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Original article

EFFECTS OF ACUTE ALCOHOL INTOXICATION ON TESTICULAR DNA STABILITY, GENE EXPRESSION OF CYTOCHROMES CYP3A AND CYP2E1, AND SERUM POOL OF FREE AMINO ACIDS IN RATS

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Background. Alcohol's toxic effects on the organism is a long-known medical problem. Alcohol's damaging effect is the end result of the complex interplay between ethanol metabolism, inflammation and innate immunity. Previously, we studied the long-term consequences of chronic alcoholism and demonstrated that especially profound changes were in testes on the level of proteome and genome.
Objective. This work aimed to study short-term acute alcohol intoxication (AAI) effects for rat testis DNA fragmentation, cytochromes CYP3A and CYP2E1 genes expression, and serum pool of free amino acids in rats.
Methods. Wistar albino male rats were divided into 2 groups (8 animals in each group): 1 – Control (intact rats), and 2 – AAI (rats with short-term acute alcohol intoxication). AAI was induced by repeated administration per os 40% ethanol solution in a dose 7 ml/kg body weight, for 7 days. Contents of amino acids in serum, testes mRNA CYP2E1 and CYP3A2 expression, and DNA fragmentation were evaluated.
Results. In our experiments, the development of acute alcohol intoxication (AAI) led to increased DNA fragmentation processes in the testes of adult rats compared to the control group. Additionally, in the serum of ethanol-treated rats, the levels of histidine increased by 1.67 times and glutamine by 1.13 times in correlation with this pathology. Conversely, the levels of valine, phenylalanine, as well as non-essential and essential amino acids, decreased. Furthermore, there was a statistically significant increase in the expression of CYP2E1 and CYP3A2 genes in rat testes under the conditions of AAI.
Conclusions. In conclusion, investigation of rats' short-term alcohol administration effects permitted us to obtain the picture of complex metabolomic changes at the different levels. The main outcome of rats short-term ethanol administration in our experiments seems to be to some extent similar to changes described for rats with chronic alcohol consumption. Our results demonstrated profound changes in testes affecting the state of the genome, transcription processes and the exchange of amino acids and proteins. We suggest that the revealed testicular metabolic disorders could have negative implications on cellular regulation of spermatogenesis even under short-term ethanol exposure.

Keywords: acute alcohol intoxication; DNA fragmentation; gene expression; CYP450; pool of free amino acids; testis.

Abbreviations

EtOH – ethanol,
CYP – cytochrome P450,
b.p. – base pairs.

Introduction

Alcohol toxic effects on the organism are a long-known medical problem [1]. For example alcoholic hepatitis is associated with significant morbidity and mortality (up to 1.95% of global deaths). In severe cases, patients have a very poor prognosis, with short term mortality around 30–50% [2]. In recent years, a huge amount of data related to ethanols' biological, psychological, neurological effects has been accumulated [3].

Of particular concern is the accumulation of experimental data indicating the ability of ethanol to cause large-scale disruptions to the genome and proteome of various organisms. In particular, the toxic effect of alcohol leads to DNA molecules structure various damages, including an increase in its molecules' single-strand breaks number [4]. Changes also occur in the processes of gene expression of a number of proteins, especially those involved in the formation of the cytoskeleton, ensuring endocytosis, biogenesis and functioning of ribosomes,

nuclear transport and stress-related reactions [5, 6]. Mechanisms of apoptosis [7], DNA methylation and histone modification, as well as regulation of microRNA expression [6], are disrupted.

