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Regulation of uric acid metabolism and excretion

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ABSTRACT

Purines perform many important functions in the cell, being the formation of the monomeric precursors of nucleic acids DNA and RNA the most relevant one. Purines which also contribute to modulate energy metabolism and signal transduction, are structural components of some coenzymes and have been shown to play important roles in the physiology of platelets, muscles and neurotransmission. All cells require a balanced quantity of purines for growth, proliferation and survival. Under physiological conditions the enzymes involved in the purine metabolism maintain in the cell a balanced ratio between their synthesis and degradation. In humans the final compound of purines catabolism is uric acid. All other mammals possess the enzyme uricase that converts uric acid to allantoin that is easily eliminated through urine. Overproduction of uric acid, generated from the metabolism of purines, has been proven to play emerging roles in human disease. In fact the increase of serum uric acid is inversely associated with disease severity and especially with cardiovascular disease states. This review describes the enzymatic pathways involved in the degradation of purines, getting into their structure and biochemistry until the uric acid formation.

1. Introduction

Uric acid production and metabolism are complex processes involving various factors that regulate hepatic production, as well as renal and gut excretion of this compound. Uric acid is the end product of an exogenous pool of purines and endogenous purine metabolism. The exogenous pool varies significantly with diet, and animal proteins contribute significantly to this purine pool. The endogenous production of uric acid is mainly from the liver, intestines and other tissues like muscles, kidneys and the vascular endothelium [1].

Uric acid is a $C_5H_4N_4O_3$ (7,9-dihydro-1H-purine-2,6,8(3H)-trione) heterocyclic organic compound with a molecular weight of 168 Da. Many enzymes are involved in the conversion of the two purine nucleic acids, adenine and guanine, to uric acid. Initially, adenosine monophosphate (AMP) is converted to inosine via two different mechanisms; either first removing an amino group by deaminase to form inosine monophosphate (IMP) followed by dephosphorylation with nucleotidase to form inosine, or by first removing a phosphate group by nucleotidase to form adenosine followed by deamination to form inosine. Guanine monophosphate (GMP) is converted to guanosine by nucleotidase. The nucleosides, inosine and guanosine, are further converted to purine base hypoxanthine and guanine, respectively, by purine nucleoside phosphorylase (PNP). Hypoxanthine is then oxidized to form xanthine by xanthine-oxidase (XO), and guanine is deaminated to form xanthine by guanine deaminase. Xanthine is again oxidized by xanthine oxidase to form the final product, uric acid. Fig. 1 shows the enzymatic pathway for the purines degradation. At physiologic pH, uric acid is a weak acid with a pKα of 5.8. Uric acid exists majorly as urate, the salt of uric acid. As urate concentration increases in blood, uric acid crystal formation increases. The normal reference interval of uric acid in human blood is 1.5 to 6.0 mg/dL in women and 2.5 to 7.0 mg/dL in men. The solubility of uric acid in water is low, and in humans, the average concentration of uric acid in blood is close to the solubility limit (6.8 mg/dL). When the level of uric acid is higher than 6.8 mg/dL, crystals of uric acid form as monosodium urate (MSU). Humans cannot oxidize uric acid to the more soluble compound allantoin due to the lack of uricase enzyme. Normally, most daily uric acid disposal occurs via the kidneys [2].

Uric acid concentration might be measured in serum, plasma, urine and in exhaled breath condensate. Determination of uric acid concentration includes phosphotungistic acid methods (PTA), uricase methods, high-performance liquid chromatography methods, dry chemistry systems and biosensor methods. Prior to determination of urate in urine, alkalinization of urine might be necessary, because of urate crystallize at pH lower than 5.75 [3]. The production and catabolism of purines are relatively constant between 300 and 400 mg per day. The kidneys eliminate approximately two-thirds, while the gastrointestinal tract eliminates one-third of the uric acid load. Almost all uric acid is filtered from glomeruli, while post-glomerular reabsorption and secretion regulate the amount of uric acid excretion. The proximal tubule is the site of uric acid reabsorption and secretion, and approximately 90% is reabsorbed into blood. This is primarily accomplished at the proximal tubular level by transporters that exchange intracellular anions for

