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RESEARCH ARTICLE

Lithium ascorbate as a protector of human blood biomolecules under ethanol impact

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ABSTRACT

Background: Alcoholism is an acute social problem worldwide for negative impact on health. This problem is closely associated with social stress and drinking traditions. Previously, it was established exhaustion of blood antioxidant in alcoholism patients. It requires development and investigation of substances with protective properties under ethanol impact, on both cells and biomolecules. **Aims and Objectives:** Lithium salts are widely used in medicine as mood stabilizers for mental pathologies, including alcoholism. In this work, we investigate lithium ascorbate for the protection of human blood plasma lipids and proteins under ethanol impact. **Materials and Methods:** Well-known antioxidants - carnosine and ascorbic acid - were used as reference drugs. Heparinized venous blood was used as an experimental substrate. The ethanol and tested substances in the form of water (physiological)solutions were added to blood *in vitro*. Protection effects were measured and estimated as concentrations of carbonyls proteins and products of lipid peroxidation in blood plasma. **Results:** It was shown similar protective action of lithium ascorbate and carnosine on human plasma biomolecules against damaging action of ethanol. No protective effect revealed for ascorbic acid. **Conclusion:** Lithium ascorbate and carnosine were proved to possess the protection effects for blood lipids and proteins under ethanol impact. Normothymic and blood-protective properties are desirable for psychotropic drugs, and thus, lithium salts could be considered as prospective agents in the treatment of addictions and other pathologies associated with oxidative stress.

KEY WORDS: Lithium Ascorbate; Normothymic; Carnosine; Ethanol; TBA-reactive Products; Carbonyls Proteins; Blood Protection; Alcoholism

INTRODUCTION

Alcohol abuse and alcoholism are one of major health and social problems in the world for the severity of the impact and the negative impact on health, especially for young.[1] This problem is closely associated with social stress and drinking traditions and applied to different nationalities.[2] Exposure to ethanol has a negative impact on the functioning of all organs and systems, resulting in multiple biochemical abnormalities. Recent research confirms a reduction of antioxidant activity of blood serum takes place in the alcoholism pathogenesis.[3] In a large extent, it depends on the consumption of antioxidants and overall nutrition level.[4] From other side, oxidative stress and free radicals play an important role in the pathogenesis of many somatic and psychopathological processes, including depressive disorder[5] and alcoholism.[6] It was shown that oxidative stress contributes to the formation of comorbid somatic complications in patients with alcoholism.[7]

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It is known that ethanol (alcohol)and its metabolic products have a damaging effect on biomolecules.[7] There is a direct correlation between the level of oxidation of blood plasma proteins (carbonylation)and the severity and expressivity of withdrawal symptoms. The damaging effect of ethanol on the blood plasma biomolecules directly affects the complex homeostatic functions of blood. This requires the development of methods for the protection of the blood macromolecules against damaging agents, including ethanol.[8]

The oxidation of biomolecules plays a significant role in the clinical course of alcoholism, so study the effects of drugs on this parameter is highly important as well as the development of new drugs with protective properties against the effects of ethanol. In this study, we carried out the study of lithium ascorbate, as well as a number of known antioxidants with proven effect as a protective agent. Commonly, lithium salts used to treat bipolar psychosis and affective disorders. Presumably, applications of the new lithium salt with antioxidant activity significantly improve the effectiveness of lithium drugs. The following water-soluble compounds - lithium ascorbate, carnosine, and ascorbic acid as a standard antioxidant were used for comparative study of blood compounds protection against ethanol.

The antioxidant and electrochemical properties of lithium compounds have been studied in the previous works.[9,10] Recently, it was shown a low toxicity and hemo-protective properties of lithium ascorbate, which is an important advantage for medical application.[10]

MATERIALS AND METHODS

The following substances have been used in the experiment: Lithium ascorbate (synthesized in laboratory), carnosine - the dipeptide β-alanyl-L-histidine (Yonezawa Hamari Chemicals, Japan), as reference drug and ascorbic acid (Sigma-Aldrich, Germany)as a standard and widely used antioxidant (Figure 1). Lithium ascorbate was prepared *ex tempore* in reaction with the calculated amount of lithium carbonate and ascorbic acid. The product of reaction was salted out from water solution by ethanol. Obtained crystalline powder was dried and used in further experiments. The composition of lithium ascorbate was confirmed by atomic emission spectrometry, infrared spectroscopy, and thermogravimetry.

