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Analysis Of The Influence Of Alcohol-Induced Stress On The Quantitative And Qualitative Composition Of Amino Acids Related To S-Adenosylmethionine And Glutathione Transformations

Abstract

Studies of preparations that decrease oxidative stress and, as a consequence, that can prevent or reduce the development of alcoholic liver disease are relevant. A wide range of drugs, the bioprotective effect of which is studied, in its action is associated with natural antioxidant systems. Therefore, the study of the features of these systems is necessary for the effective development of bio protectors. The aim is to analyze changes in the quantitative and qualitative composition of amino acids involved in antioxidant mechanisms in the presence of alcohol-induced stress in rats. In the presence of alcohol-induced oxidative stress, there are changes in the quantitative and qualitative composition of amino acids (methionine, serine, taurine), which are involved in the mechanisms of antioxidant protection - cycles of S-adenosylmethionine and glutathione. A slight increase in methionine levels in the blood serum of animals of the experimental group and disruption of the recovery cycle of methionine under alcohol-induced oxidative stress are arguments for the ineffectiveness of S-adenosylmethionine as a bioprotective substance. The same decrease in the level of serine (by 15%) and taurine (by 13%), and analysis of literature data, may be indicate the "secondary" nature of glutathione as an antioxidant compared to taurine.

INTRODUCTION

It is known that alcohol consumption causes oxidative stress in body tissues - in particular in liver tissues. In hepatocytes, oxidative stress directly damages mitochondria, causing cell death; in Kupffer cells, it increases their sensitivity to lipopolysaccharide. Thus, strategies based on reducing oxidative stress can prevent or reduce the development of alcoholic liver disease (Nagy et al., 2016). Some of the remedies for oxidative stress that are being studied today are drugs S-adenosylmethionine (SAM), glutathione (GSH), methionine, taurine, betaine, and others. The action of the above drugs on the body in the presence of oxidative stress is directly related to and relies on the action of natural antioxidant mechanisms - the cycles of SAM and GSH. However, our research suggests the different effects of these mechanisms and their individual components under the action of oxidative stress. Therefore, this study aimed to analyze the effect of alcohol-induced stress on the quantitative and qualitative composition of amino acids that are associated with the transformation of bio protectors.

MATERIAL and METHODS

The experiments were performed on ma le rats with a live weight of 180–220 g, from which 3 groups were formed (7 animals each): a control group, a group that used an aqueous solution of ethanol (1st experimental group), a group that used an aqueous solution of ethanol and bio protector (2nd experimental group). For 28 days, all rats received the standard food "Purina rodent chow". Animals of the control, 1st, and 2nd experimental groups were also given per os ad libitum water, the aqueous solutions of ethanol A (30% v / v; 8 g / kg body weight) and B (A + Sulfur-containing bio protector betaine in a final concentration of 1%), respectively. At the end of the experimental period, the animals were euthanized by deep chloroform anesthesia. Animal experiments conducted in compliance with the requirements of the Law of Ukraine “On Protection of Animals from Cruelty” (Article 230 of 2006), “General Ethical Principles of Animal Experiments” approved by the National Congress of Bioethics and by The European Convention on the Protection of vertebrate animals, which used in experiments and other scientific purposes (Strasbourg, 1986) (Zakon et al.,2006). The study of amino acid levels in the serum of rats was performed on an amino acid analyzer T-339, (Prague, Czech Republic), and such oxidoreductases as lactate dehydrogenase (LDH, EC 1.1.1.27), superoxide dismutase (SOD, EC 1.15.1.1) and catalase (EC 1.11.1.6), according to the described methods (Kalachnyuk et al., 2011; Korolyuk et al., 1988). The content of TBA-active compounds (malonic dialdihydride, MDA) was determined by reaction with thiobarbituric acid (Stal’naya and Garishvili, 1977). Statistical analysis of the data was performed according to Student's criteria using the computer program "Microsoft Excel-2003".

RESULTS and DISCUSSIONS

Decreased SOD and catalase activity and increased MDA (in the blood serum and liver tissues of the 1st experimental group of rats, respectively) indicate the presence of alcohol-induced oxidative stress. The activity of LDH in the serum of rats increases almost 2 times (Table 1). It indicates the functional and structural changes in liver tissues. In animals of the 2nd experimental group, under the use of bio protector, these indicators were close to control ones. Also in the blood serum of the 1st experimental group of animals, there were changes in the level of amino acids associated with the SAM and GSH cycles, namely: an increase in methionine and a decrease in serine, cystine (dipeptide consists of two molecules of cysteine), taurine.

