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ACTIVITY OF CYTOSOLIC ISOENZYMES OF ENDOGENOUS ALDEHYDES CATABOLISM UNDER THE CONDITIONS OF ACETAMINOPHEN-INDUCED HEPATITIS ON THE BACKGROUND OF PROTEIN DEFICIENCY

O. M. VOLOSHCHUK, G. P. KOPYLCHUK, Y. I. MISHYNA

Fedkovych Chernivtsi National University, Ukraine;
e-mail: o.voloschuk@chnu.edu.ua

The research deals with the determination of the activity of aldehyde dehydrogenase (EC 1.2.1.3), aldehyde reductase (EC 1.1.1.21), the content of TBA-active products and protein carbonyl derivatives in the rat liver cytosolic fraction under the conditions of acetaminophen-induced hepatitis and alimentary deprivation of protein. The researches were conducted on white rats of 90-100 g body mass aged 2-2.5 months. There were used 36 rats, which according to the experimental model were separated into 4 groups: I – animals receiving full-value semi-synthetic ration (C); II – animals receiving low-protein ration (LPR); III – animals with acetaminophen-induced liver injury receiving complete ration (H); IV – animals with acetaminophen-induced liver injury that were previously maintained on semi-synthetic low-protein ration (LPR+H). The acetaminophen-induced liver injury was modeled by per os administration of 2% starch suspension of acetaminophen in daily dose 1250 mg/kg (0,5 LD_{50}) of the body weight. Cytosolic fraction was obtained by differential centrifugation at the temperature 0-3 ^0C in the solution which contained sucrose, EDTA and tris-HCl buffer. Aldehyde dehydrogenase and aldehyde reductase activities were determined spectrophotometrically by the tempo of regeneration of NAD^+ and oxidation of NADH respectively. Enzymatic activity was calculated using the molar extinction coefficient of according nicotinamide coenzymes. The concentration of TBA-active products was assessed by the reaction with thiobarbituric acid and forming the colored complex. The level of the oxidative protein modification assessed via amount of 2.4-dinitrophenylhydrazone derivatives, produced in reactions of oxidized amino acid residues with 2.4-dinitrophenylhydrazine. The most pronounced decrease in the activity of enzymes utilizing endogenous aldehydes is observed in the liver cytosolic fraction of animals with toxic liver injury maintained under the conditions of alimentary protein deficiency. The established fact is explained as by the disturbances of enzyme structural-functional organization and its synthesis, as by changes of the ratio between redox forms of the nicotinamide coenzymes. Meanwhile, the accumulation of TBA-active products and protein carbonyl-derivates in the liver cytosolic fraction of animals of this experimental group was established. In this case, a statistically significance of a difference between the concentration of these products in protein-deficient and control rats under the current experimental conditions was not detected. The accumulation of aldehyde products of lipid and protein oxidative damage on the background of the reduction in the activity of enzymes providing aldehyde catabolism may be considered as a possible mechanism underling hepatocyte dysfunction under the conditions of toxic damage in protein-deficient animals.

Key words: alimentary protein deficiency, hepatotoxicity, cytosol, aldehyde dehydrogenase, aldehyde reductase, TBA-active products, protein carbonyl derivatives

Introduction. The intensification of free radical processes is one of the central mechanisms of non-specific liver cell damage under the influence of stress factors of various etiologies, including some drugs [1, 2]. The implementation effect of free radical reactions is mediated by the accumulation of carbonyl products in cells, among which the main are endogenous aldehydes. Carbonyl free radical oxidation products act as the original messengers of cell damage. However, whereas carbonyl products have highly reactive ability, they demonstrate the most pronounced cytotoxic and genotoxic properties [3]. Therefore, there is an aldehyde dehydrogenase pathway of endogenous aldehydes catabolism, which function is the oxidation of aldehydes to carboxylic acids, and aldehyde reductase pathway that catalyzes the reduction of endogenous aldehydes to alcohols [4]. Neutralization of carbonyl metabolites is considered as a mechanism of protecting cells from alteration under the different pathological conditions involving oxidative stress. Our previous research showed the pronounced intensification of free radical processes in the liver cytosolic fraction under the toxic liver injury on the background of alimentary protein deficiency [5].

Therefore, the aim of the current work was to determine the activity of aldehyde dehydrogenase (EC 1.2.1.3), aldehyde reductase (EC 1.1.1.21), the content of TBA-active products and protein carbonyl derivatives in the rat liver cytosolic fraction under the conditions of acetaminophen-induced hepatitis on the background of dietary protein deprivation.

