RAT LIVER ARGINASE SYSTEM UNDER ACETAMINOPHEN-INDUCED TOXIC INJURY AND PROTEIN DEPRIVATION

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Arginase activity and L-arginine content in both cytosolic and mitochondrial fractions of rat liver cells under the conditions of toxic injury on the background of protein deprivation was studied. The most significant reduction of arginase activity in liver cells and depletion of L-arginine pool was found in rats with toxic acetaminophen-induced liver injury maintained on the ration balanced by all nutrients as well as in protein deficiency rats. It was concluded that reduction of the arginase activity in the cytosolic fraction of rat liver cells, combined with simultaneous decrease of L-arginine content, may be considered as one of the mechanisms of ornithine cycle disturbance. The decline of activity of mitochondrial isoform of arginase II, for certain, is related with activation of NO-synthase system.

K e y w o r d s: arginase, L-arginine, acetaminophen, toxic injury, alimentary deprivation of protein.

Protein deficiency becomes quiet common at present due to a constant increase of carbohydrates and fats content in a ration combined with the reduction of protein content or replacement of a full-value dietary protein with a low-value soya protein [1]; vegetarianism, dietary restrictions in different diseases, irrational artificial feeding [2].

Quite often a restriction in exogenous proteins supply is accompanied by the deficiency of essential amino acids in the organism and intensified hydrolysis of the endogenous proteins, resulting in the negative nitrogen balance and subsequent development of acute or chronic inflammatory processes in the liver [3-5].

At the same time an open access to a wide variety of medications contributes to the problem of their common haphazard and irrational use for the correction of pathological states without appropriate maintenance of dosage regimen. A common consequence of uncontrolled self-treatment is drug-induced liver injury.

There are numerous literary data about methods of experimental modeling of the acute or chronic injury arising from the use of toxic factors with various mechanisms of prolonged effect [6]. Model of acetaminophen injury is one of the most widely used [7]. Acetaminophen (paracetamol) is a drug with analgesic/antipyretic activity. Its overdose as well as its use on the background of some triggers influence, like deficiency of essential nutrients, may lead to the development of liver failure [8].

Mechanism of liver tissue injury is realized through the formation of a highly reactive toxic metabolite. The most of drug dose is conjugated with glucuronic acid or sulfate and is excreted from the body. Another part of acetaminophen is metabolized by cytochrome P450 system, with the formation of highly reactive derivative N-acetyl-p-benzochinonimin, which rapidly reacts with the reduced glutathione (GSH) leading to the depletion of its resources in the liver [9].

The issue of arginine metabolism and its influence on the hepatobiliary system is widely discussed nowadays [10-12]. In a human body L-arginine may be synthesized in liver provided by the balanced ration with both quantitative and qualitative composition of a dietary protein being of fundamental importance [13, 14].

In contrast to the most of amino acids, arginine is essential not only for protein biosynthesis but also for the formation of several regulatory and signaling molecules [15].

Arginine level is controlled by the enzyme arginase (EC 3.5.3.1) with its highest activity observed in the liver. There are two isoforms of this enzyme, which differ from each other by the intracellular localization and metabolism [16, 17].

Arginase I is mostly found in the liver whereas arginase II is active in all the tissues, especially in kidneys. While both enzymes are responsible for the urea synthesis, arginase I (cytosolic enzyme) functions in the urea cycle and is involved in the ornithine synthesis and later – formation of polyamines, whereas mitochondrial arginase II regulates cellular concentration of L-arginine/ornithine and also nitric oxide (NO) synthesis [18-19].

Limited data concerning the investigation of arginase activity in cells under the normal conditions and in various pathological states of the organism, particularly toxic injuries, are available to date.

The aim of current research was to study the arginase activity and L-arginine content in both mitochondrial and cytosolic liver fractions under the conditions of toxic injury against the background of protein deficiency.