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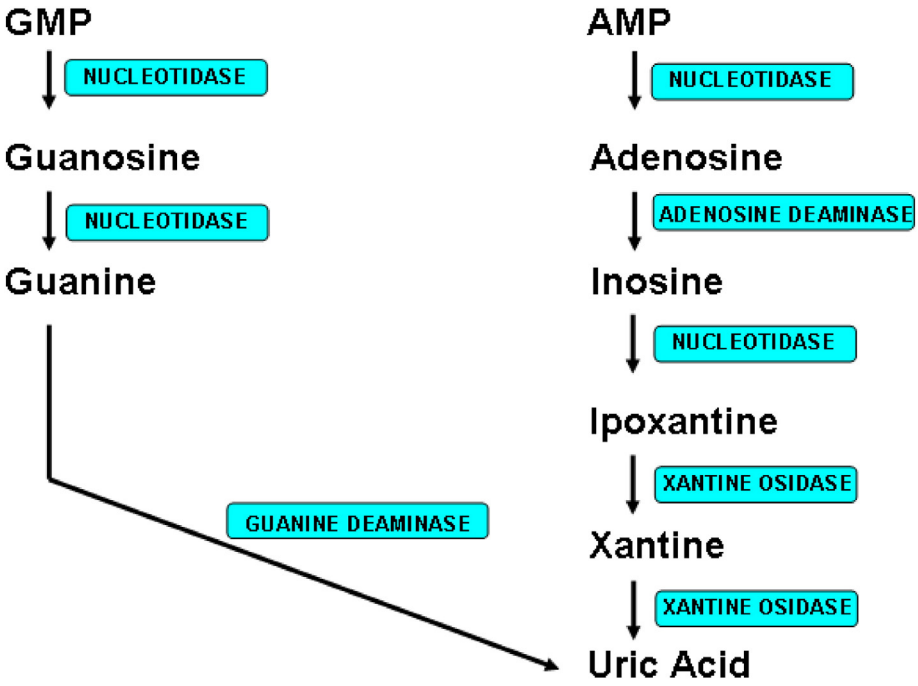

Fig. 1. Enzymatic degradation of purines in humans.

uric acid. Almost all reabsorption of uric acid occurs at the S1 segment of the proximal tubule. In the S2 segment of the proximal tubule, uric acid is secreted to a greater extent than that which undergoes reabsorption. Post-secretory reabsorption occurs at a more distal site of the proximal tubule, and approximately 10% of the filtered uric acid appears in the urine [1]. Hyperuricemia is a key risk factor for the development of gout, renal dysfunction, hypertension, hyperlipidemia, diabetes and obesity. Hyperuricemia occurs as a result of the increased uric acid production, the impaired renal uric acid excretion, or a combination of the two [4]. It is characterized by high uric acid level in the blood, causing deposition of urate crystals in the joints and kidneys [5]. Generally, hyperuricemia in adults is defined as a blood uric acid concentration greater than 7.0 mg/dL in men and 6.0 mg/dL in women. In normal humans, uric acid is excreted in urine. However, uric acid excretion may be impaired by kidney disease, leading to hyperuricemia [2].

Three urate transporters, URAT1/SLC22A12, GLUT9/SLC2A9, and ABCG2/BCRP, have been reported to play important roles in the regulation of serum uric acid (SUA), and their dysfunctions cause urate transport disorders. Among them, common dysfunction of ABCG2 exporter has proved to be a major cause of hyperuricemia and gout. Furthermore, renal hypouricemia is caused by increased renal urate excretion [6]. The molecular identification of URAT1 as the dominant apical urate exchanger of the human proximal tubule was a landmark event in the physiology of urate homeostasis. The URAT1 protein is encoded by the SLC22A12 gene, part of the large SLC22 family of organic ion transporters. URAT1 is a member of the organic anion transporter (OAT) branch of this gene family. Heterologous expression in Xenopus oocytes indicates that human URAT1 is capable of urate transport (^{14}C-labeled urate uptake), with a Km of 371 ± 28 μM. The basolateral entry of urate into renal proximal tubule cells is driven at least partially by the outwardly directed gradient for dicarboxylates such as α-ketoglutarate (α-KG), which in turn is generated by Na^+-dependent uptake via SLC13A1. Thus, in renal basolateral membrane vesicles, urate exchange is significantly trans-stimulated by α-KG. OAT1 and OAT3 appear to exchange urate with divalent anions such as α-KG, suggesting that they are suited to basolateral entry of urate, driven by intracellular α-KG, during urate secretion. Genetic variation in human ABCG2, an ATP-driven efflux pump, has emerged as a major factor in human hyperuricemia. A loss of or reduction in ABCG2-mediated renal urate secretion would lead to increased renal urate reabsorption, given that reduced renal excretion of urate is considered to be the underlying hyperuricemic mechanism in the vast majority of gout patients [7].