Heparinized venous blood of 15 healthy men (average age 37.9 ± 3.9 years)was used as biosubstrate for the experiment.

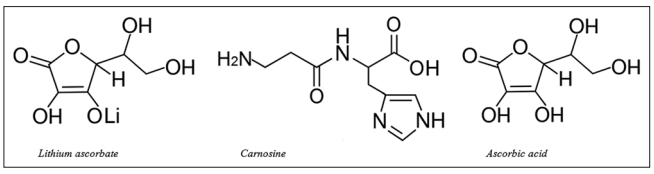

Figure 1: Structural formulas of investigated compounds

All donors were without chronic somatic diseases and not drinking alcohol, at least, the past 10 days before the study. Informed consents of all donors were obtained before blood sample collection.

All studied substances were dissolved in a saline (0.9% NaCl) before testing. Solutions of substances in a volume of 0.15 ml were added to the appropriate blood samples containing 1 ml of blood. After that, blood samples were mixed and added with 0.1 ml of saline (in sample without ethanol) or ethanol in saline to a final concentration of 0.5% (in ethanol-treated samples). Negative control samples were added with saline in a volume of 0.25 ml. Positive control samples were added with saline in a volume of 0.15 ml and 0.1 ml ethanol in saline to a final concentration of 0.5% ethanol in blood samples. Initial concentrations of the test components were adjusted so that the final concentration in the blood was 0.6 mmol/l. Concentration of 0.6 mmol/l is selected to determine the protective effect of lithium salts in a concentration corresponding to a therapeutic blood concentration of lithium in psychiatric practice. Typically, during the treatment of mental patients, the level of lithium is controlled in the range of 0.6 mmol/l, taking care not to exceed the critical level of 1.2 mmol/l. The reference drugs were also tested at a comparable concentration of 0.6 mmol/l.

After addition of test substances, the blood samples were incubated at 37°C for 1 h and centrifuged at 3000 g for 15 min for separation of blood plasma. The obtained plasma samples were evaluated for concentrations of protein carbonyls and products of lipid peroxidation. Protein carbonyls were measured after treatment of the proteins with 2,4-dinitrophenylhydrazine according to known method.[11] Products of lipid peroxidation were detected in reaction with thiobarbituric acid,[12] so these products have named as TBA-reactive products. The optical density of samples was measured by the spectrophotometer UV-VIS 1201 (Shimadzu, Japan).

Statistical analysis was performed by the "Statistica 10" software. Results are presented as means ± standard deviation. Non-parametric Wilcoxon test for dependent groups was applied to determine significant differences between data sets. Data sets with $P < 0.05$ were considered to be statistically significantly different.

RESULTS

The main results of the study are presented in Table 1. It was shown that the addition of the blood samples at 0.5% ethanol results in increasing of oxidized (carbonylated)proteins and lipid peroxides in the plasma to compare the negative control and the positive control. It is consistent with previously published data on the damaging *in vitro* effect of ethanol on blood lipids, proteins,[8] and erythrocytes.[13]

Table 1: Influence of tested substances on blood plasma protein carbonyls and TBA-reactive products under action of 0.5% ethanol

Substance	Carbonylated proteins, nmol/mg	TBA-reactive products, nmol/ml
Negative control (blood without ethanol)	0.26±0.01	3.2±0.1
Positive control (blood with ethanol)	0.33±0.02#	4.0±0.2#
Lithium ascorbate 0.6 mmol/l (blood with ethanol)	0.29±0.01*	3.5±0.1
Ascorbic acid 0.6 mmol/l (blood with ethanol)	0.30±0.02#	3.9±0.3#
Carnosin 0.6 mmol/l (blood with ethanol)	0.29±0.01*	3.5±0.1*

n=15, **P*<0.05 versus positive control, #*P*<0.05 versus negative control, presented as mean±SD, SD: Standard deviation

The final concentration of protein carbonyls and TBA-reactive products in plasma markedly decreased (compared to the positive control)in the blood samples with pre-addition of ascorbate lithium. The decrease in both indicators in samples with ascorbate lithium relative to positive control was statistically significant. A similar protective effect on blood plasma proteins and lipids from an ethanol-induced oxidative modification was observed under carnosine influence.

