ORIGINAL ARTICLE

THE EFFECTS OF L-ORNITINE AND L-ARGININE ON THE PROCESSES OF LIPID PEROXIDATION IN THE FUNCTIONAL LAYERS OF KIDNEYS ON THE BACKGROUND OF ACUTE TOXIC HEPATITIS

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ABSTRACT

The aim is to evaluate the effects of L-arginine and L-ornithine on the processes of lipid peroxidation in homogenates of renal cortex, renal medulla and renal papilla under conditions of acute toxic hepatitis.

Materials and methods: The study was performed on 40 outbred white male rats with experimental hepatitis, caused by carbon tetrachloride. The animals were divided into five groups: control group (the rats were simulated carbon tetrachloride poisoning and its correction by administering of olive oil and normal saline in equivalent doses), acute carbon tetrachloride hepatitis (single intraperitoneal injection of 50% carbon tetrachloride oil solution at the dose of 2 mlxkg^{-1} of body weight and simulation of treatment by administration of normal saline in equivalent doses), acute carbon tetrachloride hepatitis + L-ornithine (1000 mgxkg-1), acute carbon tetrachloride hepatitis + L-arginine (500 mgxkg-1) and acute carbon tetrachloride hepatitis + combination of substances.

Results: On the background of acute carbon tetrachloride intoxication it was observed the development of renal failure in experimental animals, manifested by activation of lipid peroxidation processes in homogenates of renal cortex, renal medulla and renal papilla. The administration of L-ornithine and L-arginine demonstrates positive impact on renal function and hepato-renal syndrome by stabilization of cell membranes and regeneration of functional capacity of injured renal cells.

Conclusions: The results of our study confirm both the presence of unidirectional effects and absence of toxic influences of L-ornithine and L-arginine on renal cells under the conditions of acute carbon tetrachloride intoxication, which are the most important requirements for modern drugs for the treatment of hepato-renal syndrome.

KEY WORDS: carbon tetrachloride, liver, kidney, hepato-renal syndrome, L-ornithine, L-arginine

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INTRODUCTION

The disorders of hepatobiliary system occupy a significant place in the clinic of internal diseases. The liver, as an organ that plays a leading role in the regulation of metabolism, body integrity, homeostasis support, xenobiotic neutralization, is the target organ of foreign compounds, a significant percentage of which have selective hepatotoxicity. Among them, industrial poisons and medicines take one of the significant places. According to modern data, in Ukraine, 70-80% of chronic liver diseases occurs as a result of previous viral hepatitis, 20-30% — develops due to toxic injuries [1]. Impaired elimination of toxic metabolites, associated with renal parenchyma damage, leads to their retention in the organism and worsening of intoxication, which contributes to the development of acute or chronic hepatorenal insufficiency. Clinical manifestations of combined toxic liver and kidney damage occur in about 30% of acute toxicities. In a study of patients with alcoholic hepatitis, for example, hepatorenal syndrome occurred in 28 of 101 patients [2].

A promising direction for the correction of liver toxicity is the use of drugs, containing natural amino acids, which, entering into metabolism, have a positive impact on hepatic functional recovery. The effectiveness of L-ornithine and L-arginine administration in liver pathology has been shown in a number of papers [3, 4]. However, the disruption of lipid peroxidation processes in the renal tissues on the background of carbon tetrachloride intoxication has not been sufficiently studied; there is no data about the effect of combined use of above-mentioned biologically active substances in order to inhibit activated peroxidation reactions, triggered by compounds with high toxicity.

THE AIM

The aim of the study is to evaluate the effect of L-arginine and L-ornithine on the processes of lipid peroxidation (POL) in functional layers of the kidney under conditions of acute intoxication with carbon tetrachloride.

