

HIGH THIAMINE DOSE RESTORES LEVELS OF SPECIFIC ASTROGLIAL PROTEINS IN RAT BRAIN ASTROCYTES AFFECTED BY CHRONIC ETHANOL CONSUMPTION

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Long-term ethyl alcohol consumption induces a deficiency of essential nutrient thiamine (vitamin B₁) and profoundly impairs metabolic processes in nervous tissue, resulting in structural and functional alterations in the central nervous system (CNS). This study was performed to evaluate protective effects of thiamine acute dose on the level of glial fibrillary acidic protein (GFAP), a sensitive marker of astroglia, and B₁-related enzyme thiamine pyrophosphokinase (TPK) activity in brain of rats chronically exposed to ethanol. The rats were divided into three groups as follows: i) control group; ii) rats given 15% ethanol solution as drinking water for 9 months (EtOH group), iii) EtOH rats given thiamine per os in a dose of 2.0 mg/kg one day before experiment termination (n = 4 in each group). GFAP levels were analyzed in cerebellum, brain cortex and hippocampus by western blot and immunohistochemistry. Brain TPK activity was measured with the use of the yeast apopyruvate decarboxylase apoenzyme (apoPDC). Thiamine concentration in liver was estimated with the use of thiochrome method. It was demonstrated that GFAP content was dramatically reduced in all studied brain regions of EtOH-exposed rats (approximately by 60%, $P < 0.05$) compared with control rats indicating profound astroglial dysfunction. Thiamine treatment was shown to recover GFAP levels up to 80% vs. control value in the brain of EtOH-exposed rats ($P < 0.05$). Ethanol consumption resulted in 3.7-fold decrease in liver thiamine content and 1.4-fold decrease in brain TPK activity, as compared with control ($P < 0.05$). Thiamine treatment of EtOH-exposed rats significantly elevated B₁ liver level, however, had no effect on brain TPK activity. Our data suggest that thiamine deficit can play an important role in alcohol-induced damage to brain astroglia. It is emerged that high-dose thiamine administration can represent effective treatment option against chronic effects of ethanol impact on brain structures.

Key words: *chronic ethanol consumption, astrocytes, glial fibrillary acidic protein (GFAP), thiamine (vitamin B₁) deficit.*

Ethyl alcohol is the most used toxicant worldwide. In 2012, over three million deaths were attributed to alcohol consumption, corresponding to 5.9% of the global total or one in every twenty deaths. In addition, 5.1% of the global burden of disease and injury, as measured in disability-adjusted life years, were attributable to alcohol abuse [1]. It is well known that one of the manifestations of alcohol-induced encephalopathy is thiamine (vitamin B₁) deficiency. Thiamine is a water-soluble vitamin, essential nutrition factor for humans and mammals. Thiamine deficiency (TD) accompanies almost

all of known neurodegenerative diseases, including Wernicke-Korsakoff syndrome (WKS), Leigh disease, Alzheimer disease, etc. TD is the critical factor in Wernicke-Korsakoff syndrome development in humans affected by chronic alcoholism [2, 3]. It has been estimated that approximately 80% of alcoholics express TD [4], which if left untreated can lead to Wernicke encephalopathy and ultimately progress into WKS. Over 75% of cases of TD remain undiagnosed, particularly in alcoholics [5]. Furthermore, it has been estimated that approximately 13% of alcoholics have brain abnormalities associated with TD [6].

Long-lasting ethanol consumption causes inhibition of thiamine pyrophosphokinase, an enzyme responsible for intracellular thiamine phosphorylation, resulting in the formation of its bioactive form, thiamine diphosphate (TDP). Chronic ethanol intoxication reduces the TDP formation level thus inhibiting mitochondrial dehydrogenases and transketolases activity in the brain tissues of patients with WKS. Such inhibition results in nerve cells mitochondrial dysfunction and oxidative stress development [7]. It is important to note that TD accompanies most neurodegenerative pathologies not only related to alcohol consumption, but it also can initiate their development [8].

