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NEW DETAILS REGARDING XOR AS A PART OF THE BALANCED OXIDATIVE AND ANTIOXIDATIVE SYSTEMS

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Running title. Pyridoxine as an inhibitor for XOR.

The article is devoted to the analysis of the known oxidative and antioxidative systems of the cells with the special emphasize on the role of Xanthine Oxidoreductase (XOR) in these processes. Previous our experiments proved that XOR might have an undoubtable impact on the processes of cells development and proliferation as the enzyme responsible for the formation of reactive oxygen spices (ROS) as well as the final enzymes in the chain of purine catabolism, regulating it by the feed-back mechanism. Also, XOR and generated by its ROS influence on the formation of hydrogen peroxide of mitochondria and might be inhibited not only by the well-known synthetic compound - allopurinol but also might express favor to pyridoxine, which is the subcomponents of vitamin B complex.

Xanthine Oxidoreductase - purine - catabolism - reactive oxygen spices - pyridoxine - allopurinol

Հոդվածը նվիրված է հայտնի օքսիդատիվ և հակաօքսիդանտային համակարգերին: Հատուկ ուշադրություն է հատկացված քսանտինօքսիդոռեդուկտազի (ՔՕՌ) դերի պարզաբանմանը այդ գործընթացում: Մեր անցյալ աշխատանքներում մենք ցույց ենք տվել, որ ՔՕՌ անկասկած ունի մեծ ազդեցություն բջջային զարգացման և պրոլիֆերացիայի վրա որպես պուրինային կատաբոլիզմի վերջնական ֆերմենտ, որը կարգավորում է այդ գործընթացը հակառակ կապի հիմունքով: Բացի այդ, ՔՕՌ և սինթեզված այս ֆերմենտի կողմից ազատ ռադիկալները կարող են ազդել ջրածնի պերօքսիդի գոյացման վրա միտոքոնդրիումներում: Այս գործընթացը կարող է արգելակվել ոչ միայն սինթետիկ միացության՝ ալոպուրինոլի օգնությամբ, բայց նաև պիրիդոքսինի կիրառմամբ, որը հանդիսանում է վիտամինային B կոմպլեքսի բաղադրամաս:

Քսանտինօքսիդոռեդուկտազ - պուրին - կատաբոլիզմ - պիրիդոքսին - ալոպուրինոլ

Статья посвящена анализу известных оксидативных и антиоксидантных систем. Особое внимание уделено рассмотрению роли КОР (ксантиноксидоредуктазы) в этих процессах. В предыдущих наших работах мы показали, что КОР имеет несомненное влияние на процессы развития и пролиферации клеток, как конечный фермент пуринового катаболизма, регулирующий его по принципу обратной регуляции. Более

того, КОР и генерированные им свободные радикалы могут влиять на формирование пероксида водорода в митохондриях. Этот процесс может быть не только ингибирован синтетическим соединением – аллопуринолом, но может быть подавлен пиридоксином, который является субкомпонентом витаминного В комплекса.

Ксантиноксидоредуктазы - пурин - катаболизм - пиридоксин- аллопуринол

Description of free radical producing systems in the organism

ROS (reactive oxygen species) generation is the vitally important process. However, oxidative stress is determined as a misbalance between the formation of ROS and the utilization of the last one. Generation of ROS in the normally functioning cells occurs in the organelles, as it was suggested previously, and transfers into the cytoplasm. Overwhelming amount of ROS might trigger the non-reversible cell death.

It is supposed that mitochondria are the major reservoirs for ROS generation in most mammalian cells.

The respiratory chain is mainly localized in the inner membrane of the mitochondria and it is proved that the complexes I and III are the main responsible components of the chain for the production of the free radicals [1-4].

It is necessarily to mention, that 25% of free radicals formation occurs because of the protein folding in the ER [5]. Protein disulfide isomerase (PDI) and ER oxidoreductin 1 (ERO1) are two major enzymes responsible for oxidative protein folding in ER as well as for the catalyzing disulfide bond formation, isomerization, and reduction. In the process of oxidative protein folding, PDI receives electrons through catalyzing disulfide bond formation, and is converted to the reduced form, which then transfers electrons to ERO1 to recycle itself [6]. The synthesis of the ROS by PDI/ERO1 is regulated process, which might be controlled by the supply of the flavin adenine nucleotide for ERO1 [7]. Because the folding and misfolding process of the protein is the highly energy dependant, thus, the depletion of the ATP might trigger the formation of ATP in mitochondria and, consequently, the formation of ROS [8].