MATERIALS AND METHODS

The study of the therapeutic effect of amino acids L-ornithine and L-arginine on the processes of POL in

Table 1. Combined effect of L-ornithine and L-arginine on the state of POL in homogenates of renal cortex, renal medulla and renal papilla under the conditions of acute toxic hepatitis (M±m)

Index	Control group (n=8)	Hepatitis (n=5)	Hepatitis + L-ornithine (n=7)	Hepatitis + L-arginine (n=6)	Hepatitis + combination (n=8)
DC (renal cortex), au×g^{-1}	0,448± 0,011	0,745± 0,060$^{\#\#\#}$	0,655± 0,039$^{\#\#\#}$	0,555± 0,035$^{\#*}$ p_1>0,05	0,507± 0,036** p_1<0,05 p_2>0,05
DC (renal medulla), au×g^{-1}	0,421± 0,010	0,930± 0,058$^{\#\#\#}$	0,700± 0,050$^{\#\#\#*}$	0,605± 0,042$^{\#\#**}$ p_1>0,05	0,458± 0,037*** p_1<0,01 p_2<0,05
DC (renal papilla), au×g^{-1}	0,443± 0,007	0,815± 0,032$^{\#\#\#}$	0,752± 0,053$^{\#\#\#}$	0,757± 0,040$^{\#\#\#}$ p_1>0,05	0,600± 0,044$^{\#\#**}$ p_1<0,05 p_2<0,05
TBA-reactive substances (renal cortex), mcmol×kg^{-1}	1,033± 0,029	2,013± 0,094$^{\#\#\#}$	1,252± 0,077$^{\#***}$	1,668± 0,121$^{\#\#\#}$ p_1<0,05	1,388± 0,080$^{\#\#\#***}$ p_1>0,05 p_2>0,05
TBA-reactive substances (renal medulla), mcmol×kg^{-1}	1,123± 0,022	1,850± 0,106$^{\#\#\#}$	1,303± 0,093**	1,730± 0,119$^{\#\#\#}$ p_1<0,05	1,550± 0,065$^{\#\#\#*}$ p_1<0,05 p_2>0,05
TBA-reactive substances (renal papilla), mcmol×kg^{-1}	1,065± 0,037	1,930± 0,070$^{\#\#\#}$	1,192± 0,065***	1,875± 0,111$^{\#\#\#}$ p_1<0,001	1,352± 0,104$^{\#}$ p_1>0,05 p_2<0,01

Notes:

1. # – statistical significance of indexes compared with control group (# – p<0,05; ## – p<0,01; ### – p<0,001);
2. * – statistical significance of indexes compared with group of animals with hepatitis (* – p<0,05; ** – p<0,01; *** – p<0,001);
3. p1 – statistical significance of indexes compared with group of animals with hepatitis, treated with L-ornithine;
4. p2 – statistical significance of indexes compared with group of animals with hepatitis, treated with L-arginine.

kidney tissues under the conditions of toxic hepatitis was performed on 40 outbred white male rats, with the weight of 180-220 g, which were kept under vivarium conditions on a standard diet. All manipulations on the animals were carried out in accordance with the provisions of the "European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes" (ETS No. 123, Strasbourg, 18.03.1986), "Guide for the Care and Use of Laboratory Animals" (National Academies Press, USA, 2011), in compliance with the Law of Ukraine "On the Protection of Animals from Cruelty" No. 27, Art. 230, from 2006, with the changes, made in accordance with the Law No. 1759-VI (1759-17) from December 15, 2009. Ethics Commission of medical and biological researches, Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Protocol No. 57 from March 25, 2020) did not find any violations of moral and ethical standards during experiments on animals.

Animals were divided into 5 groups: the first group consisted of control animals, that were simulated of carbon tetrachloride poisoning and therapeutic measures by intraperitoneal administration of olive oil and normal saline in equivalent doses; the second group included rats, that were modeled of acute toxic hepatitis by a single intraperitoneal injection of 50% oil solution of carbon tetrachloride at a dose of 2 ml×kg^{-1} of body weight of the animal [5] and simulated therapeutic effects by administration of normal saline in equivalent doses; in the third group, 1 day after modeling of hepatitis, the animals received intraperitoneal treatment with L-ornithine solution at a dose of 1000 mg×kg^{-1} during seven days [6]; in the fourth group — with L-arginine solution at a dose of 500 mg×kg^{-1} [7]; in the fifth group both substances were combined.

On the eighth day after the beginning of the corrective measures, all experimental animals were euthanized by the method of total bloodletting from the heart, and biochemical tests of the kidney homogenate were performed: the levels of lipid peroxides (TBA-reactive substances and diene conjugates (DC) [8] in renal cortex, renal medulla and renal papilla were determined. Statistical analysis of the results was performed using Student's t-test.