GLUT9 (SLC2A9) membrane transporter is distinct among other members of the glucose transporters (GLUT or SLC2) family due to its substrate specificity and sequence identity. While the majority of 14 members of the GLUT superfamily transport glucose or other monosaccharides, GLUT9 was shown to transport essentially urate. Single nucleotide polymorphisms in the SLC2A9 genes have also been associated with gout, coronary artery disease, and myocardial infarction. All 14 GLUT members share common structural features such as 12 transmembrane helices, cytoplasmic amino and carboxytermini, and an N-linked glycosylation site, although the glycosylation site varies across the family. Regarding GLUT9, two isoforms, SLC2A9a and SLC2A9b, have been described encoding the two proteins hGLUT9a and b that differ only by the first 29 residues of the N-terminal domains. GLUT9a is expressed ubiquitously, while GLUT9b is restricted to the main organs involved in urate transport, such as liver and kidney. GLUT9-mediated urate transport has been characterized. It is independent of sodium, chloride and anions, but is voltage dependent and currents have been recorded at physiological pH. Altogether, the data provided so far are compatible with a transport model in which GLUT9 is a uniport, without having formally excluded all other possibilities [8].

In addition to problems with uric acid excretion due to kidney dysfunction, hyperuricemia can also result from the increased generation of uric acid. Diets heavy in purine or fructose, or exposure to lead can also contribute to high uric acid levels. Fructose is a unique sugar molecule in that it rapidly depletes ATP and increases the amounts of uric acid. In certain humans, a deficiency of enzymes resulting from genetic mutations may also cause increased blood uric acid levels. For example, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) catalyzes the formation of IMP and GMP for recycling purine bases with 5-phoshorbosyl-alpha-pyrophosphate (PRPP) as a co-substrate. Lesch–Nyhan syndrome, a rare inherited X-linked disorder caused by the deficiency of HGRPP, leads to the accumulation of purine and PRPP, which are used in the salvage pathway of hypoxanthine and guanine. The HGPRT defect results in the accumulation of hypoxanthine and guanine, which further leads to high uric acid levels. The excess PRPP also increases the rate of de novo synthesis of purine, and consequently

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promotes the production of its end degradation product, uric acid. Lesch–Nyhan syndrome is the result of the buildup of high levels of uric acid in the body beginning in infancy, which leads to severe gout, kidney dysfunction, mental retardation, neurological dysfunction, and self-mutilating behaviors [2]. High levels of blood uric acid have long been associated with gout. Gouty arthritis (gout) is a medical condition characterized by red, tender, hot, and swollen joints caused by recurrent attacks of acute inflammatory arthritis. Men have a higher risk of developing gout than women due to higher baseline levels of blood uric acid. Pathologically, gout is caused by an increase of blood uric acid levels, which leads to crystal deposits in joints, tendons, and other tissues and uric acid renal stones. Recently, gout has been linked to cardiovascular disease. Furthermore, multiple studies have also associated hyperuricemia with the precursors of cardiovascular diseases, including hypertension, metabolic syndrome, and coronary artery disease, as well as with closely related vascular diseases such as cerebrovascular disease, vascular dementia, preeclampsia, and kidney disease [2,9]. Clinical studies have found that hyperuricemia relates with elevated plasma renin activity in patients with hypertension. A model of mild hyperuricemia treated with uricase inhibitor developed hypertension after several weeks due to uric acid-mediated renal vasoconstriction with an activation of the renin–angiotensin system (RAS) [10]. A recent clinical study reports that high plasma uric acid level, partly secreted from the failing heart, is a prognostic predictor in patients with congestive heart failure. In vitro studies reveal that uric acid induces gene expression of chemokines and growth factors, such as monocyte chemoattractant protein-1 (MCP-1) and platelet-derived growth factor, and stimulates proliferation of vascular smooth muscle cells. Furthermore, uric acid induced MCP-1 expression in vascular smooth muscle cells was attenuated by antioxidants, suggesting an involvement of redox-dependent mechanism. In vascular smooth muscle cells, uric acid activates critical proinflammatory pathways and stimulates cell proliferation. In endothelial cells, uric acid decreases nitric oxide bioavailability and inhibits cell migration and proliferation, which are mediated in part by the expression of C-reactive protein. In adipocytes, the redox-dependent effects of uric acid are mediated by the activation of intracellular oxidant production via NADPH oxidase. Activation of ERK in response to uric acid has been shown in vascular smooth muscle cells and adipocytes [11]. In contrast to chronic hyperuricemia, acute increase of plasma uric acid (UA) may induce various beneficial effects to human subjects. Administration of UA increases plasma antioxidant capacity, reduces exercise associated oxidative stress in healthy subjects and restores endothelial function in patients with type 1 diabetes and regular smokers. UA, as the most abundant aqueous antioxidant, accounting for up to 60% of plasma antioxidative capacity, may involve different mechanisms of action. It is a free radical scavenger which stabilizes vitamin C in serum, mostly due to its iron chelating properties and quenches peroxynitrite, a potentially harmful oxidant, resulting in formation of a stable nitric oxide (NO) donor in vitro. At concentrations close to physiological levels in humans, UA prevents hydrogen peroxide-induced inactivation of extracellular superoxide-dismutase (ecSOD), an enzyme that scavenges superoxide anions ($\bullet O^{2-}$). Also, it has been suggested that UA counteracts oxidative damage related to atherosclerosis and aging in humans. Taken together, these findings imply that UA could act beneficially in preserving vascular function, both under physiological and pathological challenges. The pro-oxidant and pro-inflammatory actions attributed to UA could be largely the result of the conversion of xanthine dehydrogenase to xanthine oxidase and of the consequent accumulation of reactive oxygen species (ROS) which occurs in parallel with UA production as an effect of ATP degradation under ischemic conditions. In this case, the ROS by-production might cause the inflammatory reaction and the arterial wall damage which have been attributed to excess of UA [12]. Hyperuricemia has a dramatically different, protective effect in neurodegenerative disease, including Parkinson's disease (PD), multiple sclerosis, and Alzheimer's disease/dementia. For example, higher uric acid levels reduce the risk of PD and reduce the risk of disease progression. Although the associated mechanisms are likely heterogeneous, most theories incorporate some role for the well-described antioxidant effect of uric acid [8].

