Biolog. Journal of Armenia, 1 (69), 2017

BEHAVIOR OF TWO PURINE AND PYRIMIDINE METABOLISM RELATED KEY REGULATIVE ENZYMES IN NORMAL AND PATHOLOGICAL CONDITIONS

DANIELYAN K.E.[1*], SIMONYAN A.A. [1], CHAILYAN S.G. [1]

H. Buniatian Institute of Biochemistry, NASRA, 5/1 ParuyrSevak Street, Yerevan 0014, Armenia
Kristine_danielyan@biochem.sci.am

Phosphoribosyl pyrophosphate synthetase-1 (PRPS-1; EC 2.7.6.1) is widely distributed in the human tissues and serves as a regulative key enzyme for purine and pyrimidine metabolism, whereas Xanthine Oxidoreductase (XOR; EC 1.1.3.22) is the enzyme responsible for the catabolism of purine nucleotides. These enzymes might serve as the aimmolecules for triggering of regenerative processes after stroke. In case of inhibition of PRPS-1 and activation of XOR might serve as the targets for prevention of the cancer development. It was recently published in PLOS (Donini S, Garavaglia S, 2017), regulation of these enzymes prevents bioactivity of the pathogenic microorganisms. Thus, investigations of these enzymes' activities in normal and pathological conditions might be vital for the development of new treatment avenues.

Phosphoribosyl pyrophosphate Synthetase, Xanthine Oxidoreductase, experimental stroke

Фосфорибозил пирофосфатсинтаза (ФРПС-1; EC 2.7.6.1) широко распространена в тканях организма человека и служит в роли регуляторного фермента при метаболизме пуринов и пиримидинов, в то время как ксантин оксидоредуктаза (КОР; EC 1.1.3.22) является ферментом, ответственным за катаболизм пуринов. Эти ферменты могут выступать в роли основных инициирующих регенеративные процессы белков после инсульта. В случае ингибирования (ФРПС-1) и активации (КОР) могут служить в роли мишеней для предотвращения развития раковой опухоли. Недавно было опубликовано в PLOS статья о том (Donini S, Garavaglia S, 2017), что регуляция этих ферментов подавляет биоактивность патогенных микроорганизмов. Таким образом, исследование активности этих ферментов при нормальных и патологиеских условиях может стать жизненно важным для разработок новых путей лечений.

Ֆոսֆորիբոզիլ-պիրոֆոսֆատսինթազը (ՖՌՊՍ-1; EC 2.7.6.1) լայն տարածում ունի մարդու հյուսվածքներում և ծառայում է որպես կարգավորող ֆերմենտ պուրինների և պիրիմիդինների մետաբոլիզմի համար, այն դեպքում, երբ քսանտին-օքսիդոռեդուկտազը (ՔՕՌ; EC 1.1.3.22) պատասխանատու է պուրինների կատաբոլիզմի համար: Այս ֆերմենտները կարող են հանդես գալ որպես հիմնական ռեգեներատիվ գործընթացները խթանող սպիտակուցներ ինսուլտից հետո: Կարող են հանդես գալ որպես քաղցկեղների զարգացմանը խոչընդոտող թիրախներ, եթե ճնշել մեկի ակտիվությունը (ՖՌՊՍ-1) և խթանել մյուսինը (ՔՕՌ): Վերջերս PLOS-ում (Donini S, Garavaglia S, 2017) տպագրվել է այն միտքը, որ այս ֆերմենտների ակտիվության կարգավորմամբ կարելի է կանխարգելել ախտածին միկրոօրգանիզմների կենսունակությունը: Այսպիսով, այս ֆերմենտների ակտիվության ուսումնասիրությունը ֆիզիոլոգիական և ախտաբանական պայմաններում կարող են դառնալ կենսունակ կարևորություն ունեցող ուսումնասիրություններ նոր բուժման ճանապարհների փնտրտուկում:

We will be discussing two enzymes: one of them is responsible for the synthesis of purine as well as pyrimidine, the other is respomsible foe purine catabolism.

