38] Two ways of Ado formation in tissues are: the dephosphorylation of AMP by 5′-nucleotidase and the hydrolysis of S-adenosyl homocysteine formed by methylation of S-

adenosylmethionine. Another substrate of ADA, a derivative of Ado 2′-deoxyadenosine (2′-dAdo), is a deoxyribonucleoside, differing from Ado by the replacement of hydroxyl group (-OH) at 2′ position of ribose by hydrogen (-H).

The earliest studies of the physiological role of Ado have shown that it participates in the regulation of coronary blood flow, dilates blood vessels via endothelium-dependent and -independent actions. Currently, Ado is known as playing an important role in different metabolic and pathological conditions, in regulation of kidney function, in asthma and hypoxia, in cardiac ischemia, in the inflammation severity.[39] It is an endogenous immunoregulator in cancer pathogenesis,[40] important neuromodulator in the central nervous system, playing role in neuronal excitability and transmission in the hippocampus and basal ganglia.[41]

Ado is inactivated by two ways: by the capture followed by adenosine kinase catalyzed phosphorylation to AMP and by deamination to inactive inosine by the presented in the most animal tissues ADA (a subject of the present review).

Active site structure and enzymatic mechanism of ADA

Human and bovine ADA amino acid sequences are 93% identical,[42] and the human and mouse enzymes are 83% interchangeable,[43,44] Meanwhile, the authors suggested that the structures and functions of the mammalian and bacterial enzymes are similar and the amino acid residues in the active site of ADA are highly conserved[1]

For understanding the mechanism of the catalyzed by ADA enzymatic reaction, the knowledge concerning the active site structure and the amino acid residues responsible for binding of the substrates and inhibitors is necessary,[45-50] This knowledge will help in designing and synthesizing of the effective pharmaceutical preparations, applicable in treatment of the ADA-related diseases.

X-ray analysis

Many authors studied the crystal structure of ADA complexes with different ground- and transition-state inhibitors (1-deazaadenosine, 8(R)-hydroxyl-2-deoxycoformycin, EHNA, 6-hydroxyl-1,6-dihydropurine ribonucleoside).[51] It was shown that the protein molecule in these complexes is folded as an eight-stranded parallel alpha/beta barrel with deeply placed active site pocket. The X-ray analysis revealed that at the carboxy-terminal end of the β-barrel, a zinc cofactor is bound tightly, which is coordinated by D295 and three histidine residues (H15, H17 and H214). The Zn^{2+} ion is shown as essential for the catalytic function and stability of the native protein.

It was revealed also that the substrate, Ado, is bound to the active site and stabilized by nine hydrogen bonds. The five of them are the bonds between the adenine moiety with E217, D296, G84, D295 and H238 residues of the enzyme. The other four hydrogen bonds are formed by the adenosine ribose 3'-OH group with D19, 5'-OH group with H17, and 2'-OH and 3'-OH groups with the "catalytic water molecule".[52,53]

Active site of ADA

Proteins usually undergo large conformational changes upon interaction with the substrates or other ligands. Two conformations of active site of ADA were revealed: closed and open. In the absence of the substrate, the active site adopts the open form. At substrate binding, it transforms to the closed form. The closed form of the enzyme is usually observed in the complexes with the substrate analogs having the adenine moiety. It indicates that namely this form is reached after binding of the substrate. Comparing the crystal structures of different ligand bound holo- and apo-forms of the enzyme, Kinoshita and coauthors[54] hypothesized that the removal of a water molecule, bound at the bottom of the active site, might trigger the conformational change from the open to the closed form. The binding to the active site of adenosine (or any substrate like compound) moves this “trigger water” away, promoting the transition to the closed form. In fact, all substrate analogs induce the closed form. Conversely, the non-nucleoside type inhibitors, such as EHNA, occupying the “trigger water”-binding position[55] do not cause significant conformational change in the protein, which remains in the open form[53] Hence, for the open/closed conformational alteration, the occupancy at the “trigger water” position is more critical than the binding of the nucleoside framework. Recently, using a new series of ADA inhibitors, the binding mode of the most potent compound (4-decyl-pyrazolo[1,5-a]pyrimidin-7-one) was revealed.[56] The innovative computational technique shaded light on some of the factors in the interaction of these new class inhibitors with the active site of the enzyme.