2. 5′-Nucleotidase

Enzyme 5′-Nucleotidase hydrolyzes nucleotide monophosphates or deoxynucleotide monophosphates to nucleotides and deoxynucleotides more inorganic phosphate. This enzyme, together with nucleotide kinase, regulates the pool of the nucleotides in cells [13]. Seven isoforms of the enzyme 5′-Nucleotidases have been isolated and characterized, and have a different nomenclature depending on the subcellular localization. Five isoforms are cytosolic, one is located in the mitochondrial matrix and one is related to the outer plasma membrane.

The first cytosolic isoform (cN-I) is particularly expressed in the skeletal and heart muscles [14]. cN-I has been isolated and characterized from the heart of many animals including the man. The activity of this cytosolic isoform is greatly affected by pH, which, in different species, should be between 6.5 and 7.0 and divalent cations Mg^{2+}, Mn^{2+} and Co^{2+} [15]. In humans two genes are noted *NT5C1A* and *NT5C1B* that encodes for its related products cN-IA and cN-IB. The *NT5C1A* gene is located on chromosome 1 and its related protein product, cN-IA, is described as an enzyme that prefers the AMP as a substrate. In humans, the mRNA of this isoform is particularly present in the skeletal muscle, but also in the heart, in the brain, in the pancreas, in the liver, in the testes and the uterus [16]. Human cN-IA has particular relevance in the protection of the heart; in fact, in normal conditions the formation of AMP is greater, while under conditions of ischemia or hypoxia it's adenine to be produced in large quantities [17]. The increased production of adenine, in this case, is the result of the inhibition of adenosine kinase activity and the increased activity of cN-IA [18]. From the structural point of view the enzyme cN-IA appears to be a tetramer. cN-IA prefers AMP as a substrate and the human form has a K_m between 1.46 and 1.9 mM [15]. The gene *NT5C1B* codes for cN-IB and is located on chromosome 2. From the functional point of view cN-IB differs little from cN-IA and its substrate of excellence is AMP. In humans cN-IB is ubiquitously expressed and mRNA expression is particularly high in the testis and lowest in the brain and skeletal muscle; cN-IB exists as a dimer [19].

The second cytosolic isoform (cN-II) was the first nucleotidase to be described and purified. Through its activities hydrolyzes preferably 5′-IMP, 5′-GMP, 5′-deoxy-IMP and 5′-deoxy-GMP by adjusting the cell concentration of IMP and GMP [20]. cN-II gene is located on chromosome 10 [21]. Its mRNA is expressed in an ubiquitously manner with a higher expression in the pancreas, in the skeletal muscle and heart [22]. From the structural point of view cN-II appears to be a tetramer capable of forming oligomers of higher molecular weight upon addition of ATP. The enzyme works optimally at a pH value of 6.5, but in the case that you verify a transfer reaction of the phosphate group, the optimum pH result appears to be 7.0. Moreover, its activity is dependent on the presence of the cation Mg^{2+}. When Mg^{2+} is replaced by Co^{2+} and Zn^{2+} cN-II activity is much lower [20,23]. A correlation has been shown between a high activity of this citosolic enzyme and development of neurological disorders [24].