Phosphoribosylpyrophosphate synthetase-1 (PRPS-1; EC 2.7.6.1) catalyzes the phosphoribosylation of ribose 5-phosphate to 5-phosphoribosyl-1-pyrophosphate, which is necessary for the salvage pathways of purine and pyrimidine, pyridine nucleotide cofactors NAD and NADP, the amino acids histidine and tryptophan biosynthesis. Three PRPS genes have been identified: the widely expressed are PRPS1 and PRPS2 genes, and PRPS3, which is predominantly transcribed only in testis.

Most PRSPs belong to class I, which require Mg^{2+} and phosphate for enzymatic activity, but can be inhibited allosterically by ADP and possibly other nucleotides.

Class II PRSs are found specifically in plants which are not dependent on phosphate for activity and lack an allosteric site for ADP

While class I PRSs transfer the diphosphoryl group only from ATP or dATP to ribose 5-phosphate, class II PRSPs have a much broader specificity for a diphosphoryl donor, including ATP, dATP, UTP, CTP and GTP . Recently, a novel class III PRS has been identified from *Methanocaldococcusjannaschii* which is activated by phosphate and uses ATP and dATP as a diphosphoryl donor, but also lacks an allosteric site for ADP .

Mg^{2+} forms a complex with ATP (Mg–ATP) to act as the actual substrate of the enzyme although other divalent cations, such as Mn^{2+}, Ni^{2+}, Co^{2+} or Cd^{2+} can serve as substitutes for Mg^{2+} with relatively lower activity . Phosphate has multiple effects on the activity and structure of the enzyme. It usually acts as an activator for the activity of bacterial and mammalian PRSPs although SO_4^{2-} can mimic the effect of phosphate at approx. 10-fold higher concentrations .

In patients with PRPS super activity, Roessler et al. , and Becker et al. identified mutations in the PRPS-1 gene. All patients except 1 had hyperuricemia, neurodevelop-mental abnormalities, and sensorineural deafness; the other patient had only hyperuricemia and gout. Functional expression studies of all mutations showed that enzyme over activity were due to alteration of allosteric feedback mechanisms.

Thus, abnormalities in synthesis of purines might influence on the catabolism of them and induce hyperuricemia and gout. The final enzyme, responsible for the formation of uric acid is Xanthine Oxidoreductase.

Also, it is necessarily to mention, that very little is known about the PRPS-1 in the stroke or experimental stroke conditions.

Xanthine oxidoredictase (XOR; EC. 1.1.3.22) is key and primer enzyme responsible for the formation of the uric acid from xanthine and hypoxanthine.

In our previous publications we stated that regulation of XOR might have an impact on the entire catabolism of the purines. By the utility of XOR inhibitors we demonstrated in our experiments that inhibition of this enzyme might trigger cells genesis in vitro.

XOR and brain ischemia. It is very well documented that after forebrain ischemia/reperfusion it might be generated excessive amount of superoxide anion radicals. Evidence exists that allopurinol, a xanthine-oxidase inhibitor, reduces delayed cell death in animal models of perinatal asphyxia and in human patients with other forms of organ reperfusion injury. Thus, allopurinol pretreatment suppresses generation of superoxide anion radicals, making XO (xanthine oxidase) the main enzyme responsible for the oxidative damage caused after oxidative stress, early inflammation, endothelial injury. By the other group of authors it was demonstrated that XO and cyclooxygenase are mostly responsible for postanoxic damage of the brain. Also, it was demonstrated that hydrogen peroxide damage is mediated through the activity of the XO in cerebellar granule neurons obtained from 8-day old Sprague-Dawley rat pups. By the other work it was shown that superoxide anion generated by the XO as well as singlet oxygen initiated the apoptosis-like cell death whereas hydrogen peroxide, generated because of the activity of the glucose oxidase and glucose deprivation in neuronal cell culture initiated necrosis.

PRPS-1 and XOR after experimental stroke conditions, induced by intracranial injection of hydrogen peroxide.

In accordance to the literature data, allopurinol inhibits the enzyme XOR, thereby blocking the conversion of the oxypurines hypoxanthine and xanthine to uric acid. Allopurinol administration also leads to deceleration of the rate of de novo synthesis of purine nucleotides.

In our own experiments to clarify the behavior of the XOR and PRPS-1 enzymes in the pathological conditions, first, we needed to evaluate their activities in the brain of intact animals.

