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Long-term alcohol consumption provokes oxidative and nitrosative stress in albino rats brain

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Key words: long-term alcohol consumption, brain, rats

Introduction. At present alcohol has become an important socio-medical problem due to its massive uncontrollable consumption in the world. Chronic alcohol consumption causes serious consequences for entire nervous system functioning and, particularly, the brain [1], associated with metabolic processes complex violations [2]. In adult human chronic alcoholics, brain damage is characterized by cerebral and cerebellar atrophy, and impaired neuronal function within the hippocampus and frontal cortex. Besides specific alcohol-related disorders, such as Wernicke–Korsakoff syndrome, hepatic encephalopathy and pellagra, heavy alcohol consumers exhibit cognitive and motor impairments, cholinergic deficits and dementia [3]. It is estimated that 50–75 % of long-term alcoholics show cognitive impairment and structural damage to the brain, making chronic alcoholism the second leading cause of dementia behind Alzheimer's disease [3]. Neuropathological analyses have provided evidence for loss of neurons in certain regions of the brain of alcoholics [4].

Among several proposed mechanisms of brain damage at chronic alcoholism, should be mentioned accumulation of DNA damages in the absence of repair processes, resulting in genomic instability and death of neurons [3]. The processes of alcohol metabolism are also connected with generation of reactive oxygen species (ROS) and nitric oxide (NO) via induction of NADPH/xanthine oxidase, nitric oxide synthase (NOS), and cytochrome P450 2E1 (CYP2E1) [5, 6]. Additionally, ethanol activates and recruits toll-like receptors (TLR)4 within the lipid rafts of glial cells, triggering the production of inflammatory mediators and causing neuroinflammation [7].

It is very important to note that synaptic changes promoted by ethanol are mediated directly and indirectly by acetylcholine, dopamine, serotonin, glutamate, GABA and other neurotransmitters [8].

However, despite of intensive investigation of alcoholism consequences for nervous system, the full complex of such metabolic alterations remains unknown, as soon as limiting and regulating factors of alcohol metabolism in brain [9]. Based on these facts and considering that alcoholism is chronic disease highly prevalent in the world population, the present work reports the influence of chronic ethanol consumption on rats' brain NO-synthase activities, and pro- and antioxidant system parameters.

Material and methods. Wistar albino male rats, body weight (b.w.) of 150 g to 170 g, were used in the study. They were kept under a controlled temperature (from 22 °C to 24 °C), relative humidity of 40 % to 70 %, lighting (12 h light-dark cycle), and on a standard pellet feed diet («Phoenix» Ltd., Ukraine). The study was performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committee. The «Principles of laboratory animal care» (NIH publication № 85–23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the bioethics committee of theEducational and Scientific Centre «Institute of Biology», Taras Shevchenko National University of Kyiv. For the experimental (chronic alcoholism) model, reproducing male rats were selected

according to the method for measuring voluntary alcohol self-administration in rats, which provides a continuous choice between an alcohol solution and water (two bottle preference test) [10]. The six selected rats were used for chronic alcoholism modeling by replacing water with a 15 % ethanol solution during 150 days. Six intact male rats (of the same age and weight) were used as a control. From the beginning of the experiment, they were kept in the same conditions as experimental animals but were given only water ad libitum.

After 150 days, both the experimental and control rats were sacrificed by decapitation under a mild ether anesthesia. Their brains were used for investigation.

Inducible nitric oxide synthase (iNOS) and constitutive nitric oxide synthase (cNOS) activities measure in brains were performed by spectrophotometric method [11]. Concentration of protein in brain fractions was determined by the Bradford protein assay using bovine serum albumin as a standard.

Lipid peroxidation was investigated as the rate of ascorbate-induced thiobarbituric acid reactive substances (TBARS) formation [12], superoxide dismutase activity (SOD) was determined in accordance to Chevary [13], catalase activity was detected using the colorimetric ammonium molybdate method [14].

The obtained data were calculated by one-way analysis of variance (ANOVA) and expressed as the mean ± standard error of the mean (M ± S.E.M.). Data were compared using Tukey test. Differences were considered to be statistically significant at $p < 0,05$.