Materials and Methods

Experiments were conducted on white non-linear rats aged 2.5-3 months weighing 100-120 g. The research was carried out in accordance with the rules set by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and General Ethical Principles of Experiments Involving Animals approved by the First National Bioethics Congress (Kyiv, 2001).

The animals were kept in plastic cages with sand bedding and free access to water. The daily ration was regulated in accordance with principles of pair feeding [20].

The acetaminophen-induced liver injury was modeled by per os administration of 2% starch suspension of acetaminophen in daily dose 1250 mg/kg of the body weight during 2 days [21]. The animals were divided into the following experimental groups: I – animals maintained on the full-value semi-synthetic ration balanced by all nutrients – control group (C) [22]; II – animals maintained on the semi-synthetic low-protein ration (1/3 of the commonly accepted daily protein requirements) (LPR) [23]; III – animals subjected to acetaminophen-induced liver lesions receiving complete ration (H) (TI); IV – animals subjected to acetaminophen-induced liver lesions that were previously fed semi-synthetic low-protein ration (LPR+H).

Cervical dislocation was performed under the light ether anesthesia on 28th and 31th day of the experiment.

Mitochondrial fraction was separated by differential centrifugation [24]. Homogenate medium contained: 250 mM sucrose solution, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4. Nuclei and cellular fragments were precipitated by centrifugation at 700 g during 10 min. Mitochondrial fraction was precipitated at 10000 g during 10 min. Obtained sediment was washed twice with medium without EDTA.

Microsomal fraction was obtained by the method of [25]. Supernatant liquid, which remained after the receiving of microsomal fraction, was used as a cytosolic fraction in the following experiments.

Arginase activity in the subcellular fractions was determined by the formation of urea [26]. Reactive mixture contained: 2 M Tris-HCl, pH 7.5, 0.2 M $MnCl_2$, 10 M NaOH, 1 M arginine solution and 100 µg of protein. Samples were incubated at 37 °C during 30 min. Reaction was stopped by the adding of 100 µl 50% TCA. Total urea content was determined with the help of diagnostic kit "Filicit diagnostica" (Ukraine) in accordance with instruction. Arginase activity was expressed in µmol of formed urea during 1 min per 1 mg of total protein in a sample.

L-arginine content in cytosolic and mitochondrial fractions was determined by the method of [26] after precipitation of proteins with 20% TCA solution. The obtained supernatant was incubated during 20 min at 37 °C in the reactive mixture containing 20% NaOH, 0.02% α-naphtol, 10% urea, hypobromide reagent (Br_2 and 5% NaOH, correlation 1:100). Extinction of samples was measured at wavelength 500 nm. L-arginine concentration was expressed in µmol/mg of protein.

Protein content in the studied samples was determined by Lowry method [27].

Statistical analysis of research results was processed with MS Excel software using standard Student's *t*-test. Differences were considered as statistically significant at $P \leq 0.05$.

Results and Discussion

Intracellular arginases compartmentalization plays an important role in the metabolism of L-arginine. Ornithine, which was formed in a cytosol with the involvement of arginase I, is transformed into putrescine under the influence of ornithine decarboxylase, whereas in mitochondria L-arginine metabolism with the participation of arginase II and ornithine aminotransferase results in the formation of proline and L-glutamate [16-18]. It is known that the expression of arginase isoforms shifts under the influence of exogenous and endogenous factors and metabolic demands.

Research results have shown that under the conditions of low-protein diet in the cytosolic fraction of rat liver there was a two-fold decrease in arginase activity compared to control (Fig. 1). At the same time an administration of toxic acetaminophen doses to control and protein-deficiency animals causes monodirectional changes: enzyme activity is reduced in animals of the both groups (Fig. 1).

Since arginase I takes part in the process of ammonium inactivation, the established decrease in the enzyme activity is obviously caused by the inhibition of urea cycle due to the alimentary deprivation of protein [28]. On the other hand products of the arginase reaction – ornithine and urea – are able to inhibit L-arginine metabolism in the urea cycle by the principle of negative feedback, causing the reduction of cytosolic isoform enzymatic activity [18, 19].