The gene for the third 5′-Nucleotidase cytosolic (cN-III) is located on chromosome 7 and this isoform is the only one to be composed of a single monomer [25]. The cN-III catalyzes the dephosphorylation of nucleoside monophosphates pyrimidine nucleoside and has no activity on the purine substrates. The enzyme is expressed in many parts such as the human heart, the bone marrow, the liver, the testis, the colon, the stomach and the brain. The deficiency of this enzyme is associated with a form of hemolytic anemia. In particular homozygous patients with mutations in the gene for cN-III develop anemia and massive accumulation of cytidine and uridine phosphate that interfere with glycolysis erythrocyte [26]. In general, the mutations that affect this gene cause aberrant splicing and premature stop codons that abruptly interrupt the normal amino acid sequence of the relative protein [25]. Even this

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isoform, such as 5′-Nucleotidase cytosolic I and II, is linked for its activity to ion Mg^{2+} and its optimal pH is 7,5.

The gene for the fourth cytosolic isoform (cN-IV) is localized on chromosome 17 and its active enzyme is a dimeric deoxyribonucleotidase. The DNA sequence is formed by 5 exons and 4 introns [27].

The mitochondrial 5′(3′)-deoxyribonucleotidase (mdN) function is to protect the mitochondria from excessive dTTP levels. Its gene, as cN-IV, is located on chromosome 17 and consists of 5 exons and 4 introns, suggesting a common origin with an homology of 52%. Recombinant human and rat enzymes show a low activity with purine monophosphate and no activity with cytidine monophosphate. In addition to this mdN requires Mg^{2+} ion and an optimal pH of 5.0 to 5.5 [28].

Finally, we must consider the last isoform of nucleotidase cell surface-located (ecto-nucleotidases, eN). Principal functional role of ecto-nucleotidases is to hydrolyze ribo- and deoxyribonucleoside 5′-monophosphates including AMP, CMP, UMP, IMP, and GMP even if whereby AMP generally is the most effectively hydrolyzed nucleotide. In fact the production of extracellular adenosine from extracellular AMP is considered to be a major function of eN. Changes in expression of these enzyme are responsible for the availability of adenosine. Additional functions of eN are to maintain the exact amount of adenosine for cellular reuptake and purine salvage and ensure the major pathway for communication between cells [29].

3. Adenosine deaminase

Adenosine deaminase (ADA) is an important enzyme in the purine metabolism that catalyzes the deamination of both adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively, and ammonia. Adenosine is an endogenous purine nucleoside that acts as a homeostatic network regulator in all living systems via multiple AR-dependent and AR-independent (where AR is adenosine receptor) pathways. Within cells, adenosine is involved in cellular energy and purine metabolism, but it is also released to or produced in the extracellular medium where it binds to the cell membrane ARs and its action is determined by the receptor to which it binds. Adenosine plays an important role in different metabolic and pathological conditions, such as the intrarenal metabolic regulation of kidney function, in asthma and hypoxia, in cardiac ischemia, and in regulating the severity of inflammation during an immune response. There is general agreement that adenosine is an important neuromodulator in the central nervous system, playing a crucial role in neuronal excitability and synaptic/nonsynaptic transmission in the hippocampus and basal ganglia. Adenosine is also associated with Alzheimer's disease, Parkinson's disease, schizophrenia, Huntington's disease, epilepsy, drug addiction, and sleep [30].

ADA exists in all human tissues, but the highest levels and activity are found in the lymphoid system such as lymph nodes, spleen, and thymus. It is also essential for the proliferation, maturation and function of T lymphocyte cells. It is assumed that ADA plays a crucial role in development of the immune system, while its innate deficiency causes severe combined immunodeficiency (SCID). Moreover, ADA activity changes in a variety of other diseases including acquired immunodeficiency syndrome (AIDS), anemia, various lymphomas, tuberculosis, and leukemia. On the other hand, ADA regulates the levels of endogenous adenosine which results in immune system suppression by inhibiting lymphoid or myeloid cells, including neutrophils, macrophages, lymphocytes and platelets [31].