Results and discussions. Increased cellular oxidative stress and altered antioxidant pool have been implicated at alcoholism. Our data on state of brain's pro- and antioxidant system at alcoholism coincide with data of other authors. Ethanol enhances ROS production in brain through a number of pathways including increased generation of hydroxyl ethyl radicals, induction of CYP2E1, alteration of the cytokine signaling pathways for induction of iNOS and secretory phospholipase A_2 ($sPLA_2$), and production of prostanoids through the PLA_2/COX pathways. Excess of free oxygen radicals could cause brain tissue damage not only via lipid peroxydation, but also proteins denaturation, enzymes inactivation, nucleic acids damage, Ca^{2+} release and cytoskeleton destruction. Influence of free radicals mediates blood-brain barrier functions violations, endothelial dysfunctions with constant vasodilatation [15].

Results on brain nitric oxide synthases activities following 150 days alcohol administration are demonstrated in table. The level of iNOS in ethanol-treated rat's brain was within the normal limits. At the same times cNOS activity was raised 2,8 times as compared with control.

Our results on cNOS activity rates at alcoholism are in good correspondence with other authors data [16]. According to their results glutamatergic system is directly involved into mediating acute and chronic alcohol effects. Its receptors are included into NO signal way, as N-methyl-D-aspartate (NDMA) glutamatergic receptors stimulation caused Ca^{2+} exit from the depot with further calmodulin binding and neuronal NOS (nNOS) activation. nNOS gene is of key importance for regulation behavioral effects of alcohol [16].

As it was reviewed, brain tissue is most vulnerable to oxidative damage caused by its high consumption of oxy-

Table

Levels of iNOS and cNOS activities in rat's brain after 150 days 15% ethanol consumption, M ± S.E.M (n = 6)

Indices	Animal groups	
	Control	Chronic alcoholism
iNOS activity, nM $NO_2^- \cdot min^{-1} \cdot$ mg of protein^{-1}	3,53 ± 0,62	3,32 ± 0,89
cNOS activity, nM $NO_2^- \cdot min^{-1} \cdot$ mg of protein^{-1}	1,92 ± 0,36	5,45 ± 0,31*

**p < 0,05 statistically significant in comparison with control*

gen, a high metabolic rate, and low levels of antioxidant enzymes, such as SOD, glutathione peroxidase, and catalase. A large increase of lipid peroxidation levels is caused by an increase in ROS, because of the brain's high content of polyunsaturated fatty acids that are highly susceptible to oxidation [17].

Data on brain pro- and antioxidant system state are shown in figure.

Compared with the unaffected animals, ethanol-treated rats showed brain pro- and antioxidant system alteration. The chronic 15 % ethanol administration led to increase of TBARS rate formation (42 %). Under this condition the activities of antioxidant enzymes SOD and catalase were decreased 19 % and 25 % respectively.

In the current study the increased TBARS levels in ethanol-exposed rats' brain evidenced oxidative stress development. Several studies have examined the role of oxidative stress in alcohol-mediated neurotoxicity, possibly via the formation of free radicals [18–20]. The free radicals interact with other cell components, such as proteins, DNA, and lipids, to form multiple catabolic products.

An example of these is lipid peroxidation resulting in lipid hydroperoxides and aldehydes that interact with the sulfhydryl groups of proteins causing the loss of protein functionality and thus perpetuating cell damage. The increased levels of calcium and nitric oxide stimulate the production of inflammatory interleukins causing gliosis and increasing the state of oxidative stress causes damage and cell death [21], thus establishing a cycle through a chain of oxidative reactions that could involve both neurons and glia. At the conditions of our experiment we have established the increase of brain biomolecules oxidation with simultaneous decrease in the activity of antioxidant system, which could be evidence of redox balance loosing. It is known that the fate of a synapse depending in part on the redox balance means that if the oxidant-environmental cell produces an oxidative-stressed state and the reactive oxygen species (ROS) cause elimination of spines [22].