The leakage of Ca^{2+} ions into the cytoplasm also might triggers the production of ROS in mitochondria [8,9].

NADPH oxidases (NOXs) are the enzymes, functioning of which might be the key regulators in the pathological processes such as ischemia-reperfusion, diabetes, neurodegenerative diseases and atherosclerosis, as well as vessels related other diseases [10-13]. The basic catalytic subunit of NOXs contains a C terminal dehydrogenase domain featuring a binding site for NADPH and a bound FAD, as well as an N-terminal domain consisting of six transmembrane alpha helices that bind two heme groups. Once activated, cytosolic NADPH transfers its electrons to FAD, which in turn passes electrons sequentially to the two hemes and ultimately to molecular O_2, forming superoxide anion (O^{2-}) [14, 15]. Oxidants from various sources may affect the translocation of important regulatory subunits of NOX2 (and NOX1), p47phox, by oxidation of thiol groups in PKC with subsequent phosphorylation and translocation of these subunits to the membrane causing activation and superoxide formation by NOX2 (and NOX1). Likewise, those oxidants cause oxidation of thiols in protein disulfide isomerase (PDIox) leading to association with p47phox (maybe also NOXO1 or NOXA1), translocation of this dimer to the membrane, and activation of NOX2 (and NOX1).

Endothelial nitric oxide synthase (eNOS) redox switches might be based on S-glutathionylation, PKC- and protein tyrosine kinase-2 (PYK-2)–dependent phosphorylation, oxidative BH4 depletion, disruption of the zinc-sulfur cluster, as well as asymmetric dimethylarginine (ADMA) synthesis/degradation, all of which contribute to the regulation of its enzymatic activity. GSH, glutathione; GSSG, glutathione disulfide [16].

Since AT-II-induced oxidative stress is largely due to activation of mtROS formation [17-19], the conversion of XDH to XO under chronic AT-II treatment or increased levels of this vasoconstrictor (e.g., in diabetes or hypertension) could be mainly driven by mtROS formation. A direct proof for the interaction between mt ROS and XO activity was based on the improvement of cardiac complications and XO activation in a model of heart failure by therapy with the mitochondria-targeted antioxidant mitoquinone [20, 21].

Hemoglobin and myoglobins might serve as a source for the formation of free radicals. Reduction of nitrite to ·NO under hypoxic conditions serving as a putative autoregulatory mechanism for capillaries and muscle [22]. Nitric oxide synthases are also might serve as a source of the ·NO [22].

The other enzyme, which will be highlighted in the frames of this paper is Xanthine Oxidoreductase (XOR). Under normal circumstances, most amount of this enzyme exists in the form of NAD-dependent cytosolic dehydrogenase (XDH).

General description of XO enzy.

Xanthine Oxidase (XO) as well as the XDH are two enzymes responsible for the last steps of purines metabolism, hydroxylation of a wide variety pyrimidine, pterin, and aldehyde substrate. XOR enzymes have been isolated from a wide range of organisms, from bacteria to man. All of these proteins have similar molecular weights and composition of redox centers [23, 24]. The mammalian enzymes, which catalyze the hydroxylation of hypoxanthine and xanthine, the last two steps in the formation of urate, are synthesized as the dehydrogenase form. XDH exist mostly as such in the cell but can be readily converted to the oxidase form XO by oxidation of sulfhydryl residues or by proteolysis. XDH shows a preference for NADH reduction at the flavin adenine dinucleotide (FAD) reaction site, whereas XO fails to react with NADH and exclusively uses dioxygen as its substrate, leading to the formation of superoxide anion and hydrogen peroxide [24]. The active form of the enzyme is that of a homodimer of molecular mass 290 kDa, with each of the monomers acting independently in catalysis. Each subunit contains one molybdopterin cofactor, two spectroscopically distinct [2Fe-2S] centers and one FAD cofactor. The oxidation of xanthine takes place at the molybdopterin center (Mo-pt) and the electrons thus introduced are rapidly distributed to the other centers by intramolecular electron transfer [25]. The full amino acid sequences of XOR enzymes from various sources have been deduced by sequencing of the respective cDNAs or genes. They all consist of approximately 1,330 amino acids and are highly homologous with, e.g., the bovine milk enzyme (1,332 residues) showing 90% sequence identity to the human liver enzyme (1,333 residues) [26]. Also, it is necesserely to mention that that main cellular localization of XOR is cytoplasm.