Adenosine deaminase is a zinc-containing $(\beta/\alpha)_8$-barrel enzyme. The $(\beta/\alpha)_8$ or triose phosphate isomerase (TIM) barrel, consisting of eight parallel central β-strands and eight peripheral α-helices, is one of the most commonly observed protein folds. In all $(\beta/\alpha)_8$ enzymes, despite the diversification in catalytic residues and substrate specificities, the active sites are funnel-shaped pockets formed by the C-terminal ends of the eight β strands and the βα loops that link β strands with the subsequent α-helices. In contrast, the loops, locating at the back side of the barrel and linking the α-helices with the subsequent β-strands, are believed to be involved in protein stability. The spatial separation of regions important for activity and for stability is thought to be important for enzymes, which allows conformational changes during the ligand/substrate binding, catalysis or the allostery regulation process and maintains the global stable native structure of a protein [32].

ADA can adopt two very different conformations: the closed and the open forms. In the absence of substrate, ADA adopts the open form. The closed form of the enzyme is usually observed in complexes with substrate analogs possessing the adenine framework, indicating that it is reached after substrate binding. The closed form consists of a hydrophobic subsite (F0) and a hydrophilic area (S0) perfectly enclosed within a structural gate consisting of the peptide backbone of a β-strand (L1822-D185) and two leucine side chains (Leu 58 and Leu 62) from an α-helix (T57-A73). When the structural gate opens, the active site turns into the open form that conserves the S0 and F0 subsites, and shows two additional hydrophobic subsites around the gate, defined as F1 and F2. The removal of a specific water molecule binding at the bottom of the active site might be a trigger of conformational change from the open to the closed form. Adenosine, or compounds that mimic substrate binding to the active site, interferes with the "trigger water" molecule that moves away. As a consequence, the transition to the closed form is promoted and the interaction between substrate and enzyme is increased [30]. There are two different types of ADAs: ADA1 and ADA2. Both of these enzymes have been found in humans along with a gene for ADA-like protein (ADAL or ADA3) with unknown function. Although ADA1 does not express any signal sequence normally required for protein secretion by cells, the enzyme has been found in extracellular fluids. ADA2 differs from ADA1 in terms of molecular weight and catalytic parameters. In humans, the ADA1 isoenzyme is encoded by the 32 kb ADA gene on chromosome 20q and occurs as a soluble 41-kDa monomer with 363 amino acids. ADA2 is encoded by the cat eye syndrome critical region gene 1 (CECR1), a gene located on chromosome 22, member of a novel family of ADGFs (ADA-related growth factors). ADA2 has a molecular mass of approximately 100 kDa. ADA2, an enzyme more abundant in plasma than in ADA1, has a Michaelis–Menten constant Km = 2 mM, which is several orders higher than the concentration of adenosine in plasma (0.1 μM), suggesting that the rate of adenosine deamination catalyzed by ADA2 is close to zero at physiological adenosine concentrations. Only certain cell types express ADA2 in humans, whereas ADA1 is present in all cells. The optimum pH for ADA2 activity is different from ADA1 (pH 6.5 and 7.5 for ADA2 and ADA1 respectively); ADA2 displays lower sensitivity to many specific inhibitors of ADA1 and is more stable at high temperatures than ADA1. This suggests that ADA2 expresses its activity only at high levels of adenosine and low pH, conditions that are associated with tumor growth, hypoxia, and inflammation. However, at physiological concentrations of adenosine, ADA2 has an extremely low ADA activity and thus, may function differently from its catalytic form. This additional ADA-independent activity may explain the existence of two ADAs with the same catalytic activities in humans. ADA2 is a symmetrical homodimer in contrast with the monomeric ADA1. Comparison of the catalytic sites of both isoenzymes revealed large differences in the arrangement of the binding pockets, which explains the differences in the affinity of these proteins to their substrates and inhibitors. Extensive glycosylation and the presence of a conserved disulfide bond and a signal peptide in the enzymatic molecule strongly suggest that ADA2, in contrast to ADA1, is specifically designed to act in the extracellular environment according to its presence in the serum [30,33].

ADA1 plays a metabolic role not only as a key cytosolic enzyme in the purine pathway, but also as an ecto-enzyme by regulating extracellular adenosine levels [30]. In fact, although the location of ADA is mainly cytosolic, it has been found on the cell surface of many cell types, including neurons; therefore it can be considered as an ecto-enzyme. Since ADA is a peripheral membrane protein it needs integral membrane proteins to be anchored to the membrane. Apart from A_1Rs and

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A_{2B}Rs, another class of ecto-ADA-binding protein is CD26, a multifunctional transmembrane glycoprotein, acting as a receptor and a proteolytic enzyme. It has been shown that ADA anchored to the dendritic cell surface, probably by the A_{2B}R, binds to CD26 expressed on the surface of T-cells, triggering co-stimulation and enabling an enhanced immune response [34].