Astrocytes are large star-shaped cells, widely distributed throughout the brain, and performing a plethora of functions in the CNS, including the secretion or absorption of neural transmitters and maintenance of the blood-brain barrier. For example, astrocytes take up brain chemicals (i.e., neurotransmitters) that are released by neurons, and minerals such as potassium, which are generated and secreted during the brain's energy metabolism. In addition, astrocytes eliminate some substances that are toxic to neurons (i.e., neurotoxic). The proper functioning of the astrocytes and their interactions with the neurons are essential to brain function [9]. Metabolic disorders in the processes of interactions between neurons and astrocytes can cause neurodegenerative disorders and a number of other pathologies. That is why normal work and functioning of neurons in the brain depend on the activity of astrocytes. Astrocytes with pathologically modified function can release neurotoxic factors, such as pro-inflammatory cytokines and ROS, thus interfering with intercellular communications and favor pathology progression [10, 11]. Recently, it has been shown that astrocytes are the most sensitive cells to TD. Dysfunction of these cells under B1 deficit conditions plays the leading role in pathophysiological processes reflecting avitaminosis condition development [12]. Glial fibrillary acidic protein (GFAP) is routinely used as the major marker of astrocyte functional state. GFAP is the principal intermediate filaments component of astrocytic cytoskeleton. GFAP is used as a sensitive indicator of ethanol impact and has a high sensitivity for lack of thiamine. For example, short-lasting ethanol consumption during the experiment (10% ethanol solution, 4 weeks) causes astrocytes activation, which results in GFAP-positive cells number increase in brain slices obtained from experimental

animals [13]. Attempts to restore consequences of alcohol intoxication by administration of physiological doses of thiamine (0.08 and 0.4 mg/kg) during several days attenuated liver damage severity but did not completely restore the tissue state to the control level [14]. In the study [15], thiamine high dose (2.5 mg/kg) supplementation has been shown to significantly restore neurotransmitter levels exposed to ethanol during 90 days. Despite the fact that there could be different regimes of thiamine administration, which are currently well-documented in the literature [16-18], to our knowledge, the effects of the high single-dose vitamin administration are reported here for the first time.

The present study was performed to extend the previously fulfilled investigation [19], in which we have modelled the state of alimentary TD and discovered the changes in certain specific proteins in the cerebral cortex, cerebellum and hippocampus. Therefore, the present study was designed to investigate if the treatment with high-dose thiamine administration can be beneficial against ethanol neurotoxicological pattern estimated by expression of specific protein of thiamine metabolism, thiamine pyrophosphokinase (TPK), as well as verified astroglial marker, GFAP. To study the changes in brain tissue, we developed a model of chronic alcohol consumption, which could be considered as pathological condition related to TD.

Materials and Methods

Animals and experimental model. Wistar rat males were taken for modelling chronic ethanol consumption. The research was performed with the minimal number of animals necessary to obtain valid and meaningful results. Initially, animals were divided into two groups, control ($n = 4$) and ethanol-exposed (EtOH) ($n = 8$). All animals were kept on an artificial standardized diet (thiamine content was 800 µg/kg of chow). Rats from the second group were given 15% ethanol solution during nine months as the single source of drinking water. One day before the decapitation, thiamine was administrated to the part of alcoholic group (EtOH+B₁) in a dose of 2.0 mg/kg per oral gavage. Thus, we had three animal groups: control, EtOH and EtOH + B₁, $n = 4$ in each group. Choice of the dose was made based on the literature data reporting that the average daily requirement of thiamine for rats being 16 µg [20].

Brain sample preparation procedures. Rats were anesthetized with diethyl ether, decapitated

and brains were removed immediately and placed on ice. Protein samples for immunoblot analysis were obtained from the three parts of rat brain (cerebral cortex, cerebellum and hippocampus) by homogenization in cold 50 mM Tris-HCl buffer (pH 8.0), which additionally contained 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and protease inhibitor cocktail (tissue : buffer – 1 : 10, m/v). For immunohistochemistry (IHC) assay, small pieces of each brain section were collected, fixed in the cold buffered 4% paraformaldehyde solution and embedded into paraffin. The rest of the brain was homogenized in 50 mM Tris-HCl (pH 7.4) containing 0.32 M sucrose supplemented with PMSF and then used for evaluation of other biochemical parameters. Homogenates were centrifuged at 16,000 g for 45 min at 4 °C. Protein content in supernatants was measured by the Lowry method using bovine serum albumin (BSA) as a standard [21].