XO regulates purine catabolism by feedback mechanism.

There are numerous publications evidencing about the primer, regulating role of the hypoxanthine/xanthine existence and its catabolism in the row of the purine metabolic pathway [27-30].

For instance, Edwards NL et. al have performed the small clinical trial with the infusion of the radiolabeld [8-(14)C] adenine to four patients with gout as well as to the patients suffering from Lesch-Nyhan syndrome. Five days after infusion it became clear that the mean cumulative excretion of radioactivity after adenine administration to

patients not receiving and receiving (off and on) allopurinol therapy was 6.1% and 3.6% of infused radioactivity for gouty subjects and 15.9% and 20.8% for the Lesch-Nyhan patients.

By our own study with the utility of the different initial, not primer substrates for the XO we have noticed increase in the formation of the XO product – uric acid in vitro conditions (**figure 1**) [31].

Taking into the consideration, that the utility of allopurinol allows significantly to reduce activity of XOR, we have proved that XOR is the key regulative enzyme in the purine catabolism pathway. In our previous studies, we have demonstrated that inhibition of XOR activity with allopurinol might stimulate the in vitro process of cells proliferation [13], whereas the inhibition of dihydropyrimidine dehydrogenase might prevent brain derived cells from death [13]. Here, we have proved and presented the fundamental mechanism of such phenomenon, which might be utilized for regenerative processes stimulation or for the prevention of cells proliferation in the settings of cancer development.

Also, to prove whether the pure enzyme is able to react with the non-primer substrates, in this particular work we have checked the activity of purified XOR in the presence of histidine, one of the substrates, which was stimulating the activity of the enzyme very strongly.

We have noticed 10 times elevation of XOR activity in statistically significant way (control - 1.63±0.20, xanthine - 2.16±0.11, allopurinol - 1.81±0.05, $p<0.05$). In the presence of histidine we haven't noticed any activity (histidine - 1.77±0.30, allopurinol - 1.15±0.20, **figure 2**).

Influence of the generated in the cytoplasm free radicals on the activity of mitochondria

Mitochondrial dysfunctions [e.g., by nitroglycerin metabolism, MnSOD deficiency, hypoxia, and electron transport chain (ETC) inhibitors] triggers mitochondrial permeability transition pore (mPTP) opening and PKC-NADPH oxidase activation [20].

In our own work we have shown that activity of XO and ROS, generated by this enzyme might influence on the quantity of hydrogen peroxide in mitochondria (**figure 3**). Taking into the consideration, that pentylenetetrazole is able to induced epileptic seizure, which might be determined mostly by deregulation of the mitochondrial processes as well as based on the fact, evidencing about the absence of XO in the mitochondrial fractions of the cells, we have concluded from performed our experiments, that allopurinol is able to influence on the activity of the XO, localized in the cytoplasm and diminish the hydrogen peroxide formation in the mitochondria.

Thus, if the above mentioned authors have shown data regarding the influence of mitochondrial functioning on the activity of the enzymes localized in the cytoplasm, we came to the contra verse conclusion.

Antioxidant systems.

In the conditions of the oxidative stress the organism over the evolution developed protective mechanism, which include the regulation on the gene expression and biochemical levels as the first step of triggering mechanism of defense. As soon as ROS production is enhanced 3 main components are activating, which are Kelch-like ECH-associated protein 1 (Keap1), nuclear factor erythroid 2-related factor 2 (Nrf2), and antioxidant response elements (ARE). The binding of Nrf2 to the DNA sequences present in ARE induce transcription of cytoprotective, antioxidant genes including SOD, GPx, CAT, gamma-GCS, GST. In addition, several other cytoprotective genes (heme oxygenase, NQO-1) are induced. Under normal conditions (reduced intracellular conditions), Nrf2 is stabilized through binding to Keap-1 in the cytoplasm.

During enhanced ROS formation or exposure to electrophilic metabolites, the cysteine residues in Keap-1 are modified or oxidized causing the dissociation of Nrf2 and translocation to the nucleus and binding to the ARE. Depending upon the binding site present on the promoter region, the different antioxidant genes are induced [32].

Along with the above-mentioned proteins, the other family of the enzymes is responsible for the detoxification of the organism.

These antioxidant enzymes are subject to posttranslational modifications that temporally control their H_2O_2-degrading activity to represent a mechanism to govern transient changes in local H_2O_2 levels, important for redox signaling in response to specific stimuli or conditions.