Alcohol damaging effect is the end result of the complex interplay between ethanol metabolism, inflammation and innate immunity [3, 8]. In the process of metabolic transformations, reactive oxygen species are generated in the cells of the body. They, in turn, induce lipid peroxidation reactions, the products of which cause the death of cells, triggering the processes of necrosis or apoptosis. These processes are also accompanied by a high level of endotoxemia [9]. Endotoxin, various cytokines, in particular interferon-gamma and tumor necrosis factor-alpha (TNF-α) [10], chemotactic factors such as interleukin-8, are involved in the initiation and development of the systemic inflammatory response syndrome. TNF-α mediates its effects by binding to two cell surface molecules, TNF-R1 and TNF-R2 [11]. TNF-R1 is the main inducer of cytotoxicity through necrosis or apoptosis. An ethanol metabolite, acetaldehyde, and one of the end products of lipid peroxidation, malondialdehyde, bind to cellular proteins to form stable adducts. The immune response to these neoantigens leads to the development of hypertension. Non-immunological (oxidative stress, cytokine damage) and immunological factors act in combination in the pathogenesis of alcohol intoxication [12].

Based on the above ideas about the pathogenesis of organisms' alcoholic damage, various options for its pharmacotherapy have a common goal of treatment – blocking many innate immunological reactions [9, 13]. However, the effectiveness of current therapy is unsatisfactory. Obviously, some fundamental aspects of organisms' alcoholic damage development remain insufficiently studied and/or incorrectly assessed to date. New approaches based on new concepts and emerging scientific results may provide better results in the overall management of patients with this problem. The study of acute alcohol intoxication using an interdisciplinary approach, ranging from the submolecular level to the whole organism, currently seems to be the best way to prevent its harmful effects.

Previously, we studied the long-term consequences of chronic alcoholism for the state and functioning of the liver, brain, and testes of rats [14–18]. These data demonstrated that rats' chronic alcohol consumption led to complex metabolomic changes at different levels, which simultaneously characterized cell macromolecules structure, metabolism, and oxidative/nitrosative stress.

Especially profound changes were shown in testes on the level of proteome and genome. We suggested that such disorders could have negative implication on cellular regulation of spermatogenesis under long-term ethanol exposure. Accompanying them alcohol-mediated changes in collagen type I amino acid contents might have affected the spermatogenic epithelium state. The modulation of testicular cytochrome P4502E1 mRNA and protein expression could change the functioning of this isozyme in target organs and take part in the mechanism of ethanol gonadotoxicity. The correlation between the levels of CYP2E1 mRNA in testes and spermatogenesis disorders allow supposing the involvement of CYP2E1 into the non-specific pathogenetic mechanisms of male infertility under above-mentioned pathologies.

Given all of the above, the question arises as to how soon after the onset of ethanol exposure such disorders occur. Are they characteristic only of chronic alcoholism or can they occur with occasional high doses of alcohol? It was of particular interest to us to conduct a comprehensive assessment of the acute alcohol intoxication development consequences for such key aspects of each cell viability as DNA stability, the intensity of gene expression processes (especially the cytochromes P450 genes involved in the biotransformation of most drugs), and the metabolism of amino acids (as initial compounds in the proteins biosynthesis). This work aimed to study acute alcohol intoxication (AAI) consequences for rat testis DNA fragmentation, cytochromes CYP3A and CYP2E1 genes expression, and serum pool of free amino acids in rats.

Materials and Methods

Study design. A total of 16 adult Wistar albino male rats (160–180 g) were used in the study. They were kept under a controlled temperature (from 22 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 model of short-term acute alcohol intoxication (AAI) was reproduced according to the protocol [19]. AAI was induced by repeated administration per os 40% ethanol solution in dose 7 ml/kg body weight, during 7 days. Animals were divided

into 2 groups (8 animals in each group): 1 – Control (intact rats), 2 – AAI (rats with AAI).

At the end of the experiment rats were sacrificed under a mild ether anesthesia by decapitation. Prior to euthanasia blood samples from femoral vein were collected, and serum samples were separated. Testes were remouved and kept frozen at −70 °C until needed [20].