Under the use of bio protector, their level in the 2nd experimental group approached the level of the control, which indicates the involvement of these amino acids in antioxidant protection (Figure 1).

Table 1. SOD, catalase and LDH activity and MDA content in the blood serum and liver tissue of rats (in control and two experimental groups of animals; M±m, n=7)

Groups of animals →	**Control**	**Experimental**	
Biochemical parameters ↓		**1**	**2**
SOD, U mg^{-1} of protein min^{-1}	260 ±21.2	148.5±15.3**	220.2±14.2#
Catalase, U mg^{-1} of protein min^{-1}	239.8±11.3	139.3±9.1**	179.7±12 .1*#
LDH, U L^{-1}	489±18.2	990±28.3**	610±19.1**##
MDA, nmol mg^{-1} of protein	40.9±2.3	56.3±4.1*	42.1±1.8#

Note: data are statistically significant (*p<0.05 and **p<0.001) compared with the control group and #p<0.05, and ##p<0.001 compared with the 2nd experimental group, respectively.

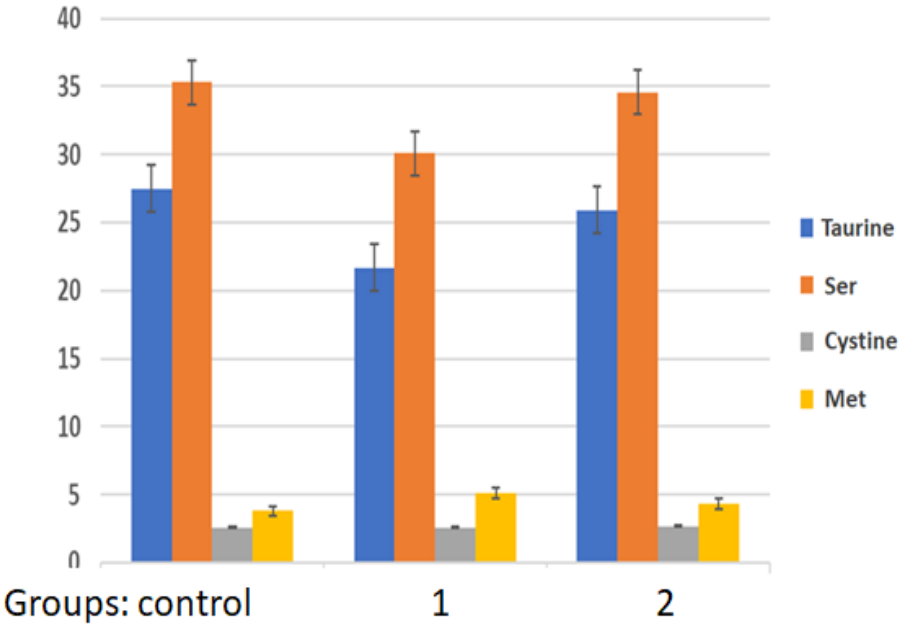

Figure 1. Level of some amino acids (μmol/L), involved in Met–transformation in the blood serum in control, experimental (1 and 2) groups of rats (M±m, n=7; *p<0.05**)**

However, it should be noted that the levels of amino acids in the blood serum of the 1st experimental group of rats changed unevenly. Cystine and methionine levels changed slightly compared to control, while taurine and serine decreased by 13% and 15%, respectively. Methionine: its level in the blood serum of the 1st experimental group compared to control increased slightly. It is a part of SAM. When interacting with toxic products, under oxidative stress, it is converted to homocysteine, which can be reduced to methionine in two ways - through folate-dependent transformation (which also involves serine) or through interaction with

betaine. However, ethanol (which is the cause of oxidative stress) inhibits methionine synthase (Figure 2, #5) - an enzyme that catalyzes the formation of methionine from homocysteine (Waly et al., 2011). A small increasing in methionine levels may be further evidence of the inactivity of the SAM-mediated antioxidant mechanism under alcohol-induced stress.