The activity of the PRPS-1 was diminished in the presence of GTP (inhibitor of PRPS-1) as well as allopurinol, which means that purines catabolism inhibition by feed-back mechanism might inhibit also the activity of PRPS-1.

Interestingly, XOR activity was inhibited by GTP, which might be explained or as the influence of key regulative enzyme PRPS-1 impact on XOR activity or overwhelming concentration of energetic source – GTP.

After comparison of the dynamic changes of XOR and PRPS-1 activities in 3 groups: peroxide intracranial injected animals serving as the control group; phosphate treated animals; allopurinol treated animals in peroxide treated as well as phosphate groups it is notable negative correlation of XOR and PRPS-1activities. Strong, over activation of the PRPS-1 and simultaneous inhibition of XOR might trigger of purine and pyrimidine nucleotides source generation, which in turn might stimulate cells proliferation.

Also, its necessarily to take into the consideration, that patients with hypoxanthine–guanine phosphoribosyl transferase (HGPRT) deficiency or Lesch-Nyhan disease (MIM 300322) having uric acid overproduction similar to PRPS-1super activity can also have mental retardation and hypotonia, as described in patients with Arts syndrome .

Thus, the phosphate related elevation of PRPS-1 activity and simultaneous decrease of XOR activity will promote synthesis of purines and will prevent above mentioned negative side effects related with overproduction of uric acid.

In allopurinol treated animals group the negative correlation was not notable.

In contrast to the intact animals brain tissue, GTP didn’t activate XOR in pathological condition; exception is the allopurinol treated animals group. Allopurinol after modifications in the organism might by itself possess with the high absorption in the conditions of very low baseline XOR activity.

It might be explained due to the formation of Xanthine Oxidase from Xanthine Dehydogenase via limited proteolysis, because XOR is the dual enzyme and in pathological conditions act mostly as the oxidase.

Allopurinol in hydrogen peroxide treated animals brain tissue was inhibiting activity of PRPS-1; exception is the allopurinol treated animals group. In pathological conditions, we might assume, that PRPS-1 might be structurally changed and behaves not as in normal, physiological conditions.

References

1. *Arnvig, K., B. Hove-Jensen and R. L. Switzer* (1990). "Purification and properties of phosphoribosyl diphosphate synthetase from Bacillus subtilis." Eur. J. Biochem.192: 195-200.
2. *Becker, M. A.* (2001). Hyperuricemia and Gout. In: Scriver, C. R.; Beaudet, A. L.; Sly, W. S.; Valle, D. (eds.) :The Metabolic and Molecular Bases of Inherited Disease. New York: McGraw-Hill.
3. *Becker, M. A., P. R. Smith, W. Taylor, R. Mustafi and R. L. Switzer* (1995). "The genetic and functional basis of purine nucleotide feedback-resistant phosphoribosyl pyrophosphate synthetase superactivity." J. Clin. Invest.96: 2133-2141.
4. *Danielyan, K. E.* (2013). "Dependence of Cells Survival from Xanthine Oxidase and Dihydopyrimidine Dehydrogenase Correlative Activities in Human Brain Derived Cell Culture." Central Nervous System Agents in Medicinal Chemistry13(2): 108-113.
5. *Danielyan, K. E.* (2014). "New details regarding XOR as a part of the balanced oxidative and antioxidative systems." Biological Journal of ArmeniaLXVI (1): 30-43.