In contrast, in the samples with ascorbic acid, there was no significant decrease in the level of carbonyls of proteins, as well as of TBA-reactive products, as regard to positive control. Statistically, there was no difference between positive control and samples with ascorbic acid.

Thus, in the presence of lithium ascorbate as well as carnosine, the addition of ethanol does not result in damage to plasma biomolecules, since concentration of carbonylated proteins and TBA-reactive products in the samples was not significantly different from negative control sample. However, ascorbic acid showed a moderate protective effect. In the samples treated with ascorbic acid, both carbonylated proteins and lipid peroxides concentrations slightly exceeded the negative control but were much less than positive control values (Table 1).

DISCUSSION

The questions related to direct biological effects of substances remain discussable due to plenty of different interaction in living cells and condition of experiments. These problems are, especially, complex in an attempt to extrapolate *in vitro* results to *in vivo* condition. To minimize negative influence, here, we apply blood to experiment immediately after collection. All substances were tested in standard medical concentrations, close to those in patients' blood under treatment. According to results, lithium ascorbate effectively protects the blood proteins and lipids from the damaging effects of ethanol. It is known fact that lithium has cytoprotective properties due to influence on specific enzymes and stimulation of genes expression. Lithium also induces indirect antioxidant effects[9,14,15] and provides complex protective effects on biological structures in cell cultures. The protective influence of lithium ions partly explains a decrease in carbonylated protein concentration. Probably, lithium ions in these experiments have an additional effect *in vitro*, which leads to an increase in the protective effect on the biomolecules of the blood plasma from the oxidative damage induced by ethanol. This can play a role in the positive effects of lithium in the therapy of alcoholism. Noteworthy, the effect of lithium *in vivo* is mediated, among other things, by a marked effect on the enzyme systems of cells, and the likely effect on the expression of neuroprotective proteins.[16] However, there is yet no full explanation for this phenomenon. From other side, ascorbate has significant antioxidant properties and reduces radical oxidation of substances. Recent work revealed inhibition of oxidative stress and apoptosis in cortical cells[17] and concluded that the protective effect of ascorbate might be through attenuation of reactive oxygen species production and autophagy. However, some authors showed apoptosis-induction properties under the influence of ascorbate, involved an increase in the levels of p53, p21, cellular Ca^{2+}, and activation of caspase 3.[18] Regarding the extracellular environment, we revealed a significant protective influence of lithium ascorbate on blood biomolecules (Table 1). This effect could be linked to various oxidation level of lithium ascorbate compared to ascorbic acid. In some cases, the oxidation of ascorbate produces a hydrogen peroxide through the formation of ascorbate radical in cell cultures.[19] The rate of oxidation of ascorbate is strongly depending on the level of catalytically active metals, first of all, tissue iron. Redox metals may play an important role in oxidative damage of biomolecules that eventually lead to different results. Recently, authors (Ma and Davidson)showed that ascorbate protects the diheme enzyme, MauG, against self-inflicted oxidative damage and revealed the novel mechanism by which ascorbate mitigates oxidative damage to heme-dependent enzymes and redox proteins in nature.[20] Contradictory results of ascorbate application from growth stimulation to induction of cell death, however, mainly depend on experimental structure, dosage, cell type, and form of ascorbic salts.