The catalytic mechanism of ADA

The catalytic mechanism of the adenosine deamination reaction was proposed by the combination of the results of crystallographic,[52,53] site-directed mutagenesis[57-59] and quantum mechanics study.[60,61] The reaction is viewed as follows: at the first step, Zn^{2+} acting as a powerful electrophile, activates the catalytic water molecule creating a hydroxide nucleophile. 6-OH in C6 position interacts with Zn^{2+} and the polar groups of H238 and D295, forming the tetrahedral intermediate conformation. Then, in the formed tetrahedral intermediate, proton is transferred from Zn^{2+}-OH to 6-NH_2. In this step, H238 serves as a proton shuttling group. It was shown that namely the proton transfer is a rate-limiting step in the ADA catalyzed deamination reaction. In the final step, the C6 amino group abstracts proton and form the reaction product, inosine, liberating ammonia. Finally, the inosine, coordinated by hydrogen

bonds to G184, E217 and D296, is released and replaced by a new water molecule.[57-61]

The tryptophan residues are essential for ADA activity

Some residues that do not coordinate directly the substrate, but environ the active site, seemed to be important for the substrate-enzyme interaction. For example, the work[62] reported the importance of cystein residues for the catalytic activity of ADA. Based on the tryptophan fluorescence decreasing in ADA from calf thymus after binding of the ground and transition state substrate analogs, Philips et al. suggested that one or more tryptophan residues (Trp) should be adjacent to the active site of the enzyme.[63] The data of Kurz et al.[64] confirmed that the conformational changes in complex of ADA with the substrate analogs bring to the resonance energy transfer from Trp to the ligand. Comparison of amino acid sequence of murine and human ADAs showed that two Trp residues (W264 and W272) are conserved in the C262 involving block[1] and are located on 11 Å close to the active site.[59] This distance is too far for the participation of Trps in the substrate binding, but it is enough for their interaction with the residues responsible for stabilization of the enzyme-substrate complex.

The X-ray studies did not reveal the Trp residues in the proximity of the active site of ADA. Unfortunately, the importance of Trp residues for activity cannot be studied by site-directed mutagenesis because there is not any amino acid, which is sterically close to the Trp molecule and can be used for its genetic replacement.[65] Hence, it seemed that only chemical modification is a useful approach in the checking of the probable participation of Trps in the ADA catalyzed reaction. Therefore, we studied the importance of Trp residues for catalytic properties of ADA from bovine brain and spleen using chemical modification. The observed loss of the enzyme activity correlated with the oxidation of Trp residues by N-bromosuccinimide (NBS).[66,67] The active site binding inhibitors (analogs of adenosine, 1-deaza Ado, 7-deazaAdo) protected the Trp residues from oxidation by NBS. The analysis of the results using the graphical method of Tsou revealed that two Trp residues were essential for the ADA activity. The fact that 7-deazaAdo, a poor inhibitor of ADA, protected two essential Trps from oxidation by NBS showed that this compound binds in the active site of the enzyme, probably, with a lower affinity compared with the substrate. In the presence of EHNA, a strong inhibitor of ADA, and its derivatives, 1-deazaEHNA and 3-deazaEHNA, the full inactivation of the enzyme by NBS is achieved after oxidation of four Trps. This fact is in accordance with the knowledge about the different places of binding on the enzyme of adenosine and EHNA derivatives.[68] This observation supports the hypothesis that the nonyl chain of EHNA analogues binds to the hydrophobic region near the active site of the enzyme,[69] and the inhibition by EHNA and its derivatives might be due to structural adaptation of the enzyme accompanying the binding of these inhibitors.[69] Frieden et al.[70] proposed the interaction of adenine moiety of EHNA with the active site of ADA as an initial binding step, followed by a rearrangement of either enzyme or inhibitor to form a stable complex, from which the inhibitor dissociates slowly. The local conformational changes led to the oxidation of more Trp residues than in the cases of adenosine analogs. However, it is worth to note that 3-deazaEHNA bonded to the spleen and brain ADA in a manner which maximally prevented the modification of Trp residues: the longer lag period of ADA inactivation by NBS in the presence of this compound was observed.[66]