6. *Danielyan, K. E. and G. A. Kevorkian* "Xanthine oxidase activity regulates human embryonic brain cells growth." Biopolym. Cell27(5): 350-353.
7. *de Brouwer, A. P., H. van Bokhoven, S. B. Nabuurs, W. F. Arts, J. Christodoulou and J. Duley* (2010). "PRPS1 mutations: four distinct syndromes and potential treatment." Am J Hum Genet86(4): 506-518.
8. *Eriksen, T. A., A. Kadziola, A. K. Bentsen, K. W. Harlow and S. Larsen* (2000). "Structural basis for the function of Bacillus subtilis phosphoribosyl-pyrophosphate synthetase." Nat. Struct. Biol. 7: 303-308.
9. *Fatokun, A. A., T. W. Stone and R. A. Smith* (2007). "Hydrogen peroxide mediates damage by xanthine and xanthine oxidase in cerebellar granule neuronal cultures." Neurosci Lett416(1): 34-38.
10. *Fox, I. H. and W. N. Kelley* (1971). " Human phosphoribosylpyrophosphate synthetase. Distribution, purification, and properties." J. Biol. Chem. 246: 5739-5748.
11. *Gibson, K. J., K. R. Schubert and R. L. Switzer* (1982). "Binding of the substrates and the allosteric inhibitor adenosine 5′-diphosphate to phosphoribosyl pyrophosphate synthetase from Salmonella typhimurium." J. Biol. Chem. 257: 2391-2396.
12. *Gyongyan, S. A., T. G. Manucharyan, K. E. Danielyan, G. A. Kevorkyan and S. G. Chailyan* (2013). "Xanthine oxidoreductase is a key enzyme of purine catabolism regulation." Electronic Journal of Natiral Science2(21): 17-21.
13. *Hove-Jensen, B.* (1988). "Mutation in the phosphoribosylpyrophosphate synthetase gene (prs) that results in simultaneous requirements for purine and pyrimidine nucleosides, nicotinamide nucleotide, histidine and tryptophan in Escherichia coli." J. Bacteriol.170: 1148-1152.
14. *Hove-Jensen, B.* (1989). "Phosphoribosylpyrophosphate (PRPP)-less mutants of Escherichia coli." Mol. Microbiol.3: 1487-1492.
15. *Hove-Jensen, B., K. W. Harlow, C. J. King and R. L. Switzer* (1986). "Phosphoribosylpyrophosphate synthetase of Escherichia coli. Properties of the purified enzyme and primary structure of the prs gene." J. Biol. Chem.261: 6765-6771.
16. *Kadziola, A., C. H. Jepsen, E. Johansson, J. McGuire, S. Larsen and B. Hove-Jensen* (2005). "Novel class III phosphoribosyl diphosphate synthase structure and properties of the tetrameric, phosphate-activated, non-allosterically inhibited enzyme from Methanocaldococcus jannaschii." J. Mol. Biol.354: 815-828.
17. *Krath, B. N., T. A. Eriksen, T. S. Poulsen and B. Hove-Jensen* (1999). "Cloning and sequencing of cDNAs specifying a novel class of phosphoribosyl diphosphate synthase in Arabidopsis thaliana." Biochim. Biophys. Acta. : 1430:1403-1408.
18. *Krath, B. N. and B. Hove-Jensen* (2001). "Class II recombinant phosphoribosyl diphosphate synthase from spinach. Phosphate independence and diphosphoryl donor specificity." J. Biol. Chem. 276: 17851-17856.
19. *Liu, X. Z., D. Xie, H. J. Yuan, A. P. de Brouwe, J. Christodoulou and D. Yan* (2013). "Hearing loss and PRPS1 mutations: Wide spectrum of phenotypes and potential therapy." Int J Audiol. 52(1): 23-28.
20. *Meyer, L. J. and M. A. Becker* (1977). "Human erythrocyte phosphoribosylpyrophosphate synthetase. Dependence of activity on state of subunit association." J. Biol. Chem.252: 3919-3925.
21. *Mink, R. B., A. J. Dutka and J. M. Hallenbeck* (1991). "Allopurinol pretreatment improves evoked response recovery following global cerebral ischemia in dogs." Stroke22: 660-665.
22. *Ono, T., R. Tsuruta, M. Fujita, H. S. Aki, S. Kutsuna, Y. Kawamura, J. Wakatsuki, T. Aoki, C. Kobayashi, S. Kasaoka, I. Maruyama, M. Yuasa and T. Maekawa* (2009). "Xanthine oxidase is one of the major sources of superoxide anion radicals in blood after reperfusion in rats with forebrain ischemia/reperfusion." Brain Res1305: 158-167.
23. *Roessler, B. J., J. M. Nosal, P. R. Smith, S. A. Heidler, T. D. Palella, R. L. Switzer and M. A. Becker* (1993). " Human X-linked phosphoribosyl pyrophosphate synthetase superactivity is associated with distinct point mutations in the PRPS1 gene." J. Biol. Chem. 268: 26476-26481.
24. *Roessler, B. J., T. D. Palella, S. Heidler and M. A. Becker* (1991). "Identification of distinct PRPS1 mutations in two patients with X-linked phosphoribosyl pyrophosphate synthetase superactivity. (Abstract)." Clin. Res. 39: 267A.