Thus, the established decrease of arginase activity in the liver cell cytosol is accompanied by the reduction of L-arginine content compared to control in all of the studied groups (Fig. 2).

According to literature data [13, 14], reduction of L-arginine cellular pool is caused by the disturbances of its delivery to cell or its intensified use in metabolic processes. As is known, a significant amount of arginine is used for the creatinine synthesis – the substrate of creatine kinase system is responsible for the deposition and transport of energy within cells in a form of creatine phosphate [15]. It may be assumed that under the conditions of protein deficiency there is an intensification of cellular creatine fund restoration system as a consequence of enhanced L-arginine utilization in the anabolic processes.

As is shown in Fig. 2, administration of acetaminophen in toxic doses is accompanied by the significant decrease of L-arginine concentration in
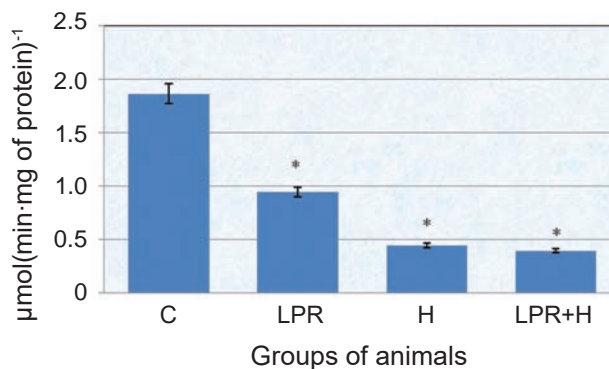

Fig. 1. Arginase activity in the cytosolic fraction of rat liver cells under the conditions of acetaminophen-induced toxic injury on the background protein deprivation. Here and in Fig. 2-4: C – rats maintained on full-value semi-synthetic ration – control group; LPR – rats maintained on low-protein ration; H – animals subjected to acetaminophen-induced liver lesions receiving complete ration; LPR+H – animals subjected to acetaminophen-induced liver lesions that were previously fed semi-synthetic low-protein ration; * significant difference compared to control, $P \le 0.05$

cytosol regardless of feeding ration, which may be linked not only with the influence of exogenous factors but also has an endogenous character. Apparently, the established fact is the result of both intensified use of arginine in the processes of protein synthesis and disturbances of its formation in ornithine cycle [28].

Concentration of intracellular L-arginine depends on its dietary intake, synthesis/resynthesis within the organism, active intracellular transport
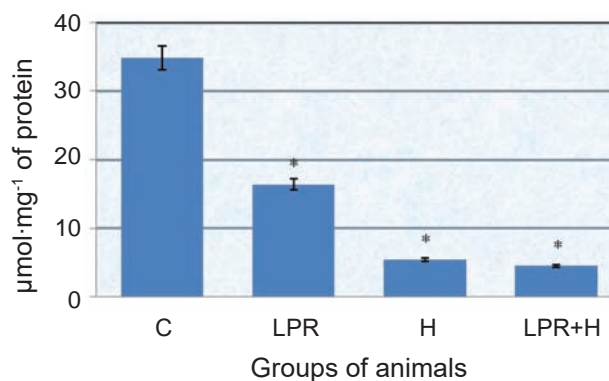

Fig. 2. L-arginine level in the cytosolic fraction of liver cells under the conditions of acetaminophen-induced toxic injury on the background protein deprivation

and activity of arginine degradation enzymes. Protein catabolism and/or resynthesis of L-arginine from citrulline in the L-citrulline cycle are capable of compensating acertain lack of L-arginine [29].

It is established [30], that L-citrulline of intestinal enterocytes of mammals is almost a unique source of endogenous arginine. In the intestinal cells citrulline is produced as the end product of glutamine/glutamate metabolism under the influence of urea cycle enzymes – carbamoyl phosphate synthase and ornithine carbamoyl transferase, and then it is carried with blood to kidneys. Kidney – is the main organ of enterocytes' citrulline transformation by arginine succinate synthase and arginine succinate lyase into arginine with subsequent transport of the latter to the liver and its hydrolysis by arginase. Uptake of the physiological arginine concentration in the liver is restricted by the decreased activity of the transport system of positively charged amino acids in hepatocytes [31].