Our findings confirmed that the integrity of Trp residues is essential for the proper catalytic behavior of ADA. The obtained data manifested that while the Trp residues were not revealed in the X-ray study of the enzyme, they are rather close to its active site and are important for the catalytic events. Moreover, the extent of Trp accessibility in the protein molecule can serve as a powerful tool to probe enzyme conformational changes induced by the binding of different ligands and may contribute to a greater knowledge of protein binding properties.

Applying the technique of selective quenching of Trp fluorescence by acrylamide, I^- and Cs^+ ions, we studied the conformation of SADA in the complexes with 1-deazaAdo and EHNA.[71] The binding of inhibitors did not influence on the main emission characteristics of Trp fluorescence in SADA. Hence, in general, the folding of the enzyme molecule was not perturbed by inhibitors. Conversely, the quenching of Trp fluorescence by charged ions, I^- and Cs^+ in SADA complexes with inhibitors was hindered compared with the quenching in free SADA. In the complex with 1-dAdo, the quenching parameters evidenced the essential worsening of the access to Trp residues for both ions. In the case of EHNA, along with worse quenching by I^-, a complete prohibition of quenching by Cs^+ was observed manifesting the substantial distortions in local environments of the fluorescing Trps and their shielding in the presence of EHNA.

The histidine residues essential for ADA activity

The role of three histidine residues in ADA activity has been elucidated in the X-ray study of the enzyme complexes with the substrate analogs.[52,53] More direct investigations were realized using the chemical modification of these residues by diethyl pyrocarbonate (DEP). The thermodynamic titration of ADA in a wide range of DEP concentrations demonstrated the modification of three important for activity His residues.[72] Our study in narrow range of DEP concentrations demonstrated the modification of six His residues. The graphical analysis by the method of Tsou revealed that only one from them was essential for ADA activity.[73] We assumed that this essential His might be just H238, the role of which is elucidated in the active

site investigations by other methods. This opinion is proved by the following considerations: 1) p*K*a of the His responsible for ADA inactivation in the presence of DEP is high, which means that this residue is close to the immediate neighborhood of two acidic amino acid residues, D295 and D296;[59] 2) in our experiments ADA inhibitors EHNA, 3-deazaEHNA and 1-deazaAdo with a rather high affinity to the enzyme, did not protect His from the action of DEP. The inhibition constants of all these compounds were not changed significantly by the modification of essential for activity His residue.[73] Bhaumik et al. demonstrated that change of H238 by E and R did not influence the enzyme affinity to the substrate and inhibitors noticeably.[74] This suggestion supported our results proving that the adenine moiety of inhibitors and the substrate did not bind to H238 which serves as a proton shuttling group of the active site.

ADA activity in different pathologies

Changes in the level of ADA activity have been observed in a number of diseases: tuberculosis,[75] acquired immunodeficiency syndrome,[76] systemic lupus erythematosus,[77] inflammatory responses,[78] rheumatoid arthritis,[79,80] hereditary hemolytic and Diamond Blackfan anemias,[81] heart diseases,[82] some types of carcinoma,[83,84] neurological disorders as multiple sclerosis[85] and meningitis.[86] It was suggested[87] that elevated ADA activity may play a role in the pathogenesis of chronic tonsillitis both by impairing tissue structure and contributing to superoxide radical (SOR) formation. The enzyme abnormalities have been reported also in: cutaneous Leishmaniasis,[88] viral hepatitis,[89] some leukemias,[90,91] neoplastic transformations,[92] myasthenia gravis,[93] Down's syndrome,[94,96] Parkinson's disease,[95] etc.