Materials and methods. The experiments were conducted on white rats of 90-100 g body mass aged 2-2.5 months. The experiment was conducted 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). The animals were separated into solitary plastic cages with sand bedding and *ad libitum* access to water. The daily rations were regulated according to principles of pair feeding. The animals were separated into the following experimental groups: I – animals receiving full-value semi-synthetic ration (C); II – animals receiving low-protein ration (LPR); III – animals with acetaminophen-induced liver injury receiving complete ration (H); IV – animals with acetaminophen-induced liver injury that were previously maintained on semi-synthetic low-protein ration (LPR+H).

The animals of the groups I and III received a standard ration containing 14% of protein (casein), 10% of fat, and 76% of carbohydrates, balanced by all the essential nutrients. The animals of the groups II and IV received isoenergetic ration containing 4.7% of protein, 10% of fat, and 85.3% of carbohydrates, calculated after recommendations of the American Institute of Nutrition [6]. The animals were maintained on the corresponding diet during four weeks. Afterwards, the acetaminophen-induced liver injury was modeled by *per os* administration of 2% starch suspension of acetaminophen in daily dose 1250 mg/kg (0,5 LD_{50}) of the body weight during 2 days [7].

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

Cytosolic fraction was obtained by differential centrifugation after the separation of mitochondria and microsomes.

Aldehyde dehydrogenase activity was determined spectrophotometrically. Enzymatic activity was calculated using the molar extinction coefficient of NAD^+ at a wave-length of 340 nm ($\varepsilon = 16.9\times10^3$ $M^{-1}\times cm^{-1}$) [8]. The enzyme activity was expressed in nmol NAD^+/min×mg of protein.

Aldehyde reductase activity was calculated using molar extinction of NADPH ($\varepsilon = 6.22\times10^3$ $M^{-1}\times cm^{-1}$) [9]. The enzyme activity was expressed in nmol NADPH/min×mg of protein.

The concentration of TBA-active products was assessed by the reaction with 2-thiobarbituric acid (TBA), occurring at high temperature in acidic environment, and forming the colored complex, determined at λ 532 nm ($\varepsilon = 1.56\times10^5$ $M^{-1}\times cm^{-1}$). The concentration of TBA-active products was expressed in nmol/mg of protein [10].

Protein carbonylation was assessed via amount of 2.4-dinitrophenylhydrazone derivatives, produced in reactions of oxidized amino acid residues with 2.4-dinitrophenylhydrazine, and expressed as nmol of carbonyl protein derivatives per mg of protein.

The protein content was determined according to the Lowry method.

The data statistics was processed with MS Excel software, and represented as mean ± deviation. The statistical significance was determined with standard Student's *t*-test.

Results and discussion. The results of study have shown that the enzymatic activity of aldehyde dehydrogenase, which catalyzes the oxidation reaction of aldehydes to carboxylic acids [11], was decreased in the liver cytosolic fraction of all experimental groups of animals compared to the control (Fig. 1). But the most pronounced change in the enzymatic activity was detected in the liver cytosolic fraction of animals with toxic liver injury maintained under the conditions of dietary protein deprivation. The cytosolic aldehyde dehydrogenase activity was decreased by more than 4 times in animals of this group (Fig. 1).

Meanwhile, we observed the decreased activity of cytosolic isoform of aldehyde reductase, which catalyzes the reduction of aldehydes to alcohols [12] in the animal liver cytosolic fraction of all experimental groups (Fig. 2). Our study established the maximal lowering of aldehyde reductase reaction in protein-deficient rats with liver toxicity.

On the one hand, the reduction in the catalytic activity of studied enzymes can be probably associated with its inhibition by reactive polar metabolite of acetaminophen – N-acetyl-*p*-benzoquinone imine which is produced by cytochrome P450 isoenzymes. In addition, whereas aldehyde reductase is a NADH-dependent enzyme, a decline of its activity may be caused by the deficiency of nicotinamide nucleotides reduced forms under the current experimental conditions [13].

Since aldehyde dehydrogenase and aldehyde reductase are key enzymes of endogenous aldehydes utilization, reduction in their activity causes the accumulation of aldehyde adducts with cellular macromolecules. For this reason a determination of TBA-active products and protein carbonyl derivatives allows analyzing the intensity of toxic endogenous aldehydes accumulation in the cell [14].