A redox-signaling role for antioxidant enzymes is exemplified by Peroxiredoxins (Prxs). Prxs show high reactivity for H_2O_2 when compared with other thiol oxidants and exhibit a rate constant ($1.3 \cdot 10^7$ M/sec) sufficiently high to outcompete catalase and Gpx for H_2O_2. During the catalytic reduction of H_2O_2, the active-site cysteine of Prxs occasionally interacts with two molecules of H_2O_2, resulting in hyperoxidation or sulfinylation ($-SO_2$) and transient inactivation of Prxs, thereby enabling H_2O_2 to target cysteines of local signaling proteins [33]. Transient recruitment of Prx2 to a growth-factor receptor provides both temporal and spatial control of local H_2O_2 concentrations at the focal point of signaling. A role of mitochondrial Prx3 for spatial control of local H_2O_2 concentration is highlighted by a study showing that depletion of Prx3 enhanced mitochondrial H_2O_2 production and apoptosis in cells stimulated with TNF-beta[34].

A screen of the S-nitrosoproteome in cultured endothelial cells identified Prx1 as an S-nitrosylated protein, raising the possibility that, in addition to control through cysteine oxidation, Prx1 activity is also subject to redox control by NO [35]. In a further layer of control, Prxs are subject to phosphorylation at Thr-90 by cyclin B-dependant kinase. In cells, Prx1 phosphorylation is observed primarily in cells during mitosis, implicating a role for a temporal increase in H_2O_2 in this phase of the cell cycle [36].

A.B. Fisher in his work states about Peroxiredoxin 6 (Prx6) antioxidative abilities. In accordance to his work data, Prx6 is the prototype and the only mammalian 1-Cys member of the Prdx family. Major differences from 2-Cys Prdxs include the use of glutathione (GSH) instead of thioredoxin as the physiological reductant, heterodimerization with pGSH S-transferase as part of the catalytic cycle, and the ability either to reduce the oxidized sn-2 fatty acyl group of phospholipids (peroxidase activity) or to hydrolyze the sn-2 ester (alkyl) bond of phospholipids (phospholipase A2 [PLA2] activity). The bifunctional protein has separate active sites for peroxidase (C47, R132, H39) and PLA2 (S32, D140, H26) activities. These activities are dependent on binding of the protein to phospholipids at acidic pH and to oxidized phospholipids at cytosolic pH. Prx6 can be phosphorylated by MAP kinases at T177, which markedly increases its PLA2 activity and broadens its pH-activity spectrum. Prx6 is primarily cytosolic but also is targeted to acidic organelles (lysosomes, lamellar bodies) by a specific targeting sequence (amino acids 31–40). Oxidant stress and keratinocyte growth factor are potent regulators of Prx6 gene expression. Prx6 has important roles in both antioxidant defense based on its ability to reduce peroxidized membrane phospholipids and in phospholipid homeostasis based on its ability to generate lysophospholipid substrate for the remodeling pathway of phospholipid synthesis [37].

Reversion of redox signaling requires reductive repair of reactive cysteines in proteins. An antioxidant enzyme responsible for the repair of oxidized protein cysteines is the 12-kDa oxidoreductase Trx [38]. Two types of Trx exist: Trx1 in the cytosol or Trx2 in the mitochondria, maintained in the reduced state via electrons donated by Trx1 or Trx2 reductase enzymes, respectively. The Trx family of proteins represents important regulators of cellular redox signaling by using Cys-32 and Cys-35 in the

conserved active- site motif (Cys-Gly-Pro-Cys) to reduce intra- or intermolecular disulfides or sulfenic acids present on proteins.

Recent findings on phenotypical alterations of mouse models with targeted disruptions of Prx genes are discussed, confirming the physiological functions of Prxs for antioxidant cell and tissue protection along with an important role as tumor suppressors [39].

As noted earlier, S-glutathionylation is an important redox-sensitive posttranslational modification that not only protects protein cysteines from irreversible oxidation but also alters protein function to control cell signaling. Grxs, members of the thioredoxin protein family, are glutathione-dependent oxidoreductase enzymes primarily responsible for reduction of S-glutathionylated proteins [38]. Two different glutaredoxins exist: Grx-1 in the cytosol and Grx-2 in the mitochondria. Grx-2, but not Grx-1, is an iron–sulfur protein [38]. The 2Fe-2S cluster bridges two molecules of Grx-2 to form an inactive dimer. Cluster destruction results in enzyme activation.