ADA in immunodeficiency

The role of ADA is best characterized in immunodeficiency. The enzyme disorders have been reported in acquired immunodeficiency syndrome (AIDS)[76] and in various diseases with autoimmune character. At ADA deficiency the levels of Ado and 2'-dAdo elevated in the blood and urine. The elevation of these ADA substrates involves disruption of differentiation, function and viability of lymphocytes resulting in lymphopenia. Hereditary deficiency of ADA is associated with the severe combined immunodeficiency syndrome (SCID). The common form of SCID is an autosomal recessive disorder caused by the defects in the activity of the enzymes catabolizing purine nucleotides. It is characterized by impaired development and function of T, B and NK cells, depressed cellular and humoral immunity.[96]

The diminished activity of ADA results in the accumulation of Ado and 2'-dAdo in the cells and, as a consequence, in the increase in the concentrations of toxic nucleotides. Particularly accumulation of dATP causes inhibition of the ribonucleotide diphosphate reductase activity, the enzyme that synthesizes DNA and RNA, required in a large amount during lymphocyte proliferation. This way initially thought to be an only cause of the immune system disordering.[97] Resta and Thompson speculated about another possibility: the activation of ARs by the accumulated extracellular adenosine can also play a role in the SCID development.[98]

Two ways are under consideration for treatment of SCID, caused by ADA-deficiency. One of them is the enzyme replacement with pegylated ADA (PEG-ADA, marketed as Adagen). The enzyme replacement therapy restored functions of both T and B cells in ADA deficient patients.[99] Actually, PEG-ADA is approved for the treatment of ADA-related SCID.

The other way is the autologous hematopoietic stem cell gene therapy. ADA deficiency is the first approved human gene therapy trial. A multicenter retrospective study of outcome of hematopoietic cell transplantation in the patients with ADA-SCID manifested humoral immunity and donor B-cell engraftment in nearly all surviving patients.[100] Gene mutations were proposed to be responsible for blocking the ADA expression and causing SCID. The mutations leading to over-expression of the enzyme may cause hemolytic anemia.

The ADA level could be used as a nonspecific marker of T-lymphocyte activation. We studied the dynamics of DPPIV and ADA activities and IgG and IgM antibodies during development of immune response in laboratory rats after immunization with human erythrocytes. The correlation between DPPIV and IgG, and between ADA1 and IgM was registered. The results suggested ADA1 participation in Th2, and DPPIV – in both Th2 and Th1 immunity pathways.[101]

We have shown that in the blood plasma of rats, injected with the known immunosuppressant cyclophosphamide, the levels of circulating ADA and DPPIV were modulated by the new immunomodulator cytokines from bovine hypothalamus: proline rich peptides PRP and dPRP.[102]

ADA in arthritis

The significant differences between the levels of ADA activity in the synovial fluids of patients with inflammatory rheumatoid arthritis (RA) and non-inflammatory osteoarthritis (OA) were registered in the work of many researchers.[103,104,105] Our investigations indicated that ADA-test can be applied as a sensitive (0.96), specific (0.89) and high efficiency (0.93) test, suitable for the fast and low-cost differential diagnosis for differentiating RA and OA in the Armenian population ($p<0.001$).[80] The cutoff value for this test in our research, 12 IU/L, differed from the value of 20 IU/L reported for the Iranian population.[104] This manifested that, prior of using any test in the diagnosis, a special investigation must be performed to define the cutoff

value and parameters of the test, applicable for the given population.