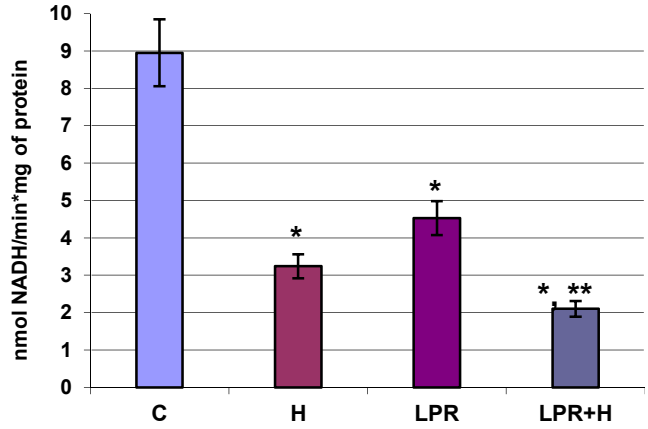

Fig. 1. The enzymatic activity of aldehyde dehydrogenase in the rat liver cytosolic fraction under the conditions of acetaminophen-induced hepatitis and alimentary protein deprivation

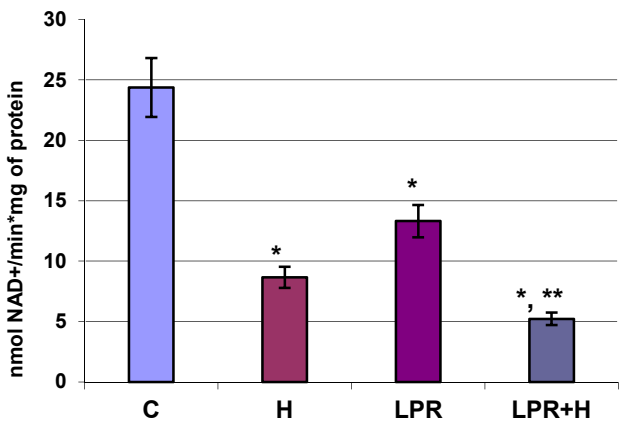

Fig. 2. The enzymatic activity of aldehyde reductase in the rat liver cytosolic fraction under the conditions of acetaminophen-induced hepatitis and alimentary protein deprivation

It is known that aldehyde dehydrogenase is involved in the utilization of endogenous aldehydes, which mostly are products of lipid peroxidation and are defined as "TBA-active products" [20]. It is also known that oxidative stress causes formation of the numerous aldehydes: saturated (ethanal, propanal, hexanal), unsaturated (acrolein, 4-hydroxy-2-nonenal and 4-hydroxy-2-hexenal) and dicarbonyls (glyoxal, methylglyoxal, malonic dialdehyde) through the polyol pathway of fatty acid peroxidation [15, 16]. These reactive carbonyl compounds are capable to non-enzymatic interaction with protein molecules, forming the irreversibly modified end products of lypoxigenation [17].

In turn, aldehyde reductase is involved in the reduction of mostly unsaturated endogenous aldehydes, which are the products of protein oxidative modifications [18].

The results of this study suggest that the most intensive accumulation of TBA-active products and protein carbonyl-derivatives observed in the liver cytosolic fraction of rats with acetaminophen-induced hepatitis, which were subjected to dietary protein deprivation (Fig. 3, 4). Intensive accumulation of TBA-active products can lead to the increase of viscosity and permeability of cell membranes, disturbances of their integrity, which causes an imbalance in the mechanisms of cellular homeostasis regulation [19, 20]. On the other hand, the protein carbonyl derivatives accumulation in the cytosol may result in the interruption of signal transduction, structural and functional changes of receptor proteins, extracellular matrix proteins, enzymes of metabolic transformations and antioxidant defense [17].

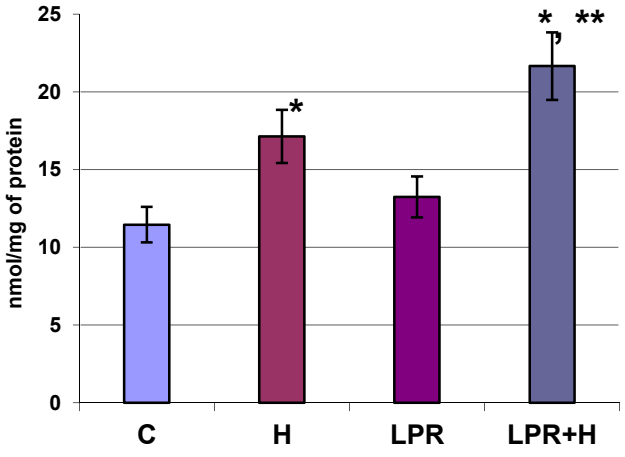

Fig. 3. TBA-active products content in the rat liver cytosolic fraction under the conditions of acetaminophen-induced hepatitis and alimentary protein deprivation

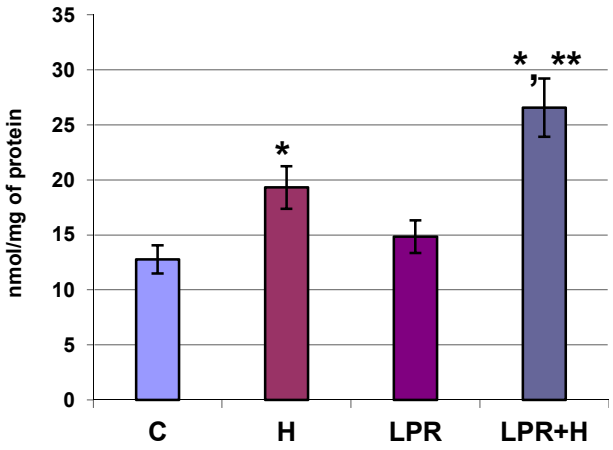

Fig. 4. Protein carbonyl derivatives content in the rat liver cytosolic fraction under the conditions of acetaminophen-induced hepatitis and alimentary protein deprivation

Conclusions.