The similar results were obtained using natural antioxidant carnosine. Carnosine also significantly reduced plasma concentration of oxidized biomolecules (Table 1). Such effects of carnosine were obtained in the findings of Yarygina and Prokopyeva,[8] where the ability of carnosine

to protect proteins and lipids of the blood plasma from the oxidative damage induced by ethanol and its main metabolite acetaldehyde was revealed. Carnosine in our study was selected as the reference drug since its antioxidant properties are well known. In a review,[4] the main properties and biological effects of the antioxidant carnosine are considered and assessed the role of ethanol and its metabolite acetaldehyde in the formation of oxidative stress in patients with alcoholism, as well as the correction of oxidative stress with carnosine in alcoholic patients. Carnosine can also have an impact on the antiradical protection system of the organism. Results of experiments on rats showed that carnosine accelerates metabolizing of cortisol and noradrenaline released into the blood of animals under stress, this showing the mediation effect of carnosine. A decrease in level of stress hormones in blood leads to decrease in severity of oxidative stress.[21]

It was shown regulating influence of carnosine on enzymes whose function is associated with free radical compounds.[22] However, such mechanisms are difficult to realize *in vitro*. Therefore, in the current study, the antioxidant effect of carnosine, as well as of other substances studied, was most likely realized because of their ability to scavenge of free radicals, thus protecting plasma molecules (proteins and lipids)from oxidative damage initiated by ethanol addition to blood samples.

It is well known that carnosine is a low molecular weight hydrophilic antioxidant of direct action[23] and also possesses anti-glycation properties[24] and anti-crosslinking effects.[25] These properties are, in fact, also a reflection of its antioxidant properties. There is also evidence that carnosine acts as quenchers of reactive and cytotoxic carbonyl species through its ability to form adducts with them. This suggested that carnosine is a protector of biomolecules from oxidative carbonyl stress.[26] Ascorbic acid is also a hydrophilic natural antioxidant of direct action. Molecular mechanisms of the antioxidant effect of ascorbic acid in the body are largely related to its ability to reduce the α-tocopheryl radical, thereby returning α-tocopherol (Vitamin E)antioxidant properties.[27,28]

Carnosine, unlike ascorbic acid, is not able to regenerate oxidized forms of Vitamin E.[29] Thus, the molecular mechanisms of the antioxidant action of these substances are different. Lithium salts possess a direct antiradical action against reactive oxygen species.[14] However, experiments with a direct comparison of the antioxidant effects of carnosine and ascorbic acid shown that these substances have a close ability to extinguish the superoxide anion action.[30]

Both carnosine and ascorbic acid have the ability to protect biological membranes from the damaging effects of peroxidation, and they are a buffer for metals of variable valence.[31] The ability of carnosine and ascorbic acid to regulate the level of mixed-valence metal ions in the organism is important property of them that confirms their antioxidant status.

It is important to note that the results accumulated to date on the effects of antioxidants are contradictory. Depending on the conditions and concentration, antioxidants may exhibit a prooxidant effect. Under certain conditions, for example, in the presence of mixed-valence metal ions, the prooxidant effect is shown by ascorbic acid,[32] as well as carnosine.[33] Specific properties of ascorbic acid could take part of its effects in cell culture due to low stability and acidity.[34] In this study, carnosine was more effective than ascorbic acid, and the protective effect of lithium ascorbate salt is comparable to that of carnosine. In the presence of lithium salt, the addition of ethanol did not increase oxidative damage to plasma biomolecules.

The results confirm a protective effect of lithium salt from the damaging effects of ethanol. The protective effect of lithium ascorbate is superior to the action of ascorbic acid. That fact is not explained only by antioxidant action. The revealed effects may indicate a number of indirect influences and advantages of the lithium salts of organic acids, taking into account adverse drug reaction of lithium carbonate.[35] Investigation and application of organic lithium salts are a way to reduce known lithium toxicity.

CONCLUSION

The experimental verification of the ability to protect biomolecules in human plasma from damaging oxidative action of ethanol using lithium ascorbate was fulfilled *in vitro*. Positive results allow predicting protective effects of lithium antioxidant *in vivo* and encouraging further experiments in this field. Protection of proteins and lipids from the damaging impact of ethanol is important effect taking into account alcoholism severe consequences. Normothymic and blood-protective properties of lithium salts could be prospective in the treatment of addictions and other pathologies associated with oxidative stress.

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