Therefore, ornithine cycle is not the main endogenous provider of L-arginine since it imports only 5-15% of plasms urea [32]. Consequently, metabolic compartmentalization of arginine under the current experimental conditions is of a determining character, since functional disturbances of intestines or kidneys (as it was indicated earlier [33]) may lead to the decrease of arginine endogenous synthesis, directly affecting its total cytosol content.

Moreover, there are data concerning the inhibition of lipid absorption by arginine though the reduction of low density lipoproteins level with simultaneous increase of high density lipoproteins concentration in blood, which diminishes the degree of fatty liver dystrophy [15]. Probably, under the conditions of acetaminophen-induced injury a reduction of this amino acid level not only results in the disturbances of desintoxication processes (first of all ammonia neutralization), but also indicates dysmetabolic changes of lipids. Numerous researches [11, 18, 19] testify to the fact that deficit of arginase I induces the II isoform of enzyme.

Obtained results point to the reduction of arginase activity in the mitochondrial fraction of liver cells in all groups of experimental animals maintained under the current conditions (Fig. 3). Simultaneously, as is shown in Fig. 3, similar tendency to the maximal reduction of enzyme activity remains under the conditions of toxic acetaminophen doses administration, independent of protein amount in the ration.
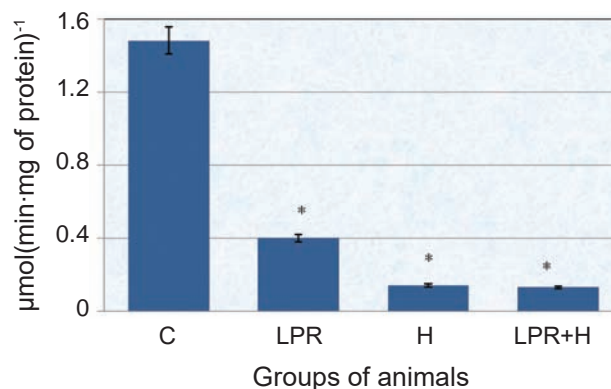

Fig. 3. Arginase activity in the mitochondrial fraction of liver cells under the conditions of acetaminophen-induced toxic injury on the background of protein deprivation

Taking into consideration the established [23, 26] interrelation of arginase II and NO-synthase activity, it may be assumed that the reduction of arginase activity in a mitochondrial fraction of liver cells is accompanied with the activation of nitrogen oxide synthesis. A balance between NO-synthase and arginase pathways of L-arginine metabolism maintains the cellular physiological pool of this amino acid and determines the intensity of NO and its metabolites production.

However the relationship between these two enzymes is much more complex than a simple competition for substrate. In particular, the intermediate product of NO synthesis is N^ω-hydroxy-L-arginine, which has a high affinity for arginase and is a strong endogenous competitive inhibitor of this enzyme [34].

It was established, that a decrease of arginine quantity in the mitochondrial fraction of liver cells is observed in all groups of experimental animals (Fig. 4), whereas the lowest indices are registered under the conditions of acetaminophen injury on the background of protein deficiency. Reduction of L-arginine bioavailability under the current experimental conditions is probably caused by the activation of NO-dependent system.