25. *Roth, D. G. and T. F. Deuel* (1974). "Stability and regulation of phosphoribosyl pyrophosphate synthetase from rat liver." J. Biol. Chem.249: 297-301.
26. *Roth, D. G., E. Shelton and T. F. Deuel* (1974). "Purification and properties of phosphoribosyl pyrophosphate synthetase from rat liver." J. Biol. Chem.249: 291-296.
27. *Switzer, R. L.* (1969). "Regulation and mechanism of phosphoribosyl pyrophosphate synthetase. I. Purification and properties of the enzyme from Salmonella typhimurium." J. Biol. Chem.244: 2854-2863.
28. *Switzer, R. L. and D. C. Sogin* (1973). "Regulation and mechanism of phosphoribosyl pyrophosphate synthetase. V. Inhibition by end products and regulation by adenosine diphosphate." J. Biol. Chem.248: 1063-1073.
29. *Valencia, A. and J. Morán* (2004). "Reactive oxygen species induce different cell death mechanisms in cultured neurons." Free Radic Biol Med36(9): 1112-1125.
30. *Widmer, R., M. Engels, P. Voss and T. Grune* (2007). "Postanoxic damage of microglial cells is mediated by xanthine oxidase and cyclooxygenase." Free Radic Res41(2): 145-152.
31. *Willemoes, M. and B. Hove-Jensen* (1997). "Binding of divalent magnesium by Escherichia coli phosphoribosyl diphosphate synthetase." Biochemistry36: 5078-5083.

Biolog. Journal of Armenia, 1 (69), 2017

INSIGHTS ON GLUCOCORTICOID RECEPTOR MODULATION THROUGH BINDING OF ARTEMISININ

S.V. GINOSYAN[1], H.V. GRABSKI[1], S.G. TIRATSUYAN[2,1]

[1]Russian-Armenian University, [2]Yerevan State University
E-mail: siranush.ginosian.93@mail.ru; hovakim_grabski@outlook.com; stiratsuyan@bk.ru;

In this work *in silico* study of the interaction of artemisinin with ligand bind domain of glucocorticoid receptor by molecular modeling methods. Artemisinins belong to the family of sesquiterpene lactones, secondary metabolites of medicinal plant Artemisia annua, which has been traditionally used in Chinese medicine. Artemisinins exhibit antioxidant, anti-inflammatory, anti-carcinogenic and other activities. Molecular docking, principal component analysis, cluster analysis have revealed three binding sites of artemisinin with ligand bind domain of glucocorticoid receptor which are very important regions. Comparative analysis was performed with dexamethasone, which is a corticosteroid medication. Thus, we have shown for the first time that artemisinin affects extremely important sites of ligand bind domain of glucocorticoid receptor, it should be noted that the first binding site of artemisinin corresponds to the interaction site of dexamethasone. This may represent a molecular basis for ligand-dependent receptor activation and the possibility of using artemisinin as a new ligand for glucocorticoid receptor.

Glucorticoid receptor – artemisinin – dexamethasone - molecular docking – cluster analysis

В данной работе изучено insilico взаимодействие артемизинина с лиганд связывающим доменом глюкокортикоидного рецептора методами молекулярного моделирования. Артемизинины относятся к семейству сесквитерпеновых лактонов, вторичных метаболитов лекарственного растения Artemisiaannua, которое традиционно используется в китайской медицине. Артемизинины проявляют антиоксидантную, противовоспалительную, антиканцерогенную и др. активности. Методами молекулярного докинга, анализа по главным компонентам и кластерного анализа выявлено три сайта связывания артемизинина с лиганд связывающим доменом глюкокортикоидного рецептора, которые являются чрезвычайно важными участками. Проведен сравнительный анализ с кортикостероидным препаратом дексаметазоном. Нами впервые показано, что артемизинин влияет на чрезвычайно важные участки лиганд связывающим доменом глюкокортикоидного рецептора, при этом первый сайт связывания артемизинина соответствует сайту взаимодействия дексаметазона. Это может представлять молекуляр-