Electrophoresis and western blot. Brain protein samples were diluted with 4 × Laemmli sample buffer, containing 125 mM Tris-HCl (pH 6.8), 20% glycerol, 10% SDS, 0.2 M dithiothreitol, 0.01% bromophenol blue, and heated for 5 min at 95 °C. Samples were loaded onto 10% PAGE (100 µg protein per track) and separated in the Bio-Rad vertical chamber with the use of electrode buffer (25 mM Tris-HCl, pH 8.3, 195 mM glycine, and 0.1% SDS). After separation, proteins were transferred onto the nitrocellulose membranes by electroblot in a buffer, containing 10 mM NaHCO₃, 3 mM Na₂CO₃ and 20% ethanol for 2 h at a voltage of 100 V. To block sites of non-specific antibody binding, the membranes were incubated in 5% dry milk PBS, containing 0.1% Tween-20 (PBST), for 2 h at 37 °C. Then, the membranes were probed with the rabbit anti-GFAP antibodies (Santa Cruz Biotech, USA, cat. no. sc-9065, the kind gift of Prof. V. S. Nedzvetsky, Dnipro National University) for 16 h at 4 °C. Non-bound antibodies were removed by washing with PBST (5 times for 5 min each) and then incubated with the appropriate secondary anti-rabbit antibodies conjugated with horseradish peroxidase (Abcam, USA, cat. no. ab6721). After incubation, membranes were washed in PBST for 5 min each. Immunoreactivity was developed by enhanced chemiluminescence with luminol (0.25 M luminol diluted in DMSO, 0.09 coumaric acid diluted in DMSO, 0.1 M Tris pH 8.5, and 0.0035% H₂O₂) for 5 sec using radiographic films (Konica Minolta, Japan). After scanning, blots were quantitated densitometrically and values of GFAP immunoreactivity were expressed as arbitrary units.

Immunohistochemistry (IHC). Paraffin sections were gradually deparaffinised in xylene for 3 min twice, xylene-ethanol mixture (1:1), rehydrated in descending grades of ethanol (96 %, 75 %, and 50%) 3 min each, and then brought to double distilled water for 5 min. For antigen retrieval, sections were heated in microwave oven in 25 mM tris-HCl buffer, pH 7.4, containing 5 mM EDTA and 0.25% Triton X-100, for 1 min at 600 W. Sections were rinsed with PBS, blocked in 3% BSA for 1 h at 37 °C. After rinsing in PBS, sections were incubated with the primary anti-GFAP antibodies (1:100) at 4 °C overnight. Sections were rinsed with PBS three times, followed by 1 h incubation with secondary FITC-conjugated antibody (1 : 250) at 37 °C. After rinsing the sections in PBS twice, the nuclei were counterstained with Hoechst 33258 (1 µg/ml PBS) for 5 min at room temperature, then thoroughly rinsed in PBS and double distilled water. Non-specific binding control slices were incubated without primary antibody. Immunolabeling and DNA staining were visualized by confocal laser scanning fluorescence microscopy. Images were collected and processed using Zeiss ZEN 2009 Light Edition software. Quantitative analysis of GFAP immunostaining was performed with the use of ImageJ software version 1.47h. Quantification of IHC labelled tissue was calculated as a ratio between green fluorescent signal (GFAP) to blue fluorescent signal (nuclei) (G/B).