As the cluster is destroyed by ROS or RNS, the iron–sulfur cluster is considered a redox sensor that ensures Grx-2 activation during oxidative stress [40]. In contrast, Grx-1 is inactivated by S-nitrosylation of critical cysteines [40]. Recent work is beginning to address the roles of Grx in endothelial cells. For example, flow-induced activation of eNOS depends on the thiol-transferase activity of Grx-1 [41]. Upregulation of Grx-1 activity in response to TNF-beta participated in the cytotoxic action of the cytokine toward endothelial cells [42]. These studies demonstrate that Grx activity in endothelial cells can have beneficial or deleterious actions.

Most of the vasculature diseases related with the ROS generation are treated with the antioxidants, which are not too effective (vitamins C and E, and β-carotene). However, it is necesserely to mention that these compounds are able chemically remove from the model systems of vascular diseases excessive amount of ROS [43].

Explanations why vitamin E is ineffective during the clinical trials are perfectly suggested and described by Drummond G.R et all [44]. In accordance to him, the first most important reason why the clinical trail failed is the wide diversity of the diseases of the patients involved, particularly vascular diseases and the also the application of the vitamins was too late for the initiation of significant protection.

Along with the existing known antioxidants, we are proposing, proving and stating about the antioxidant abilities of pyridoxine, one of the subcomponents of vitamin B complex. We were also able to prove that pyridoxine, by suppressing the XO activity, might influence on the proliferative activity of the human brain derived primer cell culture [45].

We have determined the growth of the cells as well as their death in the presence of single components of vitamin B complex: nicotinamide, ribiflavine, pyridoxine and thiamine (1668,50±189,51, 1738,33±196,60; 2556,17±355,68, 2179,00±223,55, resp.). As a positive control in these experimental series bFGF was used. It is clear from the experiments that in comparison with the 2 types of negative controls: number of the cells on the 1st (820,14±50,07) and 4th (1562,94±146,45) days, as well as positive control-growth of the cells in the presence of bFGF (2131,08±144,59); the most effective component of vitamin B complex was pyridoxine and thiamine. In comparison with the nicotinamidee and riboflavin, these two components vividly have increased the growth of the cells even on the day 4th, (**figure 4)** [45].

It was also measured the size of the cells and cell bodies**.** It became clear for us that the most prominent compound, which induced the development of the small cells fraction and their enlargement in the statistically significant way in comparison with the control group.

Taking into the consideration the fact that the most of the components of vitamin B complex separately has the impact on the prevention of reactive oxygen spices formation, influence on the cells growth and maturation, we have evaluated the activity of XO in the human brain derived cells in the presence of above mentioned subcomponents [46, 47]. In the first set of the experiments we have evaluated the XO activity in the presence of applied several concentrations of allopurinol 0,36 ug; 0,72 ug; 1,44 ug; 2,7 ug; 5,4 ug. ($0.0393\pm3.9277^{e-3}$, 0.0531 ± 0.0236, $0.0252\pm9.1646^{e-3}$, 0.0296 ± 0.0105, $0.0157\pm2.6185^{e-3}$, $0.0202\pm1.3092^{e-3}$, $p<0,05$ between the control, and 5,4 ug of allopurinol). As it is clear from the **figure 5** A. allopurinol was inhibiting the activity of XO in the concentration dependent manner.

During the next set of the experiments, we have evaluated the influence of the vitamin B complex subcomponents on the activity of XO. In the **figure 5** B it is presented the % of the XO inhibition in the presence of the subcomponents of B complex. The calculations are based on the quantity of detected uric acid normalized to 1 mg f the protein. Percentile of inhibition/ non inhibition of all subcomponents of vitamin B complex, thiamine, pyridoxine, riboflavin, nicotinamidee, (-201,39±32,76, 160,00±60,00, -120,91±39,091, -152,73±107,27, resp.) were in statistically significant way different from the control sample (31,0343±6,9222, $p<0,05$), which is the percentile of inhibition of XO in the presence of allopurinol and absence of subcomponents.

In our previous experiments we have shown that vitamin B complex initiates the cells' growth and maturation. Results presented in this article indicate that the all components of vitamin B complex are responsible for these functions. During the early period of the growth, important components responsible for these functions were thiamine and pyridoxine, riboflavin as well as nicotinamidee. However, the most vivid difference might be detected in the groups treated with the pyridoxine and thiamine [48].