Thus, the accumulation of aldehyde products of lipid and protein oxidative damage in the cytosolic fraction against the background decrease in the activity of enzymes providing aldehyde catabolism may be considered as a possible mechanism underling the liver cells dysfunction under the conditions of toxic liver injury in protein-deficiency animals.

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АКТИВНІСТЬ ЦИТОЗОЛЬНИХ ІЗОФОРМ ЕНЗИМІВ КАТАБОЛІЗМУ ЕНДОГЕННИХ АЛЬДЕГІДІВ ЗА УМОВ ТОКСИЧНОГО УРАЖЕННЯ НА ФОНІ БІЛКОВОЇ НЕДОСТАТНОСТІ

О. М. Волощук, Г. П. Копильчук, Ю. І. Мішина

У роботі визначено активність альдегіддегідрогенази (КФ 1.2.1.3), альдегідредуктази (КФ 1.1.1.21), а також вміст ТБК-активних продуктів і карбонільних похідних протеїнів у цитозольній фракції печінки щурів за умов ацетамінофен-індукованого гепатиту та аліментарної білкової недостатності. Дослідження проведено на білих безпородних щурах масою 90 – 100 г, віком 2 – 2,5 місяці. У експерименті було використано 36 щурів, яких згідно з моделлю дослідження розділили на 4 групи: I група – щурі, які перебували на повноцінному напівсинтетичному раціоні (К); II група – щурі, які перебували на низькопротеїновому раціоні (НПР); III – щурі з токсичним ураженням печінки, які перебували на повноцінному раціоні (ТУ); IV – щурі з ацетамінофен-індукованим ураженням печінки, які попередньо перебували на напівсинтетичному низькопротеїновому раціоні (НПР+ТУ). Моделювання ацетамінофен-індукованого токсичного ураження печінки проводили шляхом введення peros дослідним тваринам парацетамолу з розрахунку 1250 мг/кг (0,5 LD_{50}) маси тварин у вигляді суспензії в 2% розчині крохмального гелю. Виділення цитозольної фракції проводили методом диференційного центрифугування за температури 0-3 ^{0}C в середовищі гомогенізації, що містило сахарозу, ЕДТА та трис-HCl буфер. Активність альдегіддегідрогенази та альдегідредуктази визначали спектрофотометричним методом за швидкістю відновлення NAD^+ і окислення NADH відповідно. Ензиматичну активність даних ферментів розраховували з урахуванням коефіцієнту молярної екстинкції відповідних форм нікотинамідних коферментів. Вміст ТБК-активних продуктів визначали за концентрацією забарвленого триметинового комплексу, утвореного в реакції з тіобарбітуровою кислотою. Ступінь окислювальних модифікацій білків оцінювали за кількістю гідразонових аддуктів, що утворюються при зв'язуванні карбонільних груп протеїнів із 2,4-динітрофенілгідразином. Встановлено, що найвираженіше зниження активностей досліджуваних ензимів утилізації ендогенних альдегідів спостерігається у цитозольній фракції печінки тварин з токсичним ураженням, які утримувалися за умов дефіциту харчового протеїну. Встановлений факт можна пояснити як імовірним порушенням структурно-функціональної організації ферментів та їх синтезу, так і зміною у співвідношенні редокс-форм нікотинамідних коферментів за даних експериментальних умов. Водночас у цитозольній фракції печінки тварин даної групи встановлене накопичення ТБК-активних продуктів та протеїнових карбоніл-дериватів. При цьому у білок-дефіцитних щурів статистично достовірної різниці за рівнем даних продуктів порівняно з показниками контролю не встановлено. Зроблено висновок, що показане накопичення альдегідних продуктів окислювального пошкодження ліпідів та протеїнів на фоні зниження активності ферментів, які забезпечують їх катаболізм, може лежати в основі одного з механізмів дисфункції клітин печінки за умов токсичного ураження, індукованого на фоні дефіциту харчового протеїну.

Ключові слова: аліментарна нестача протеїну, гепатотоксичність, цитозоль, альдегіддегідрогеназа, альдегідредуктаза, ТБК-активні продукти, карбонільні похідні протеїнів

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