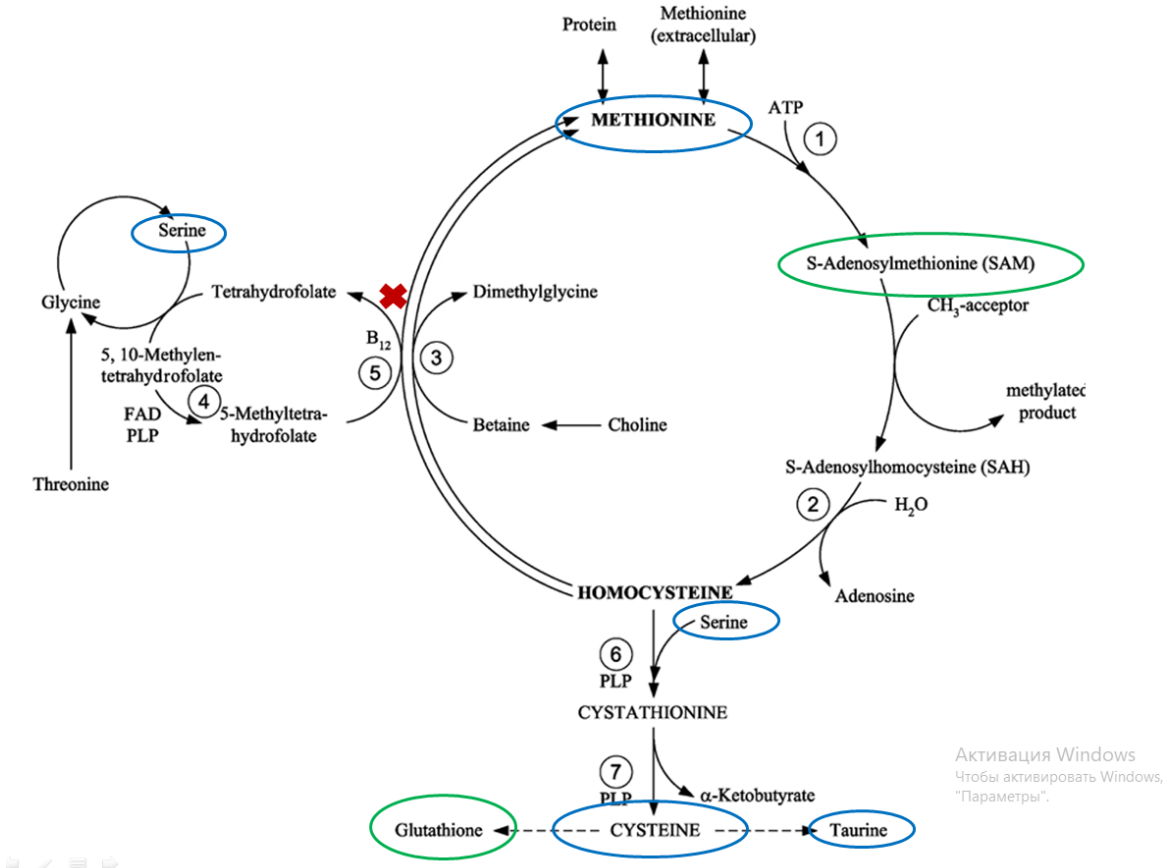

Figure 2. Methionine cycle and intermediates (adapted, Methionine)

S-Adenosylmethionine (SAM) is one of the most important bio protector and natural antioxidant. Two genes (MAT1A and MAT2A) encode an important enzyme, methionine adenosyltransferase (MAT), which catalyzes the biosynthesis of S-adenosylmethionine (SAM), a major methyl donor and, in the liver, a precursor of glutathione. MAT1A is expressed primarily in the liver, while MAT2A is widespread. MAT2A is induced in the liver during periods of rapid growth and differentiation. In human hepatocellular carcinoma (HCC), MAT1A is replaced by MAT2A. MAT2A expression is associated with lower SAM levels and faster growth, while treatment with exogenous SAM inhibits growth Rats treated with ethanol intragastrically for 9 weeks also showed relative changes in hepatic MAT expression, decreased SAM levels, c-myc hypomethylation, increased c-myc expression, and increased accumulation of DNA strand breaks. MAT in the liver due to a decrease in MAT1A expression and inactivation of isoenzymes encoded by MAT1A, which leads to a decrease in SAMe biosynthesis (Lu et al., 2002; Lu and Mato, 2005). It can be assumed that antioxidant protection is not the main function of SAM. The use of SAM as consumable leads to disruption of genetic