Thiamine level in liver. Thiamine levels in livers were measured using the thiochrome method [22]. Briefly, rat livers were mechanically homogenized, thiamine and its phosphates were extracted by boiling in an acidic medium (30 ml 0.1 M HCl per sample). To perform thiamine phosphoric ester hydrolysis, samples were incubated for 18 h with acid phosphatase (pH_{opt.} 4.5), and the precipitate was separated by centrifugation. The supernatants were applied to an ion-exchange resin IRC-50, and after washing, thiamine was eluted with 0.1 N HCl. Free thiamine fraction was oxidated to thiochrome and extracted to the organic phase by shaking with butanol for 2 min. Organic phase was discarded into clean tubes. Thiochrome fluorescence was measured at the wavelength of 360/430 nm.

Estimation of thiamine pyrophosphokinase (TPK) activity in rat brains. TPK activity in brain homogenates was determined as described elsewhere. The principle of estimation was based on the measurement of TDP content formed after incubation of thiamine with a TPK-containing preparation.

In the samples, the content of TDP was estimated with the help of the earlier proposed quick test using the apoenzyme of yeast apopyruvate decarboxylase (apoPDC) [23]. After incubation with thiamine, aliquots of brain homogenates were incubated in a phosphate buffer with the addition of apoenzyme for 30 min. Enzymatic estimation of TDP is based on recombination of the latter as a co-enzyme with apoPDC and further determination with an excess of pyruvate in the presence of alcohol dehydrogenase. The reaction was recorded according to oxidation of NADH. Apopyruvate decarboxylase for estimation of TDP was obtained from beer yeast (*Saccharomyces carlsbergensis*) and stored as a sulphate paste at -20°C . The apoenzyme was obtained from the paste immediately before the experiment. The amount of the formed TDP was calculated according to the calibration curve plotted with the use of a standard TDP reagent.

Statistical analysis. The experimental data were processed by statistical analysis using one-way ANOVA followed by the Tukey post-hoc test with the use of Origin Pro 8.6. The value of $P < 0.05$ was considered statistically significant. Quantitative results are expressed as mean \pm standard error of mean ($M \pm SEM$).

Results and Discussion

Changes in thiamine metabolism: thiamine content in liver and TPK activity in brain tissues. As shown in the Table, there was 3.7-fold decrease in thiamine content in livers of EtOH-exposed group compared to the control group ($P = 0.015373$). Thiamine treatment did not restore thiamine level to the control value, though, thiamine concentration was

Ethanol-induced changes in thiamine content in liver ($\mu\text{g/g}$ of tissue) and thiaminpyrophosphokinase (TPK) activity in brain (nmoles per 1 mg of protein)

Group	Thiamine levels in rat livers, $\mu\text{g/g}$ wet tissue	TPK activity in rat brain, nM/ mg protein
Control	3.70 ± 0.73	0.080 ± 0.007
EtOH	0.82 ± 0.07 * $P = 0.015373$	0.056 ± 0.002 * $P = 0.021833$
EtOH+B ₁	1.90 ± 0.08 * $P = 0.041624$ * $P = 0.001653$	0.052 ± 0.004 * $P = 0.003805$

*Compare to control, #compare to EtOH

twice as higher in EtOH+B₁ group than that for the alcoholized animals without vitamin administration.

Along with elevation of liver thiamine levels as a result of high dose thiamine, TPK activity in brain homogenates appeared to be significantly reduced in EtOH group (1.4-fold vs. control group, $P = 0.021833$). However, thiamine administration had no effect on TPK activity in brains of rats exposed to ethanol.

Changes in GFAP levels. The results of immunoblot, presented in Fig. 1, indicate that chronic ethanol consumption by rats from EtOH group resulted in approximately 60 % reduction in GFAP levels in all studied brain regions compared to the control group ($P < 0.05$). Injection of the single dose of thiamine (2.0 mg/kg) to ethanol-exposed rats (EtOH + B₁ group) partially restored content of this specific astroglial marker. Levels of GFAP in brain cortex and cerebellum significantly increased compared to EtOH group ($P < 0.05$) due to the thiamine treatment. Among all studied brain sections, hippocampus appeared to be less sensitive to thiamine treatment, because B₁-induced elevation of GFAP expression in this region was not statistically distinct from that of the EtOH group value ($P = 0.82593$).