Cytochrome P-450 isoforms study. The rats' testes were used for investigation of cytochrome P-450 isoforms (CYP2E1 and CYP3A2, which is ortholog of human cytochrome CYP3A4 in rat) [21] mRNA expression rates by method of reversed transcriptase polymerase chain reaction (rPCR). Isolation of total mRNA was carried out with TRI-Reagent (Sigma, USA). Synthesis of cDNA was carried out with reagents and protocol of Fermentas (Lituania). rPCR reaction mixture contents, specific primers for CYP2E1 gene amplification (forward 5′-CTTCGGGCCAGTGTTCAC-3′ and reverse 5′-CCCATATCTCAGAGTTGTGC-3′), as well as amplification protocol were chosen according to Lankford *et al.* [22]. rPCR reaction mixture, amplification protocol and following specific primers – forward 5′-TACTACAAGGGCTTAGGGAG-3′ and reverse 5′-CTTGCCTGTCTCCGCCTCTT-3′ were used for *CYP3A2* gene amplification according to Jager *et al.* [16]. rPCR with primers of β-actin (sense 5′-GCTCGTCGTCGACAACGGCTC-3′ and antisense 5′-CAAACATGAT CTGGGTCATCTTCT-3′) was carried out for internal control. All primers were synthesized by "Metabion" (Germany). Thermocycler MyCycler (BioRaD, USA) was used for amplification. Electrophoresis of PCR products (CYP2E1 – 744 b.p., CYP3A2 – 349 b.p. and β-actin – 353 b.p.) was carried out in 2% agarose gels (80 V; 1.5 h). After electrophoresis gels were stained with ethidium bromide and visualized under a UV transilluminator (BIORAD, USA). Electrophoresis data analysis was carried out with Quantity One Software (USA).

Chromatin DNA fragmentation study. DNA from the testis was isolated according to the standard protocol [23]. Chromatin DNA fragmentation (as one of the apoptosis markers) evaluation was carried out according to Bondarenko *at al.* [24]. Briefly, tissue was homogenized and digested in digestion buffer (100 mM NaCl; 10 mM Tris-HCl; 25 mM EDTA, pH 8; and 0.5% SDS) and freshly added 0.1 mg/mL proteinase K (Sigma-Aldrich Ink., USA) (1:1.2 mg/ml) with shaking at 50 °C for 15 h. RNA was degraded by incubation of the samples with 1–100 mg/mL thermostable RNase H for 1.5 h at 37 °C. DNA was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged for 10 min at 1700× *g*. The DNA was precipitated by adding 0.5 vol 7.5 M ammonium acetate and 2 vol 100% ethanol to the aqueous layer; samples were separated by centrifugation at 1700× *g* for 5 min, rinsed with 70% ethanol, and air-dried. The pellets were dissolved in TBE buffer (10 mm Tris-HCl and 1 mm EDTA, pH 8); and then were fractionated through 2% agarose gels (50–60 V; 3.5 h). After electrophoresis gels were stained with ethidium bromide and visualized under a UV transilluminator (BIORAD, USA). Electrophoresis data analysis was carried out with Quantity One Software (USA).

Serum pools of free amino acids investigation. The obtained rat serums were left for 30 min in refrigerator at 4 °C; to serum samples (1 mL) equal volumes of 3% sulfosalicylic acid were added and obtained mixtures were left for 10 min in refrigerator at 4 °C [25]. The formed sediments were removed by centrifugation (5000 g, 10 min, 4 °C). Supernatants contained serum free amino acids. Serum pools of free amino acids were analyzed by ion exchange chromatography on the amino acid analyzer AAA-881 (Czech Republic).

Statistical analysis. The obtained data were expressed as the mean ± standard error of the mean (M ± SEM) and were compared using unpaired Student's t-test to find out the level of significance between control and experimental rats using OriginPro 7.5 Software [26]. Differences were considered to be statistically significant at $p < 0.05$.

Results

In our experiments, AAI led to an intensification of DNA fragmentation processes in the testes of adult rats in comparison with control (Fig. 1). In the group of animals with AAI 14 fractions of DNA low molecular weight fragments were detected, whereas in the control there were only three (with masses approximately 900, 100 and 50 b.p.).