As the most abundant type of white blood cells that responds to infection and attacks of foreign invaders, neutrophils might possess more than one type of adenosine receptor, and adenosine regulates neutrophil function in an opposing manner through the ligation of ADA1 (immunostimulatory) and ADA2 (immunosuppressive) receptors. A number of ADA inhibitors with various degrees of potency have been reported. In one study, immunosuppressive and anti-inflammatory effects of FR234938, as a non-nucleoside inhibitor of ADA, were investigated. Moreover, deoxycoformycin, another ADA inhibitor, has been investigated in treatment of colon carcinoma cells and hematological neoplasms. By contrast, ibuprofen and medazepam effects on immune deficiency have been reported. This revealed that purine compound may act as ADA activator [31]. Thus, ADA, being a single chain protein, performs more than one function, consistent with the definition of a moonlighting protein. Moonlighting means the performance of more than one function by a single protein. This phenomenon is becoming recognized as a common phenomenon with important implications for systems biology and human health. ADA can be considered a classical example of this particular family of multifunctional proteins being, independently, an enzyme that degrades adenosine, a costimulator promoting T-cell proliferation and differentiation mainly by interacting with the differentiation cluster CD26, and an allosteric modulator of ARs that are members of the GPCR family [30].

4. Xanthine oxidase

The physiological role of xanthine oxidoreductase enzyme (XOR) is to catalyze the terminal two reactions of purine catabolism in human. In particular XOR catalyses the oxidation from hypoxanthine to xanthine and from xanthine to uric acid, with the simultaneous reduction of NAD^+ or O_2. XOR is a housekeeping and the rate-limiting enzyme in purine catabolism. This enzyme exists in two forms: xanthine dehydrogenase (XDH), which prefers NAD^+ as electrons acceptor and xanthine oxidase (XO), which prefers O_2. When the oxygen is the final electron acceptor electrons bind unstably to oxygen forming hydrogen peroxide (H_2O_2) and superoxide anion ($O^{\bullet -}$). These reactive oxygen species (ROS) produced by XOR, are responsible for cytotoxicity in physiological and pathological conditions [35]. However it is important to understand that under certain conditions also XDH reduces O_2 to generate ROS. This happens when the NAD^+ levels are low. The XOR molecule belongs to the metallo flavoprotein family and is a homodimer of 145–150 kDa subunits in which each subunit corresponds to one catalytic center composed of three redox domains [36]. The first and largest domain contains the cofactor molybdenum, the intermediate domain contains flavin adenine dinucleotide (FAD) cofactor and the last and smallest domain contains the two iron sulfur centers [37]. In each reaction there occurs an oxidative hydroxylation with electrons transfer from molybdenum to other reaction centers. The molybdenum center is the site of purine oxidation while NAD^+ or O_2 reduction continues at the FAD. The two Fe/S clusters provide the conduit for electron flux between the Molybdenum center and the FAD. Fig. 2 shows a representative pattern of XDH and XOR. The human gene for XOR is located on the short arm of chromosome 2 and contains 36 exons [38]. Many