The size of the embryonic bodies, as well as smaller cells, was sensitive to the all components of vitamin B subcomponents. At day 12^{th} the size of the small cells' fraction treated with the subcomponets of vitamin B complex was smaller in comparison with the control groups. Instead, the average size of the embryonic bodies in comparison with day 4^{th} increased by 20% in the groups treated with the subcomponents, indicating on the fact that small cells were gathering together and forming larger embryonic bodies.

Our previous results [49] indicated that the early inhibition of the XO in the human brain derived cell culture utilizing allopurinol were initiating the increase in the number of the cells in comparison with the later stage of inhibition.

In comparison with the all other subcomponents of vitamin B complex, only in pyridoxine containting samples XO activity was specifically inhibited by allopurinol. Moreover, pyridoxine by itself was inhibiting formation of uric acid. In all the other samples XO activity wasn't inhibited with no any concentrations of allpurinol, probably due to the initiation of the alternative ways of uric acid formation.

Similarly, pyridoxine as allopurinol addition into the cells medium during the early stages of the treatment was initiating the increase in the number of the cells, whereas in the late stages that process was suppressed. During the late stages the most effective components were riboflavin and nicotinamidee.

Moreover we have studied the influence of the different concentrations of pyridoxine on the activity of the purified XO (**figure 6**) and concluded that the effective impact of the compound is dose-dependent.

Equal influence in the cell culture has the allopurinol. We have proved our propose by the number of the data summarized in the publications [50,51].

Conclusion.

Along with the existing systems of ROS generation we are highlighting XOR role in this processes and pointing on its impact on the hydrogen peroxide pool of mitochondria. As and final enzyme of purine catabolism XOR is able to stand as regulating enzyme and its' inhibition by known allopuriol as well as by newly delineated by us native compound – pyridoxine (**figure** 7).

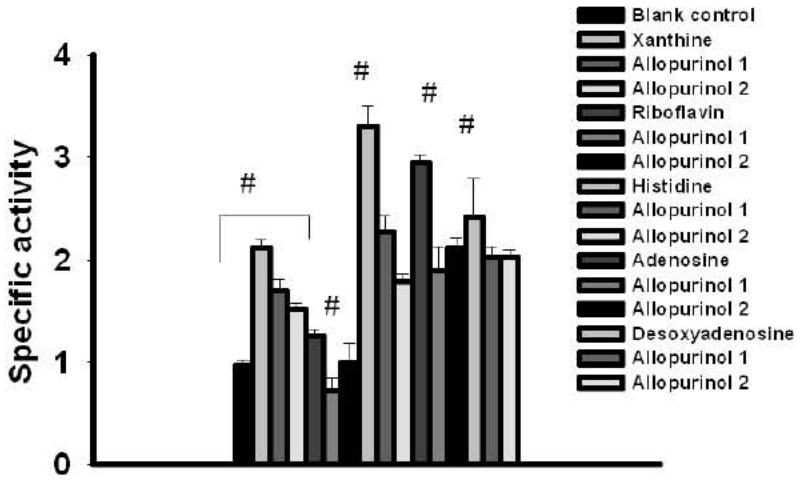

Fig. 1. Evaluation of XO specific activity in the prsence of none-primer subtsrtaes.
The experiments were performed with the utility of the spectrophotometer Perkin-Elmer Coleman Model6|20 and 6|35 Junior II Spectrophotometer (λ=660 nm) for all type of the spectrophotometric investigations. The first black column represents the 11 specific activity of XOR, in the presence of xanthine (0.06 ug/ml). The all other substrates were applied in the same quantity. During the experiments were used allopurinol for the inhibition of XO activity in the concentration equal to the concentration of the substrates (Allopurinol 1) or higher twice (Allopurinol 2). It was used ONE-WAY-ANOVA, t-test for delineation of statistical significance of the experimental results for all type of the investigations. Results were considered statistically significant when $p<0,05$.