processes that disturbs the stability of the organism. Such a solution is not the most profitable from the point of view of evolution. Glutathione (GSH) is a ubiquitous intracellular peptide with diverse functions that include detoxification, antioxidant defense, maintenance of thiol status, and modulation of cell proliferation. The major determinants of GSH synthesis are the availability of cysteine, the sulfur amino acid precursor, and the activity of the rate-limiting enzyme, glutamate-cysteine ligase (GCL). GCL is composed of a catalytic (GCLC) and modifier (GCLM) subunits and they are regulated at multiple levels and at times differentially. The second enzyme of GSH synthesis, GSH synthase (GS) is also regulated in a coordinated manner as GCL subunits and its up-regulation can further enhance the capacity of the cell to synthesize GSH. GSH synthesis is regulated primarily by gamma-glutamylcysteine synthetase activity, cysteine availability, and GSH feedback inhibition. Oxidative stress is well known to induce the expression of GSH synthetic enzymes (Guoyao et al., 2004; Shelly, 2008). Serine: its level in the blood serum of the 1st experimental group of rats decreased by 15%, indicating the involvement of antioxidant systems with which this amino acid is associated, in combating oxidative stress. Antioxidant systems with which serine is associated include SAM, GSH, and taurine (S-containing amino acid with antioxidant properties). Given the inhibition of methionine synthase, it is likely that in this case 15% of serine deficiency is associated with the synthesis of cysteine - a source of GSH and taurine. Serine deficiency alone does not lead to oxidative stress, but oxidative stress and inflammation are exacerbated by serine deficiency (Wang et al, 2020). It is important to note that serine supplementation restores level of GSH and reduces the accumulation of reactive oxygen species. But when glutathione synthesis was suppressed, such effects were not observed (He et al., 2020). Need to clarify - could to inhibition of glutathione synthesis suppress taurine synthesis in this experiment? Cystine: its level in the blood serum of the 1st experimental group of rats compared to control almost did not decrease, which can be explained by a 15% decrease in serine levels. Taurine: its level in the blood serum of the 1st experimental group of rats compared to control decreased by 13%. Known that taurine relieves alcohol-induced oxidative stress (Goc et al., 2019), inhibits the decrease in GSH levels in As-induced stress due to the action of arsenic on the body Li et al., 2019). Taurine improves lipid metabolism increases resistance to oxidative stress - it decreases the level of holesterol and triacylglycerols in serum of blood (Wang et al., 2020). Taurine can be used in combating chronic oxidative stress caused by high carbohydrate diet and endoplasmic reticulum stress - 2% taurine supplementation improved antioxidant status by reducing malondialdehyde content, increasing catalase activity and overall antioxidant capacity (Zhang et al., 2021). Taurine supplementation markedly increases the hepatic glutathione (GSH) levels, compared to the levels in the stress group. In addition, activities of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were improved in the taurine-treated group (Lee et al., 2019). This could mean that taurine plays a more important role in protection against oxidative stress than GSH. The taurines could alleviate the hepatic oxidative stress, with the presence of lower content of malondialdehyde ($P < 0.05$), higher content of glutathione, and an increased glutathione peroxidase (GSH-Px) activity (Han et al., 2020). So given the reduction of taurine by 13%, which almost corresponds to a decrease in 15% of serine, which is a precursor to GSH and taurine, suggests that taurine is a "first-line" antioxidant system - compared to GSH, and in the absence of SAM. The level the investigated amino acids in the blood serum

of animals under betaine effect has tendency to change in direction to their control values. Bio protector use averts significantly decrease levels of taurine and serine and prevents increasing the methionine level in the blood serum under effect of alcohol-induced oxidative stress.

CONCLUSION

In the presence of alcohol-induced oxidative stress, there are changes in the quantitative and qualitative composition of amino acids (methionine, serine, cysteine, taurine), which are involved in the mechanisms of antioxidant protection (cycles of SAM and GSH, which are part of the methionine cycle). Analysis of experimental and literature data suggests that the body's antioxidant systems are not equally effective because of there are more involved (taurine), less involved (GSH), and generally not involved under certain factors (SAM; for ethanol-induced oxidative stress). Further study of this issue will enable a more effective selection of strategies for protection against oxidative stress, allowing the focus of research in promising areas, which will have a significant impact on the development of bioprotective preparations.

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