As is known, NO-dependent synthesis of essential NO ("basal rate") is determined by the activation of constitutive Ca^{2+}-dependent NOS isoforms (cNOS), whereas synthesis of additional amounts of nitrous oxide under the pathological conditions is realized with the involvement of inducible NO-synthase (iNOS) [35].
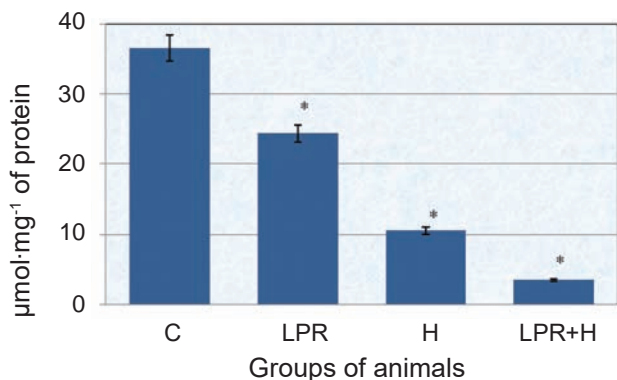
Fig. 4. L-arginine level in the mitochondrial fraction of liver cells under the conditions of acetaminophen-induced toxic injury on the background of protein deprivation

Considering all the foregoing, it may be assumed that competition between NO-synthase isoforms and arginase for the common substrate causes the depletion of this amino acid reserves either on the background of protein deficiency or after the administration of acetaminophen in toxic doses.

Thus, the reduction of arginase activity in the cytosolic fraction of liver cells with simultaneous decrease of L-arginine level may be considered as one of the mechanisms of ornithine cycle disturbances, while the decrease in activity of mitochondrial isoform arginase II is obviously caused by the activation of NO-synthase system. Toxic liver injury under the current experimental conditions is accompanied by the maximal decrease of arginase activity in liver cells and depletion of L-arginine pool.

АКТИВНІСТЬ АРГІНАЗНОЇ СИСТЕМИ В ПЕЧІНЦІ ЩУРІВ ЗА ТОКСИЧНОГО УРАЖЕННЯ АЦЕТАМІНОФЕНОМ І НЕСТАЧІ ПРОТЕЇНУ

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У роботі досліджували активність аргінази та вміст L-аргініну в цитозольній і мітохондріальній фракціях клітин печінки щурів за умов токсичного ураження на тлі нестачі протеїну. Встановлено, що в умовах токсичного ураження, індукованого ацетаміфеном, у тварин, які отримували харчовий раціон, збалансований за всіма нутрієнтами, та в протеїнодефіцитних щурів спостерігалося максимальне зниження активності аргінази та вмісту L-аргініну в клітинах печінки. Зроблено висновок про те, що зниження активності аргінази в цитозольній фракції з одночасним зменшенням вмісту L-аргініну може розглядатися як один із механізмів порушення роботи орнітинового циклу. Водночас, зниження активності мітохондріальної ізоформи аргінази II, вірогідно, пов'язане з активацією NO-синтазної системи.

К л ю ч о в і   с л о в а: аргіназа, L-аргінін, ацетаміфен, токсичне ураження, аліментарна нестача протеїну.

АКТИВНОСТЬ АРГИНАЗНОЙ СИСТЕМЫ В ПЕЧЕНИ КРЫС ПРИ ТОКСИЧЕСКОМ ПОРАЖЕНИИ АЦЕТАМИНОФЕНОМ И НЕДОСТАТОЧНОСТИ ПРОТЕИНА

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В работе исследовали аргиназную активность и содержание L-аргинина в цитозольной и митохондриальной фракциях клеток печени крыс в условиях токсического гепатита на фоне недостаточности протеина. Установлено, что при токсическом поражении, индуцированном ацетаминофеном, у животных, получавших пищевой рацион, сбалансированный по всем нутриентам, и у протеинодефицитных крыс наблюдалось максимальное статистически достоверное снижение активности аргиназы в клетках печени и истощение пула L-аргинина. Сделан вывод о том, что снижение аргиназной активности в цитозольной фракции клеток печени крыс с одновременным уменьшением содержания L-аргинина может рассматриваться как один из механизмов нарушения работы орнитинового цикла. В то же время, дисфункционирование

митохондриальной изоформы аргиназы II, по-видимому, связано с активацией NO-синтазной системы.

К л ю ч е в ы е с л о в а: аргиназа, L-аргинин, ацетаминофен, токсическое поражение, алиментарная недостаточность протеина.

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