GFAP immunostaining. The results of ICH assay presented in Fig. 2 basically confirm data of immunoblots and demonstrate robust decrease of GFAP immunoreactivity in rat brain induced by chronic ethyl alcohol consumption. Based on reduced GFAP specific immunostaining, we assumed that ethanol caused detrimental effects on metabolic activity of brain astrocytes. However, it is evidently seen that thiamine high dose administration to EtOH-exposed rats partially recovered astrocytic marker expression. As a proof of immediate beneficial action of thiamine on protein synthesis in astrocytes suppressed by ethyl alcohol, we showed two-fold elevation of G/B ratio in the cortex of EtOH-B₁ group rats as compared to EtOH-exposed rats ($P = 0.0023$). The similar changes were demonstrated for cerebellum, but they had a character of trend and did not quite achieve the level of statistical significance (1.5 fold increase, $P = 0.03641$).

Ethyl alcohol is considered to be the most consumed neurotoxin worldwide, while chronic alcohol intoxication or alcohol overuse have been found to alter cerebral structure and functions through promoting inflammatory processes, increases DNA damage, and induction of oxidative stress [24]. In addition, chronic alcohol intake is accompanied by

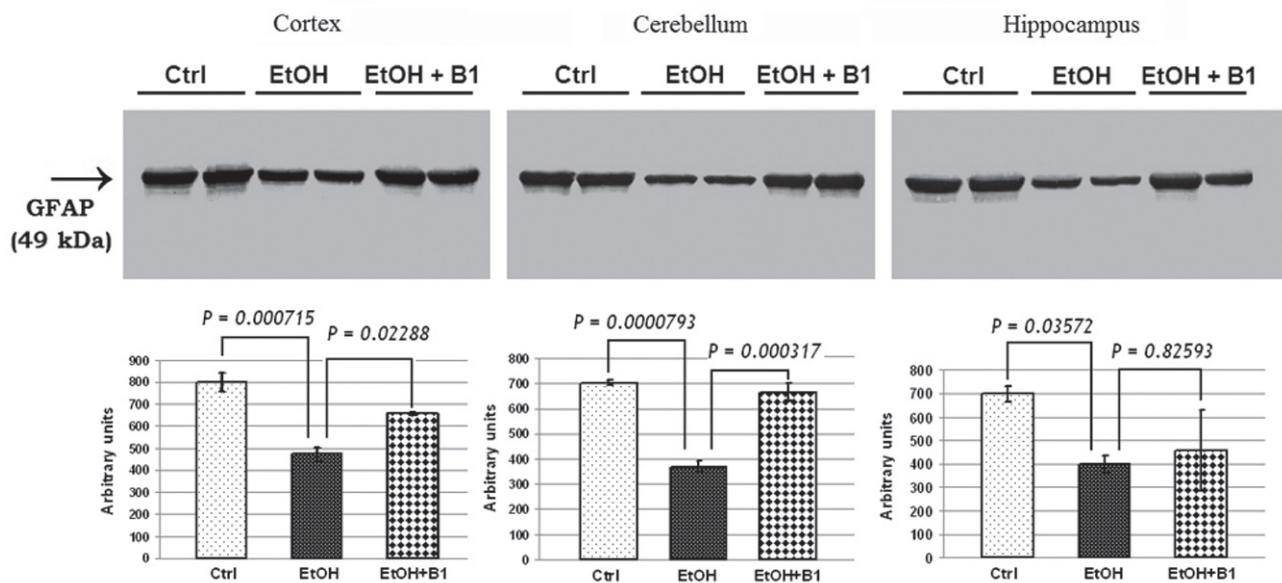


Fig. 1. Immunoblots and results of densitometry analysis of glial fibrillary acidic protein (GFAP) from different rat brain sections of control (Ctrl), ethanol-exposed (EtOH), and thiamine-treated alcoholised (EtOH + B₁) animals