ADA in aluminum toxicosis

We studied the *in vivo* influence of widespread in the life aluminum on the levels of ADA, DPPII and DPPIV activities in the brain and blood of rat. Two types of the experimental toxicosis have been applied: acute (one time injection of 5% water solution of $AlCl_3$) and chronic (*ad libitum* water containing 0.45% $AlCl_3$). The intoxication by aluminum chloride was accompanied with the ADA activity decreasing in three parts of rat brain (whole brain, medulla oblongata, cerebellum) [106]. On 30-th day after $AlCl_3$ injection in the acute toxicosis, the activity of ADA in these parts of brain decreased by 20–40%. At the chronic toxicosis, the decreasing by lower extent (11-28%) was observed. The ADA activity decreasing might be explained by the effect of substrate inhibition by adenosine, which can increase at neurodegenerative processes: adenosine protects CNS at hypoxia, ischemia, epilepsy, etc. Ado concentration at these pathologies sharply increases in the extracellular fluid of CNS, indicating its participation in the defense mechanism of neuronal cells against death.[107] Probably, the same mechanism can be involved in the inhibition of ADA for maintaining the demanded high level of the anti-inflammatory adenosine. At the acute toxicosis (one time $AlCl_3$ injection) the decreasing of ADA activity by 48–50% of the control in the erythrocytes of animal blood was observed also.[106]

Therapeutic application of ADA inhibitors

During the last decades, extensive researches have been conducted on searching the ADA inhibitors promising in regulation of the concentration of Ado in tissues. As ADA regulates both the intra- and extra-cellular concentrations of Ado, the decrease or increase of its level might trigger some pathological conditions. Hence, ADA inhibitors are potential therapeutic agents for treatment of various pathologies. The understanding of interaction of ADA with the substrates and inhibitors at the molecular level is required to develop the modern generation of pharmaceutical agents for treatment of ADA-dependent health disorders.

Extracellular Ado is a key physiological regulator of immune cell function.[107] Human ADA is very intensively investigated at the medical field because of diseases induced by lack or excess of its activity. Obtained knowledge is useful in application in the therapy of the ADA related illness. ADA inhibitors can have beneficial effects in hypertension[108] and in myocardial ischemia.[109] The pharmacological inhibition of ADA is regarded as a therapeutic approach to counteract inflammation in several pathological conditions[110] and may be useful in therapeutic management of intestinal inflammatory disorders.[111] Recently, it has been suggested that inhibitors of ADA could be used in the treatment of perinatal hypoxia–ischemia brain injury.[112]

Activity of ADA in synovial fluid of patients at inflammatory joint diseases is significantly higher than at non inflammatory diseases. The elevated enzyme reduces the concentration of the anti-inflammatory Ado and contributes to the inflammation development. We have shown that EHNA and Ado derivatives inhibited *ex vivo* the ADA activity in synovial fluid with the efficiency similar to the inhibition of ADA, purified from human biological fluids.[113]

Many adenosine analogues represent therapeutical agents in chemotherapy, cancer, immunology, virology and parasitology. Much effort is directed to development of inhibitors which can be beneficial and cost-effective for the treatment of different types of cancer affecting the immune system, such as leukemia and lymphoma.[114,115]

Some Ado analogs, being potential therapeutic agents, might be inactivated due to metabolizing to inosine analogs by ADA. So, the modification and the rational design of novel ADA inhibitors can prolong the activity of such agents. Nowadays, only two purine analogs, pentostatin (2'-deoxycorfomycin) and cladribine (2-chlorodeoxyadenosine) were approved by Food and Drug Administration (FDA) for treatment of hairy cell leukemia.[116]

Pentostatin, an Ado analog nucleoside, is an anticancer chemotherapeutic drug, which inhibits ADA interfering with the cell's ability to process DNA. It is used to treat hairy cell leukemia, B-cell chronic lymphocytic leukemia and steroid-refractory acute and chronic graft-versus-host disease. Cladribine, purine analog, is a chemotherapy agent that targets lymphocytes and selectively suppresses the immune system. It is activated only by lymphocytes, and non-activated cladribine is removed quickly from all other cells. Cladribine mimics the nucleoside adenosine and inhibits ADA, but is resistant to deamination by enzyme because of its chlorinated purine ring structure.

However, most of potent ADA inhibitors have poor pharmacokinetics. They bind to the enzyme tightly, making the enzyme activity nearly irreversible, and/or exhibit unacceptable toxicity,[48,117,72,117] Therefore, the researchers focused on the investigations directed to the designing and developing of less toxic, stable, reversible and effective inhibitors of ADA.[118]

Altogether, inhibitors of ADA can be divided into next categories: 1) ground-state inhibitors whose structures are similar to that of the Ado; 2) transition-state inhibitors that resemble the tetrahedral intermediate formed during enzymatic reaction and 3) a recent generation of non-nucleoside-type ADA inhibitors and derivatives,[119,120] which have been synthesized through structure-based drug design.