In rats with this model of the AAI, the percentage of DNA fragmentation grew almost 4.7 times. Six fractions were fragments with masses of 40 to 100 base pairs, whereas relatively longer fragments were represented by 4 fractions (350–500 b.p.) and 4 fractions (850–1000 b.p.).

Results of CYP2E1 mRNA expression comparative study in the testes of control animals and rats with AAI are shown in Figs. 2 and 3. Statistically significant increase in CYP2E1 gene expression was noted under the conditions of AAI (Fig. 2).

In the testes of rats with AAI at the same time, statistically significant changes were also detected in the level of mRNA expression of the *CYP3A2* gene (Fig. 3).

The study of the effects of AAI on the rat serum pool of free amino acids (the Table) showed that the contents of histidine (1.67 times) and glutamine (1.13 times) were increased with this pathology.

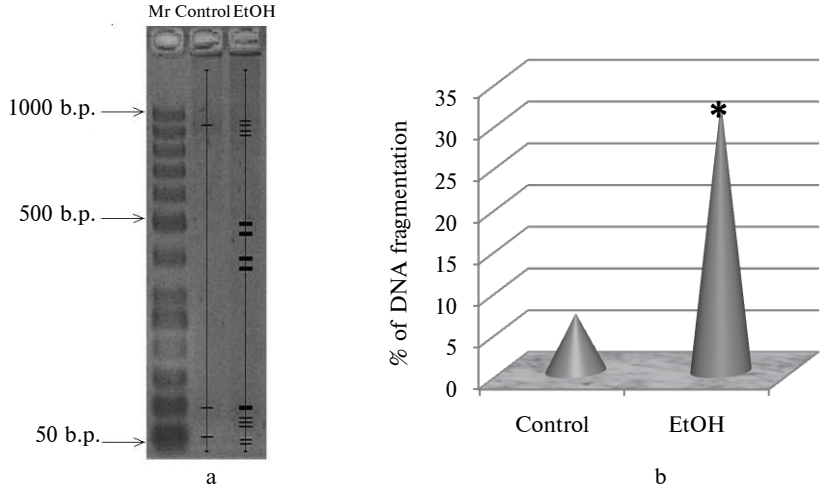

Figure 1: (a) Electrophoregram of DNA fragmentation in rats' testes with acute alcohol intoxication (Control – control group, EtOH – model of AAI); (b) relative % of rat testes DNA fragmentation in rats with AAI. * – $p < 0.05$ in comparison with control

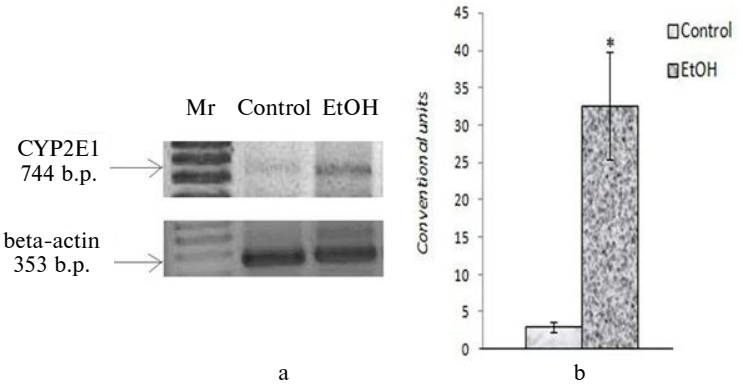

Figure 2: (a) Electrophoregram of CYP2E1 gene rPCR products (744 p.p.) and reference-gene β-actin (353 b.p.) rPCR products in the testes of control animals and rats with acute alcohol intoxication (Mr – DNA marker; Control – control group, EtOH – model of AAI; $n = 8$); (b) average rate of CYP2E1 mRNA expression in the testes of control animals and rats with AAI (Control – control group, EtOH – model of AAI; $n = 8$, signal intensity of β-actin was taken as 100%). * – $p < 0.05$ in comparison with control