Ethanol can react with the OH· radical to form the alpha-hydroxyethyl radical, which is considered to be less toxic. It also can stimulate H_2O_2 degradation through catalase activation [23]. In mammalian brain catalase is the main enzyme that catalyzes the peroxidatic oxidation of ethanol to acetaldehyde, and its inhibition is associated with functional and metabolic disturbances of the central nervous system (CNS) [24, 25]. The information concerning brain catalase activity following experimental chronic alcohol intoxication is insufficient and contradictory. At these circumstances different researchers indicated increase [26], decrease [27] and absence of changes [24] in catalase activity. It could be a result of different animals' models using. We observed decrease

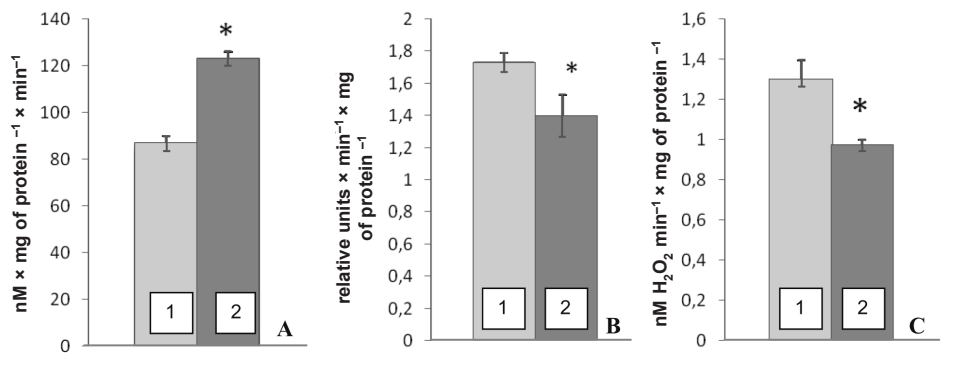

Figure. Rat brain pro- and antioxidant system state after 150 days 15% ethanol consumption (A – TBARS content, B – SOD activity, C – catalase activity; 1 – control, 2 – chronic alcoholism) (M ± S.E.M., n = 6 for each group)

**P < 0,05 statistically significant in comparison with control*

of brain catalase activity in rats chronically exposed to 15 % ethanol solution. Other authors suggest that catalase is likely to be a more major contributor to ethanol oxidation in the brain [28]. Inhibition of brain catalase activity in mice inhibits many of the pharmacologic effects of ethanol, and inhibition or stimulation of catalase activity, respectively, reduces or increases the formation of acetaldehyde in brain homogenates [28].

In our experiments chronic ethanol self-administration significantly decreased SOD activitiy in the brain of experimental animals. Such inhibition of SOD activity by ethanol may allow an accumulation of cytotoxic O^{2-} radicals; this may account for nervous system disorders during alcohol intoxication [29].

We suggest that the decrease in SOD and catalase activities may indicate an oxidative modification of the enzymatic proteins caused by free radicals which are generated during ethanol and acetaldehyde metabolism.

Thus, in the brain of rats due to chronic alcohol intoxication, as a result of the accumulation of reactive oxygen and nitrogen, and antioxidant system depletion, the oxidative stress is developed. Such changes are of particular importance for brain cells characterized by low contents of external and endogenous antioxidants as compared with other tissues. These alterations (together with high levels of polyunsaturated fatty acids) make CNS extremely susceptible to free radical damage.

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O. Kharchenko

Long-term alcohol consumption provokes oxidative and nitrosative stress in albino rats brain

Despite of intensive investigation of alcoholism consequences for nervous system, the full complex of such metabolic alterations in brain remains unknown. *The aim of present work* was to study the influence of chronic ethanol consumption on rats' brain NO-synthase activities, and pro- and antioxidant system parameters.

Wistar albino male rats (body weight 160–200 g) were divided into two groups: I – experimental chronic alcoholism, II – intact animals.

Inducible nitric oxide synthase (iNOS) and constitutive nitric oxide synthase (cNOS) activities measure in brains were performed by spectrophotometric method. Lipid peroxidation was investigated as the rate of ascorbate-induced thiobarbituric acid reactive substances (TBARS) formation, superoxide dismutase activity (SOD) was determined in accordance to Chevary, catalase activity was detected using the colorimetric ammonium molybdate method.

Results of the study suggest that under chronic alcohol consumption during 150 days in rats brain cNOS activity increased 2,8 times and TBARS formation rates rose 42 %. At the same time SOD and catalase activities decreased 19 % and 25 % respectively.