Natural inhibitors of ADA

Plant preparations have been sources of drugs centuries ago, and many of the currently available drugs have been derived from them. Herbal medicines generally have fewer side effects than synthetic compounds, and their effectiveness can be improved by modern pharmacological methods. Recent years, the natural sources are of increasing interest since they have a remarkable spectrum of biological activities. Several methods, such as high throughput screening, docking and QSAR analysis are applied in drug discovery. The selected designed compounds are studied in biological tests.

Many plant extracts and their constituents can induce biological effects through inhibition of ADA activity. It was found that extracts from Syzigium cumini, Sillybum marianum and Curcuma longa could significantly inhibit the ADA activity.[121-125] Some plant constituents such as acid sapogenins inhibit ADA with a rather low K_i.[121] The phenolic flavonoids of plants, vegetables and fruits have many pharmacological effects. The moderate inhibition of ADA by flavonoids is concentration-dependent, with the IC_{50} values in the micromolar range (about 3.0 x 10^{-5}M for kaempferol and quercetin).[124,125] The study of structure/activity relationships of ADA inhibition by these natural derivatives revealed the necessity of hydroxyl group in position 3 of chromone moiety. On the other hand, the hydroxyl group in the side phenyl ring seemed also important. Genistin, genistein, and cyanidin-3-rutinoside from different natural sources were identified as ADA inhibitors.[126] Docking simulation suggested that genistein and cyanidin-3-rutinoside can bind the active site of ADA through H-bonds and inhibit the enzyme competitively.

The inhibition of ADA seems beneficial in the treatment of such pathology as type 2 diabetes.[127] We performed the screening of the ability of water extracts of 23 Armenian Highland native plants to inhibit the enzymatic activity of DPPIV and ADA.[128] 1 percent extracts from 12 plants inhibited the ADA activity by more than 40%. Among them, the extract from St. John's wort (*Hypericum perforatum*) inhibited ADA most effectively (by 88.3%±7.4; IC_{50}=1.99±0.3 mg/ml). The extracts from blackberry, melilot, oregano, clove, cinnamon, green and black tea were highly effective in ADA inhibition too. The obtained results shown that the selected plants might be combined with the known antidiabetic drugs to improve their efficiency in the diabetes treatment.

In the synovial fluids of rheumatoid arthritis patients, the inhibition of the ADA activity by ethanol extracts from 4 Armenian Highland plants and 6 fractions from these extracts were studied *ex vivo*.[113] The low IC_{50} values of the studied plant preparations in ADA inhibition allowed us to propose them as prospective sources of the new anti-rheumatoid (anti-inflammatory) agents.

Known drugs as inhibitors of ADA

A number of approved pharmaceutics, having application as sedative, antidepressant, anxiolytic, analgetic, etc., also are provided with the ADA modulating ability, which obviously enhance their therapeutic value. For instance, such known and widely used drugs as methotrexate, lidoflazine, dipyridamole, trazodone, phenylbutazone, allopurinol, acyclovir, theophylline, etc. effectively inhibit ADA activity (Ki ~ 3.0-30.0 x 10^{-5}M).[129-131]

A combination of an anticancer phytochemical, cordycepin (immunomodulator and radical scavenger), with EHNA, a well-known strong and specific inhibitor of ADA,[132] would increase the efficacy of the drug therapy.

CONCLUSION

All these information represent important starting points for optimization of strategies in development of new ADA inhibitors as potential active drugs in inflammation, malignant, cerebral and other diseases.

The interaction of different inhibitors with ADA, the revealing of major differences of substrate specificity, the potency and selectivity of different inhibitors, and the conformational changes in the enzyme after substrate or ligand binding will give clue for synthesizing new compounds for future drug evaluation.

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