RESULTS

Acute intoxication with carbon tetrachloride led to the activation of POL in the renal tissues (Table 1), which was manifested by a marked increasing of DC level. Thus, after the simulation of toxic hepatitis, the level of this marker was 1.66 times ($p<0.001$) higher in renal cortex, 2.20 times ($p<0.001$) higher in renal medulla, and 1.84 times ($p<0.001$) higher in renal papilla as compared to control group. After a seven-day correction with L-ornithine, no significant difference was observed between the levels of DC in renal cortex and renal papilla, comparing to the animals without any correction. But, at the same time, the level of DC in renal medulla decreased by 32.85% ($p<0.05$), while remaining at 66.27% higher than in control group ($p<0.001$).

As a result of therapeutic use of L-arginine, the level of DC in renal cortex decreased by 25.50% ($p<0.05$), compared to the animals without correction, but remained 23.88% ($p<0.05$) higher than in control group of animals. In the renal medulla, this index decreased by 65.05% ($p<0.01$) and exceeded the control by 43.70% ($p<0.01$).

After seven days of administration of the combination of amino acids, the level of DC in renal cortex was 68.05% ($p<0.01$) lower than in animals without correction and was not statistically significantly different, than in control group ($p>0.05$). This result was better than with the isolated administration of L-ornithine (the level of DC decreased by 22.59%, $p_1<0.05$), but it wasn't statistically significant different after administration of L-arginine ($p_2>0.05$). Under the influence of combined correction, the level of DC in renal medulla decreased by 2.03 times relative to the group of animals without correction, which was 34.57% better than when using L-ornithine ($p_1<0.01$) and 24.29% better than using L-arginine ($p_2<0.01$). Under these conditions, the following changes in the processes of POL were observed in the renal papilla after combination therapy: the level of DC decreased by 73.62% relative to the group of animals without correction ($p<0.01$) and remained 35.44% higher than in the control group of rats ($p<0.01$). This index was 20.21% and 20.73% lower, respectively, then with isolated administration of L-ornithine ($p_1<0.05$) and L-arginine ($p_2<0.05$).

On the 8th day after administration of carbon tetrachloride, significant increasing of the levels of TBA-reactive substances was observed in renal cortex, renal medulla and renal papilla (Table 1). Comparing to the control group, this index increased by 94.86%, 60.70% and 81.22% ($p<0,001$), respectively, indicating a pronounced nephrotoxic effect of this compound.

Under the influence of corrective treatment on the investigated pathological process with L-ornithine, the following changes were observed in renal cortex: the level of TBA-reactive substances decreased by 62.19% ($p<0.001$) compared with animals without correction, which remained 17.49% ($p<0.05$) higher than in control group. In renal medulla this index decreased by 41.98% ($p<0.01$) and reached the level of control group after the treatment with L-ornithine. According to the content of TBA-reactive substances in renal papilla, the following should be noted: under the influence of L-ornithine this index decreased by 61.91% ($p<0.001$) comparing to the group of animals without correction, which was not statistically significantly different from the control level ($p> 0.05$).

Therapeutic use of L-arginine did not lead to significant changes in the content of TBA-reactive substances relative to the group of animals without correction. Comparing with the control group of animals, in renal cortex this index exceeded the control level by 61.47% ($p<0.001$), in renal medulla — by 54.05% ($p<0.001$), and in renal papilla — by 76.05% ($p<0.001$). The results, obtained in renal cortex and renal medulla, were 33.22 % and 32.77% lower, respectively, than after the administration of L-ornithine ($p_1<0.05$), and 63.57% higher than in the renal papilla ($p_1<0.001$).

The combined use of these amino acids with therapeutic purposes in rats with acute toxic hepatitis contributed to the following changes in the content of TBA-reactive substances in the kidney tissues: in renal cortex this index decreased by 68.95% ($p<0.001$) relative to animals without correction, in renal medulla — by 70.05% ($p<0.05$), and in renal papilla the differences were not statistically significant ($p>0.05$). At the same time, the concentration of TBA-reactive substances in renal cortex remained 34.36%, ($p<0.001$) higher, comparing to the control group, in renal medulla — 38.02% ($p<0.001$), and in renal papilla — 26.94% ($p<0.05$) higher, respectively. Comparison of data in all experimental groups with each other showed that after the combine use of investigated amino acids, the content of TBA-reactive substances in renal cortex was not significantly different from that, obtained after individual administration L-ornithine and L-arginine. In renal medulla this index became statistically significantly higher, than with the introduction of L-ornithine (18.95%, $p_1<0.05$), and in renal papilla — 72.10% lower, than after administration of L-arginine ($p_2<0.01$).