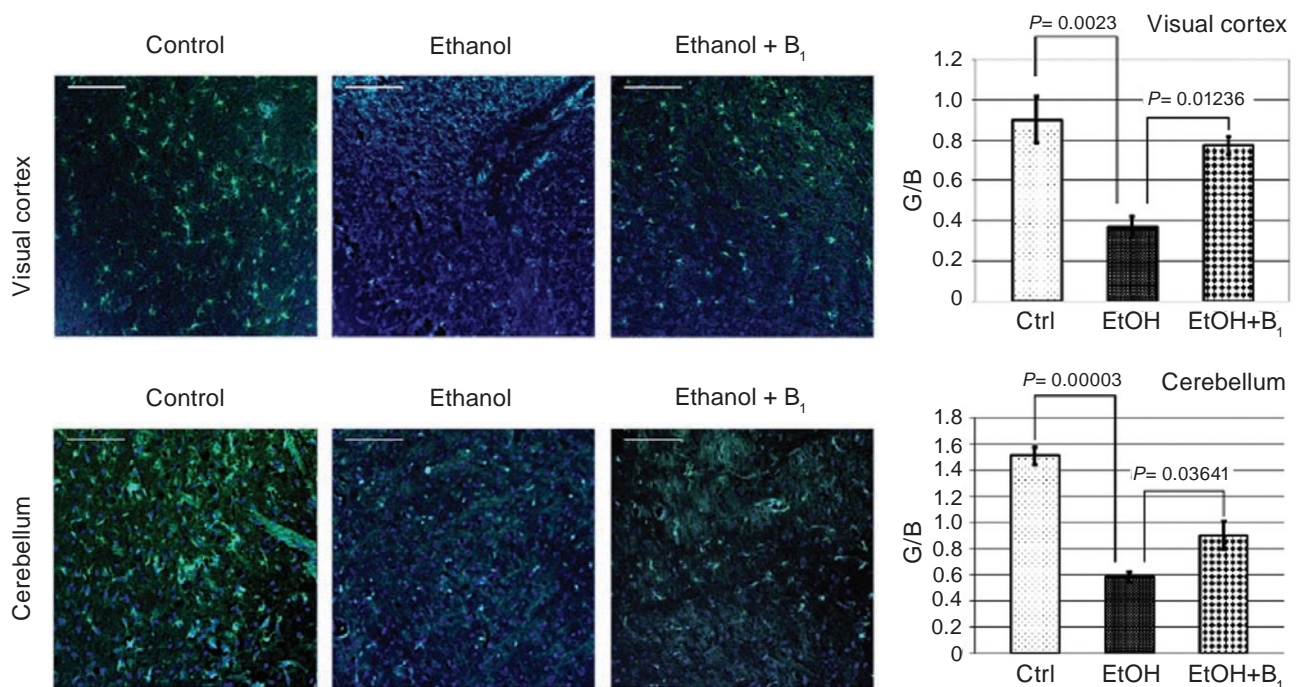


Fig. 2. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) in visual cortex and cerebellum slices and results of quantitative analysis (green – GFAP immunostaining, blue – Hoechst nuclear counterstaining). Bar – 100 μ m

thiamine deficiency (TD) and may lead to Wernicke encephalopathy. Moreover, TD has been shown to be one of the risk factors for chronic alcoholic encephalopathy development [25]. Thus, animal models of

TD are widely used for neurodegenerative conditions studying, however there are limited literature data covering the damage degree of astrocytes in different brain regions during TD-associated states. As-