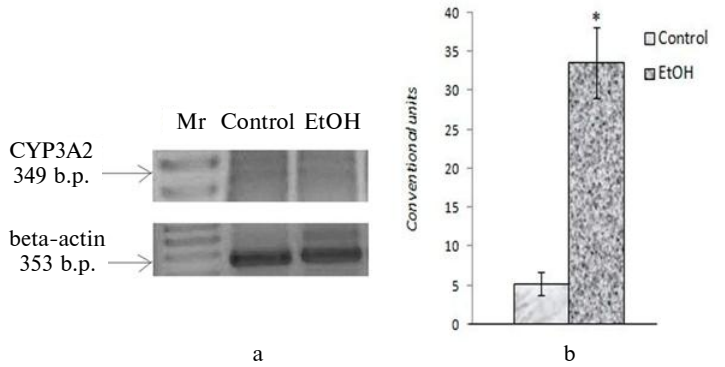

Figure 3: (a) Electrophoregram of CYP3A2 gene rPCR products (349 p.p.) and reference-gene β-actin (353 b.p.) rPCR products in the testes of control animals and rats with acute alcohol intoxication (Mr – DNA marker; Control – control group, EtOH – model of AAI; $n = 8$); (b) average rate of CYP3A2 mRNA expression in the testes of control animals and rats with AAI (Mr – DNA marker; Control – control group, EtOH – model of AAI; $n = 8$, signal intensity of β-actin was taken as 100%). * – $p < 0.05$ in comparison with control

Table: Serum pool of free amino acids in rat with acute alcohol intoxication (AAI) (mg×100 mL^{-1} of serum, mean ± SEM, $n = 8$)

Amino acid	Control	AAI
Lysine	4.20 ± 0.65	4.14 ± 0.33
Histidine	0.40 ± 0.06	0.67 ± 0.01*
Arginine	2.80 ± 0.41	3.10 ± 0.20
Aspartic acid	0.57 ± 0.04	0.50 ± 0.03
Threonine	1.12 ± 0.16	0.87 ± 0.08
Serene	2.10 ± 0.20	1.62 ± 0.10
Glutamine	4.00 ± 0.12	4.53 ± 0.08*
Proline	1.75 ± 0.14	1.73 ± 0.10
Glycine	2.63 ± 0.20	2.58 ± 0.10
Alanine	4.22 ± 0.21	3.69 ± 0.11
Valine	1.60 ± 0.05	1.41 ± 0.03*
Methionine	0.41 ± 0.08	0.40 ± 0.09
Isoleucine	0.65 ± 0.06	0.64 ± 0.04
Leucine	1.10 ± 0.09	1.00 ± 0.03
Tyrosine	1.14 ± 0.10	1.12 ± 0.09
Phenylalanine	0.81 ± 0.05	0.62 ± 0.05*
Total amount of amino acids	29.50	28.66
Total amount of non-essential amino acids	15.27	13.65
Total amount of essential amino acids	14.23	13.05
Non-essential / Essential amino acids	1.073	1.046

* – $p < 0.05$ in comparison with control.

At the same time, the contents of valine, and phenylalanine, as soon as the contents of non-essential amino acids, essential amino acids, and total amount of amino acids were lowered.

Discussion

The stability of chromatin DNA is a key aspect of maintaining cell viability. DNA should be considered as an important molecular target for toxicants [14] which induce different endonucleases for its lethal splitting and inhibit processes of DNA repair by nuclear DNA-polymerases. The level and character of DNA fragmentation serve as markers of apoptotic processes in the organism [27]. Acute alcohol intoxication development is accompanied by apoptotic processes activation [28]. Apoptotic changes inevitably lead to disturbance in DNA fragmentation processes [27]. Our results on DNA fragmentation levels in testes of ethanol-treated rats are in good correspondence with our previous results with chronic alcoholism model [13] and other authors' data [29].