DISCUSSION

As can be seen from the above, the results of our investigation showed that the modeling of acute toxic hepatitis is accompanied by synchronous injury of renal parenchyma, which is manifested by the development of hepato-renal syndrome with a significant increase in the content of primary and secondary products of LPO in homogenates of renal cortex, renal medulla and renal papilla. The obtained data are consistent with the results of studies of other authors [9, 10, 11]. The use of L-ornithine and L-arginine for the corrective purposes is accompanied by a pronounced positive effect. Moreover, it should be noted, that evaluating the changes of DC content, it was observed, that therapeutic potency was more significant after combined use of investigated amino acids, while L-ornithine administration was more effective relative to the content of secondary products of LPO. According to present knowledge, L-ornithine has the ability to enhance the regeneration processes in the affected cells of the body by stimulation the synthesis of spermine and spermidine. However, many scientists believe that this effect is possible

only with the normalization of liver hemodynamics [12, 13], which in this case is obviously related to the stimulation of nitric oxide (NO) synthesis in endothelial cells. The physiological role of NO is the relaxation of the smooth muscle of the blood vessels, which leads to improvement of microcirculation and hepatic circulation. The link between the ornithine cycle and the nitric oxide cycle is the amino acid L-arginine, which is a direct precursor of L-ornithine and a substrate for NO-synthase, an enzyme that catalyzes the synthesis of nitric oxide. As a result, it can be assumed that this complex of amino acid substances contributes to the formation of the required pool of L-arginine, as a precursor of NO, which under experimental conditions obviously contributes to the improvement of microcirculation, leading to a faster restoration of cell integrity and functional capacity of liver cells. As a lipophilic molecule, NO easily diffuses through cell membranes into the neighboring cells (e.g. from endothelial to monocytes of vessels), where cyclic guanozine monophosphate decreases the concentration of free calcium and activates myosin light chain kinase causing vessel dilatation [14, 15]. Recent study demonstrated that L-arginine supplementation in type II diabetic rats was beneficial by preserving glomerular filtration rates, presumably via increased renal endothelial nitric oxide synthase levels, that leads to renal vasodilation [16]. At the same time, L-arginine in the standard therapy facilitates the correction of lipid peroxidation processes and reduces the severity of microalbuminuria [17].

The beneficial effect of L-ornithine is also described in a number of trials in patients with hepatic encephalopathy. A 2017 randomized clinical trial compared the effect of intravenous L-ornithine versus placebo in reverting over overt hepatic encephalopathy (OHE) at day 5 of treatment in a total of 193 patients with cirrhosis. The authors conclude that L-ornithine shortens the recovery time from OHE and the duration of hospitalization [18]. A meta-analysis of L-ornithine versus placebo or other interventions (lactulose, probiotics, and/or rifaximin) included 26 randomized clinical trials involving 1783 patients [19]. L-ornithine had a beneficial effect on hepatic encephalopathy (RR 0.60, 95% CI 0.44–0.82) and was associated with reduced mortality (RR 0.42, 95% CI 0.22–0.84).

Consequently, it may be summarized that the amino acids L-arginine and L-ornithine act as synergists, potentiate each other, and their combined administration leads to the normalization of the structural and functional capacity of the liver and kidney, affected by carbon tetrachloride.

CONCLUSIONS

1. First of all, it should be noted, that the amino acids L-arginine and L-ornithine are able to alleviate the pathogenic mechanisms of toxic effects of carbon tetrachloride on the liver and kidneys.
2. These investigated substances, both individually and in combination, have the ability to reduce the activity of lipid peroxidation processes in the functional layers of the kidneys, which have a beneficial effect on the stabilization of cell membrane and the restoration of functional capacity of the affected kidney cells.
3. This indicates the absence of toxic effects of L-arginine and L-ornithine on nephrocytes, which is one of the most important requirements for modern drugs for the treatment of hepato-renal syndrome.

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