trocytes are the specific targets of both ethanol and vitamin B₁ deficit. These cells are involved in many important processes in the central nervous system (CNS), such as synaptic transmission, synapse formation and plasticity, blood-brain barrier (BBB) maintenance, neurotoxicity and nervous system repair. Therefore, dysfunction of astroglia ultimately results in the failure of CNS homeostasis and compromises defensive capabilities of the nervous tissue [26]. Besides, atrophy of astroglia may disrupt the neuro-vascular unit through decreasing endfeet coverage of brain vessels thus contributing to vascular deficits. One of the simplest strategies to recover compromised astrocytic functions may be environmental stimulation and dietary supplementations, which are aimed to improve GFAP expression and astroglial response. Numerous *in vivo* and *in vitro* researches have demonstrated that chronic consumption of ethanol leads to substantial astrocyte damage or astrocytes loss, which are primary cell changes in a brain occurring prior the neuron death [2, 27-30]. The role of astrocytes is critical in detoxification processes of ethanol itself and products of its metabolism in the brain. Influence of ethanol on the state of astrocytes has been studied in detail; the obtained data is, however, rather controversial. This is related in the first place to the fact that the pattern and direction of changes in the amount of GFAP depends on the dose and duration of this toxicant consumption. Despite the generally accepted fact that there are regional differences in the vulnerability to the neurotoxic effects of chronic ethanol intake, our data indicate that prolonged ethanol consumption in the studied regime can produce profound similar changes in most brain structures. Nevertheless, more detailed studies are needed to provide with a more comprehensive understanding of alcohol-related brain damage and possible ways of its therapeutic treatment.

Protective role of thiamine in different pathological conditions caused by alcoholism are being studied for a long time [31], but optimal dosage and regime of thiamine administration depend on the extent of ethanol-induced injury to the brain and other effects of alcohol abuse and may vary from person to person. A group of scientists studied the possibility of high doses of thiamine (100 mg per day per person) for the treatment of various neurodegenerative diseases (Parkinson's disease, Alzheimer's, ataxia Friedrich, etc.). The patients' condition was assessed according to special scales at the beginning

of treatment and in a month. The results showed a significant improvement in the absolute majority of patients, but the exact mechanism of thiamine responsiveness in such patients is unknown [32, 33]. In the previous work, we showed that the physiological (0.1 mg/kg) dose was unable to completely restore the GFAP level at B₁ hypovitaminosis. Current literature gives no evidence on thiamine-induced adverse effects even after prolonged intake in high doses [34]. From these circumstances, we addressed here if high dose thiamine administration can exert prompt protective effects against ethanol-induced damage to rat brain astroglia. To verify this, we developed a rat model of chronic alcohol abuse. Inhibition of thiamine metabolic parameters is an important indication of the developed model adequacy. Further, it is well-known that liver failure has a great significance in the development of alcoholic encephalopathy, along with alcohol specific neurotoxicity and associated vitamin B₁ deficiency [35]. Although the brain is protected from many neurotoxic substances by the blood-brain barrier, a property of blood vessels in the brain that prevents passage of many compounds from the blood into the brain tissue, nevertheless certain neurotoxins can penetrate that barrier. These substances, including ammonia, manganese ions, and other chemicals, can enter the brain at least to some extent unless they are effectively removed from the blood by the liver. Our results are in agreement with these data, demonstrating 4.5-fold reduction of thiamine concentration in livers of rats from EtOH group, as compared with control animals. Also, long-lasting ethyl alcohol consumption produced significant decrease in brain TPK activity and dramatically down-regulated GFAP expression, which can result in abrogation of astroglial functional activity in the studied brain regions. There are complex interactions of these and other factors and it is often difficult to dissect out the relative importance of each in the pathogenesis of alcohol-related brain damage. Thus, it can be assumed that alcohol-induced astroglial dysfunction is further translated into neuronal damage, which is accompanied by the impairment of coordination between neurons and glial cells. The latter may have serious consequences for neuronal survival and plays the essential role in ethanol-induced brain injury.

Thiamine overloading dose administration (2 mg/kg) had no adverse effects on studied parameters. However, its injection to ethanol-exposed rats resulted in two-fold elevation of vitamin B₁ level,

which likely contributes to the restoration of normal liver function, including utilization of ammonia.