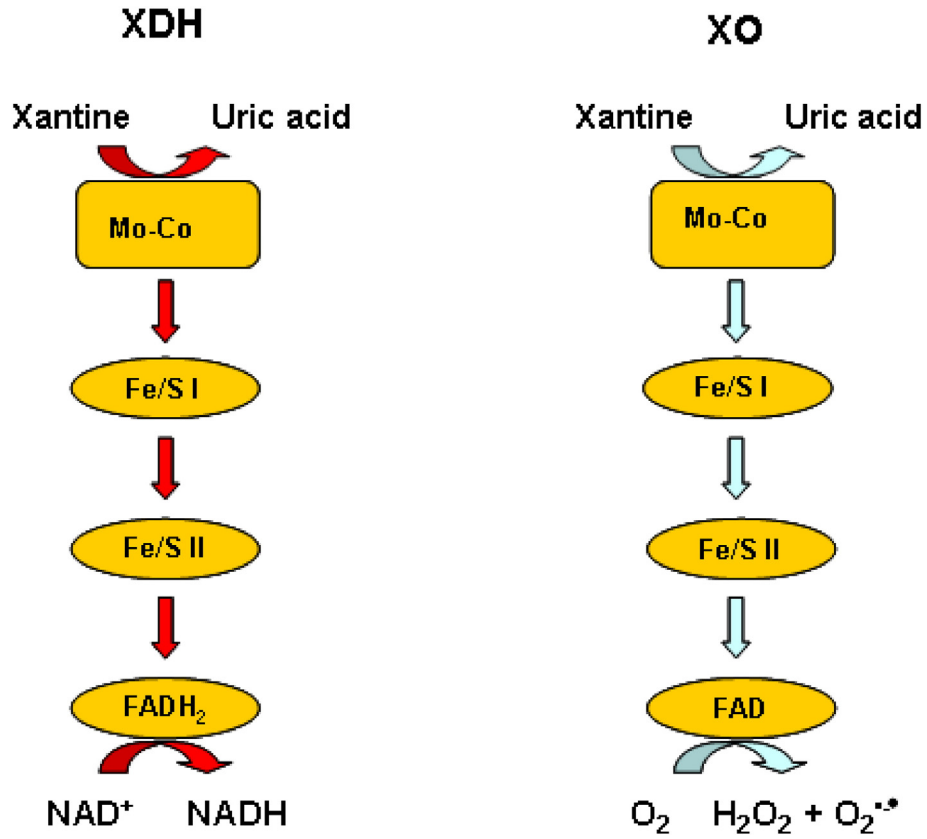

Fig. 2. In the left panel for XDH, xanthine is oxidized to uric acid and electrons transferred via Fe/S centers to the FAD where NAD^+ is reduced to NADH. In the right panel for XO, xanthine is oxidized to uric acid and electrons are transferred to the FAD where O_2 is reduced to $O^{\bullet -}$ and H_2O_2.
Modified from Ref. [35].

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studies have unequivocally demonstrated that its expression is strictly controlled by repressor proteins that limit XOR expression. The first regulation of XOR occurs at transcriptional level and its gene expression may be regulated by nutritional factors, steroid hormones, growth factors, cytokines, and insulin [39]. XOR expression decrease was observed after intake of tungsten which antagonizes molybdenum or with a diet poor in proteins. Otherwise a lack of vitamin E increased its protein expression [40]. There is also a post-transcriptional control of XOR activity consisting in a conversions between active and inactive forms [41]. In mammals XOR is distributed in some organs as liver, intestine and blood where its levels are very high and has been purified from cytosolic fractions, as peroxisomes and cellular membrane [42]. Based on protein expression, the highest activity levels of human XOR are present in the intestine and liver, while a very low activity has been detected in other human organs. Interestingly, human endothelial cells have been identified as having high levels in XOR activity. Recent studies have shown that XOR activity is undetectable in human serum, brain, heart and skeletal muscle tissues [43]. Inhibitors of XOR are used as anti-gout drugs. Allopurinol and other compounds can inhibit this enzyme because act as inhibitors that bind to the molybdenum center competitively respect to xanthine. For instance, patients with chronic heart failure and increased expression of XOR, are treated with allopurinol and this treatment led to improvement in myocardial efficiency [44]. However it's important to emphasize that the XOR-generated ROS are responsible for many biological activities including a defense against infection. XOR has antimicrobial properties inhibiting growth of bacteria in vitro and in vivo [45]. The bactericidal activity of XOR may be potentiated by the ability to produce peroxynitrite. When XOR activity produces superoxide radical, this reactive molecule can combine with nitric oxide (NO) and form peroxynitrite ($ONOO^-$), a potent non-radical oxidant species. NO is normally generated by nitric oxide synthase but XOR may also contribute to produce NO especially in hypoxic conditions. Nevertheless NO is involved also in nitration of tyrosine residues and the dysfunction of proteins due to nitration are connected to cardiovascular diseases, ischaemic injury, hypertension and heart failure. In addition to this was observed that myocardial contractile function decreases with increasing XOR activity and $ONOO^-$ generation [46]. In conclusion xanthine dehydrogenase/xanthine oxidase (XDH/XO) is responsible for the production of uric acid and ROS with pathophysiological consequences. The increase of XOR activity is connected with hypertension, dyslipidemia, diabetes, and atherosclerosis.

5. Conclusion

Elevated serum levels of uric acid has been shown to play an important role in many disease states including gout and articular degenerative disorders as well as vascular inflammation and atherosclerosis. The balance of uric acid formation and excretion is driven by several enzymatic pathways which occur via different genetically-defined isoforms being also highly regulated by pathophysiological determinants including metabolic products and free radical species. XOR represents the most relevant pathway involved in uric acid overproduction and offers significant perspectives for a better pharmacological approach for treating hyperuricemia-related vascular and non vascular disorders.

Conflict of interest

The authors declare that no conflict of interest occurs for this work.

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