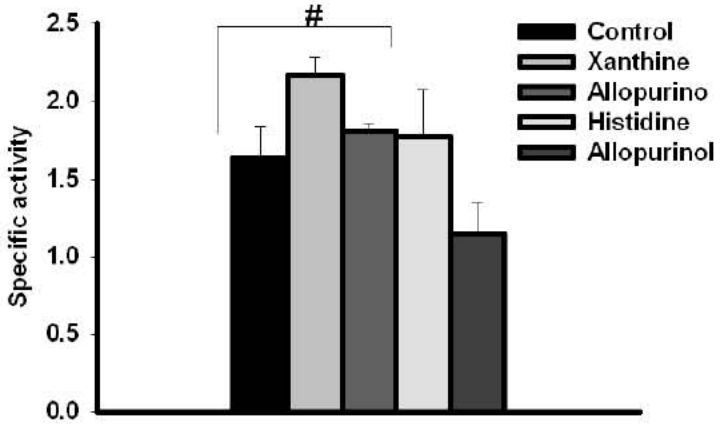

Figure 2. Determination of purified XOR activity in the presence of non-primer substrate.
The second light grey column represents the specific activity of XOR, in the presence of xanthine (0.06 ug/ml). The histidine, allopurino were applied in the same quantity.

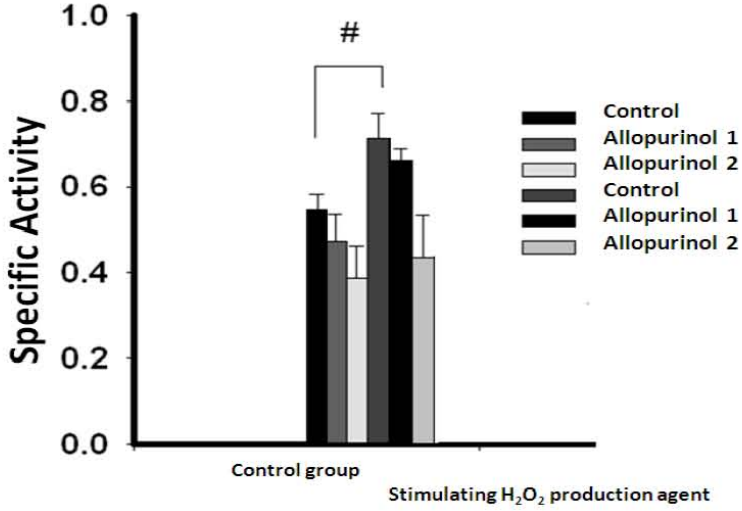

Figure 3. Influence of the allopurinol mediated XO activity on the formation of hydrogen peroxide. We had in our experiments two groups of the animals: injected with the pentylenetetrazole vs control native animals. In mitochondrial fractions we haven't detected the XO activity, however in the presence of allopurinol formation of hydrogen peroxide was elevated. It was used two concentrations of the allopurinol low and high, 0.06 ug and 0.12 ug.

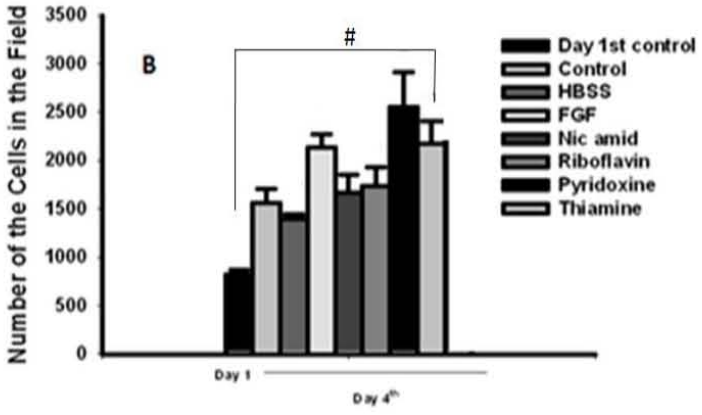

Fig.4. The influence of the single components of vitamin B complex on the growth and development of the human embryonic brain cells. A. Presented are the pictures of the brain cells. Pictures are taken on day 1st and day 4th. Polarizing microscope Bipolar PI (PZO,Warsaw,Poland; magnification 60x1.25x40) was used. The cells were grown on the glass in the Petry dishes, covered with the Poly-L-Lysine. The Neurobasal/B27 containing bFGF , nicotinamide , riboflavin , pyridoxin , thiamine or low concentration of vitamin B complex was used as cell media. The number of the cells in the field was counted using the Pixcavator program, allowing to calculate authomatically the size as well as the number of the cells.