Thus, in the brain of rats due to chronic alcohol consumption, as a result of the accumulation of reactive oxygen and nitrogen, and antioxidant system depletion, the oxidative stress is developed. Such changes are of particular importance for brain cells characterized by low contents of external and endogenous antioxidants as compared with other tissues. These alterations (together with high levels of polyunsaturated fatty acids) make central nervous system extremely susceptible to free radical damage.

Key words: long-term alcohol consumption, brain, rats

О. Харченко

Розвиток оксидативного та нітрозативного стресу в мозку білих щурів за умов тривалого споживання алкоголю

Незважаючи на інтенсивне дослідження наслідків алкоголізму для нервової системи, повний комплекс метаболічних змін у головному мозку залишається остаточно не з'ясованим.

Мета дослідження – вивчення впливу хронічного споживання етанолу на активність NO-синтази та показники про- і антиоксидантної системи в мозку щурів.

Щури-самці лінії Вістар масою 160–200 г були розподілені на дві групи: I – експериментальний хронічний алкоголізм, II – інтактні тварини.

Вимірювання індуцибельної NO-синтази (iNOC) та конститутивної NO-синтази (cNOC) у мозку проводили спектрофотометричним методом. Перекисне окиснення ліпідів було досліджено за швидкістю аскорбат-індукованого утворення продуктів реакції з тіобарбітуровою кислотою (ТБК-реактанти), активність супероксиддисмутази (СОД) визначали за Чеварі, активність каталази визначали колориметричним методом з молібдатом амонію.

У результаті проведених досліджень було показано, що за умов хронічного споживання алкоголю протягом 150 днів у мозку щурів зростала активність cNOC у 2,8 разу та швидкість утворення ТБК-реактантів на 42 % порівняно з інтактними тваринами. При цьому активність СОД та каталази знижувалися на 19 і 25 % відповідно.

Таким чином, внаслідок тривалого споживання алкоголю відбувається розвиток оксидативного стресу в мозку щурів у результаті як накопичення активних форм кисню та азоту, так і виснаження антиоксидантних систем. Особливе значення ці зміни становлять для клітин мозку, що характеризуються низьким вмістом екзогенних і ендогенних антиоксидантів порівняно з іншими тканинами, що в поєднанні з високим рівнем поліненасичених жирних кислот робить центральну нервову систему виключно сприйнятливою до вільнорадикального ушкодження.

Ключові слова: тривале споживання алкоголю, мозок, щури

О. Харченко

Развитие оксидативного и нитрозативного стресса в мозге белых крыс при длительном потреблении алкоголя

Несмотря на интенсивные исследования последствий алкоголизма для нервной системы, полный комплекс метаболических изменений в головном мозге остается окончательно не выясненным.

Цель исследования – изучение влияния хронического потребления этанола на активность NO-синтазы и показатели про- и антиоксидантной системы в мозге крыс.

Крысы-самцы линии Вистар массой 160–200 г были разделены на две группы: I – экспериментальный хронический алкоголизм, II – интактные животные. Измерение индуцибельной NO-синтазы (iNOC) и конститутивной NO-синтазы (cNOC) в мозге проводили спектрофотометрическим методом. Перекисное окисление липидов исследовали по скорости аскорбат-индуцированного образования продуктов реакции с тиобарбитуровой кислотой (ТБК-реактанты), активность супероксиддисмутазы (СОД) определяли по Чевари, активность каталазы – колориметрическим методом с молибдатом аммония.

В результате проведенных исследований было показано, что в условиях хронического потребления алкоголя в течение150 дней в мозге крыс увеличилась активность cNOC в 2,8 раза и скорость образования ТБК-реактантов на 42 % по сравнению с интактными животными. При этом активность СОД и каталазы снижались на 19 и 25 % соответственно.

Таким образом, в результате хронического потребления алкоголя происходит развитие оксидативного стресса в мозге крыс в результате как накопления активных форм кислорода и азота, так и истощения антиоксидантных систем. Особое значение эти изменения представляют для клеток мозга, характеризующихся низким содержанием экзогенных и эндогенных антиоксидантов по сравнению с другими тканями, что в сочетании с высоким уровнем полиненасыщенных жирных кислот делает центральную нервную систему исключительно восприимчивой к свободнорадикальному повреждению.

Ключевые слова: длительное потребление алкоголя, мозг, крысы

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