The most significant observations of this study indicate rapid restoration of B_1 level in livers and up-regulation of astrocyte marker expression in the thiamine-treated group. The fact that astroglia, being long under alcoholic intoxication, reacts to thiamine treatment; i.e., the GFAP level is practically restored within 24 hours might suggest that this is still a biochemical and not a chemical lesion. It could be assumed that the administration of thiamine leads to the restoration of the astroglial marker, due to its well-known antioxidant properties [36]. Indeed, there are several lines of evidence that demonstrate beneficial effects of long-term thiamine treatment against oxidative stress induced by alcohol intoxication [37]. However, in our previous work, we demonstrated that a single injection of a high dose of thiamine had no effects on the parameters of the developed oxidative stress (Pavlova et al., 2016). Also, it should be emphasized that thiamine administration did not affect TPK activity in the brain. Based on this finding, it can be hypothesized that protective effects can be realized by distinct thiamine-mediated pathways or processes, others than its coenzyme role or antioxidant properties. Although future research is needed to ensure this assumption, obtained data support hypothesis highlighting non-coenzyme function of thiamine in the CNS [39]. Finally, regardless of the fine molecular mechanism(s), thiamine-high dose administration was shown to represent effective treatment option against chronic effects of ethanol impact on astroglial pattern of brain.

In conclusion, present study demonstrated, that lack of thiamine can play an important role in alcohol-induced damage to brain astroglia. It is emerged that high-dose thiamine administration can represent effective treatment option against chronic effects of ethanol impact on brain structures. Also we hypothesized, that protective effects of administration can be provided by distinct thiamine-mediated pathways or processes, others than its coenzyme role or antioxidant properties.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiocemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

ВИСОКА ДОЗА ТІАМІНУ ВІДНОВЛЮЄ РІВЕНЬ СПЕЦИФІЧНИХ АСТРОЦИТАРНИХ ПРОТЕЇНІВ ГОЛОВНОГО МОЗКУ ЩУРІВ, СХИЛЬНИХ ДО ХРОНІЧНОГО СПОЖИВАННЯ ЕТАНОЛУ

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Довгострокове вживання етанолу спричинює дефіцит незамінної в харчуванні речовини – тіаміну (вітаміну B_1), що призводить до структурних та функціональних змін у центральній нервовій системі (ЦНС). У роботі досліджували вплив високої дози тіаміну на рівень гліально-го фібрилярного кислого протеїну (GFAP) і на активність B_1 -залежного ензиму тіамінопірофосфокінази (ТПК) в мозку щурів, схильних до хронічного споживання етанолу. Щурів було розділено на три групи: i) контрольна група; ii) щури, які отримували 15%-й розчин етанолу як єдине джерело пиття протягом 9 місяців (група EtOH), iii) щури, які отримували EtOH + перорально тіамін у дозі 2,0 мг/кг за один день до термінації експерименту ($n = 4$ в кожній групі). Рівень GFAP аналізували в мозочку, корі головного мозку і гіпокампі за допомогою вестерн-блот аналізу та імуногістохімії. Активність ТПК в головному мозку вимірювали з використанням апоензиму дріжджової апопіруватдекарбоксилази (apoPDC). Концентрацію тіаміну в печінці оцінювали тіохромним методом. Показано, що вміст GFAP різко знижувався у всіх досліджених відділах мозку щурів, які споживали EtOH (приблизно на 60%, $P < 0,05$) порівняно з контрольними щурами, що вказує на глибоку астрогліальну дисфункцію. Введення тіаміну частково відновлювало рівень GFAP до 80% порівняно з контрольним значенням з групи EtOH ($P < 0,05$). Споживання етанолу знижувало вміст тіаміну в печінці в 3,7 раза і в 1,4 раза активність ТПК в головному мозку експериментальних тварин порівняно з контролем ($P < 0,05$). Введення

тіаміну щурам із групи EtOH значно підвищувало рівень B_1 в печінці, однак не мало впливу на активність ТПК в головному мозку. Дані показують, що дефіцит тіаміну відіграє важливу роль у пошкодженні астроглії головного мозку щурів, які споживали етанол, а введення високих доз тіаміну може бути ефективним варіантом у запобіганні цих наслідків.

Ключові слова: хронічне вживання етанолу, астроцити, гліальний фібрилярний кислий протеїн (GFAP), дефіцит тіаміну (вітаміну B_1).

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