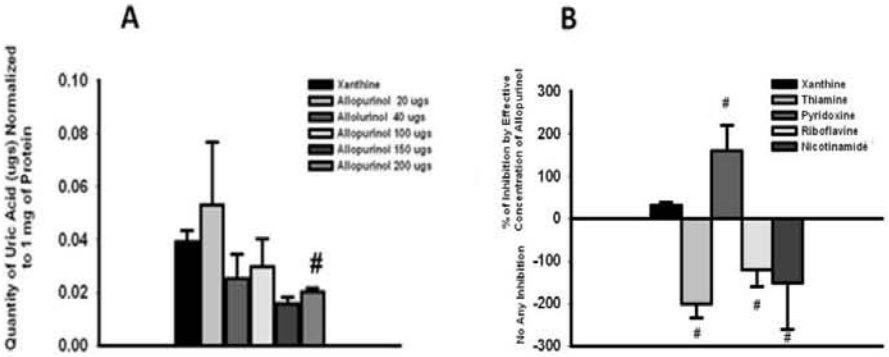

Figure 5. Assessment of XO activity by the estimation of uric acid formation in the presence of allopurinol and vitamin B complex subcomponents.

A. Inhibtion of uric acid formation in the human brain derived (E90) cells by the utility of allopurinol.
B. Persentile of inhibition of uric acid formation in the pressence of vitamin B subcomponents.

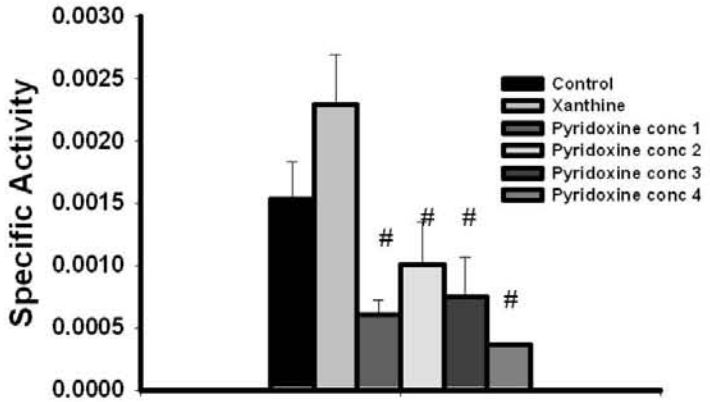

Figure 6. Influence of different concentrations of pyridoxine on the activity of XO.
The experiments were performed similar to above mentioned conditions.

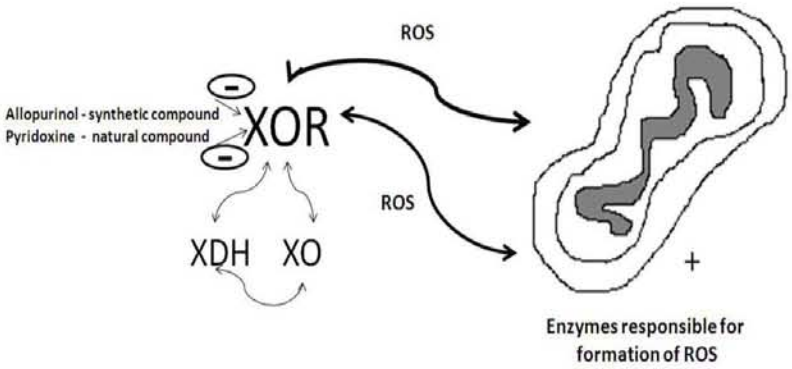

Figure 7. Schematic representation of the new details regarding XO role as the part of oxidative and antioxidative system of the cells

Abbreviation.

Xanthine Oxidoreductase (XOR)
Xanthine Oxidase (XO)
Xanthine Dehydrogenase (XDH)
Endoplasmatic Reticulum (ER)
Protein disulfide isomerase (PDI)
ER oxidoreductin 1 (ERO1
NADPH oxidases (NOXs)
Superoxide anion (O^{2-})
Flavin Adenine Dinucleotide (FAD)
Molybdopterin center (Mo-pt)
Hypoxanthine-Guanine Phosphoribosyltransferase (HGPRT)
Permeability Transition Pore (mPTP)
Electron Transport Chain (ETC)
Mitochondria-targeted antioxidants (mitoTEMPO)
Angiotensin II (AT-II)
ATP-sensitive potassium channel (KATP)
mitochondrial membrane potential (mtJ)
Zinc-sulfur clusters (ZnCys3)
Nitric Oxide Synthase (eNOS)
Asymmetric Dimethylarginine (ADMA)
Nuclear factor erythroid 2-related factor 2 (Nrf2)
Antioxidant Response Elements (ARE)
Peroxiredoxins (Prxs)

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