A comparison of our results on DNA fragmentation under short-term and chronic ethanol exposure suggests that with AAI this process was greatly intensified. If in chronic alcoholism the presence of 7 DNA fractions was noted [14], then in AAI we recorded twice as many. Moreover, the number of different types of fractions increased: both low- (with masses of 40 to 100 b.p.), middle- (350–500 b.p.) and high-molecular (850–1000 b.p.).

Thus, we can state that already at the earliest stages of ethanol toxic effect realization, the processes of DNA fragmentation are intensified. Their further weakening in chronic alcoholism is possibly associated with the inclusion of organism's adaptive defense mechanisms over time [3].

With AAI development such changes apart from the rest could be caused by disturbances in the functioning of endonucleases involved in DNA repair processes in mammalian cells, as ethanol is well known enhancer of autolysis [30], as soon as by mitochondrial dysfunctions accompanying AAI [3, 31].

Reduced stability of DNA could significantly change cell viability due to increased levels of damaged and dead cells. Apoptosis processes are incorporated into the system of germ cell development regulation. Damaged germ cells removing from seminiferous tubules via apoptosis allows to prevent their further differentiation into spermatozoa. Such damaged germ cells deletion is a critical component of genome safekeeping mechanisms [32]. Dysregulation of the fine-tuned balance may lead to the onset of testicular diseases [33].

Ethanol induces apoptosis by two different mechanisms. The first assumes mitochondrial permeability transition, the second – up-regulation of

the expression of CD95-Fas ligand. The excess accumulation of reactive oxygen species (ROS) by mitochondria during acetaldehyde metabolism intensification, is a common trigger of both mechanisms [29]. In the organism of alcoholics, apoptosis is caused by increased ROS due to increased availability of the reduced form of nicotinamide adenine dinucleotide (NADH) owing to mitochondrial acetaldehyde metabolism, and it is prevented by blocking the opening of mitochondrial permeability transition pores with cyclosporine A [29].

The ability of ethanol to disturb spermatogenesis was noted in our previous experiments [14] and also by other investigators [34]. On the other hand, when spermatogenesis is disrupted in any way, the germ cells tend to default to an apoptotic state [35]. According to other authors' results systemic changes in DNA molecule structure are directly correlated with the rates of AAI development [3, 31] and thus, apoptosis violations may cause inhibition of tissue regeneration and pathological changes deepening. The number of research suggests that germ cell death in adult rats, whether occurring spontaneously during normal spermatogenesis or accelerated for instance due to factors such as deprivation of gonadotrophic support or moderate increases in scrotal temperature, predominantly occurs through apoptosis.

The regulation of germ cell apoptosis probably occurs in a cell-type-specific manner, although the fundamental components of the apoptotic machinery may be universal [36]. Both spontaneous and increased cell death due to triggering stimuli (deprivation of intratesticular testosterone and gonadotrophins, Sertoli cell toxicants, chemotherapeutic drug, etc.) occur via apoptosis [32–36].

Maintaining certain levels of gene expression is equally important for maintaining cell viability along with controlling DNA stability. In our experiments increased DNA fragmentation levels were accompanied by changes in the expression rates of CYP2E1 and CYP3A2 (ortholog of human CYP3A4) mRNA in testes. Our data was in good agreement with our previous results [14, 15, 17] and other authors investigations [37, 38]. However, a comparison of our results regarding the direction of these changes in short-term and chronic administration of ethanol [14, 15, 17] revealed a number of fundamental differences.

As for cytochrome CYP2E1, in this case extrahepatic expression of its gene was intensified both in short-term and chronic administration of ethanol although with short-term administration of alcohol, the changes were much more pronounced: increasing by 10 times and by 3 times correspondingly. In the case of cytochrome CYP3A2, a change in the very direction of changes was noted: if with a short-term administration of alcohol, an increase in the expression of this gene was recorded by approximately 7-7-5 times, then with chronic alcoholism, inhibition of expression by approximately 3-5-4 times was already observed. Ethanol has been reported to be either an inducer or an inhibitor of CYP3A expression in dependence of ethanol exposure duration in the number of scientific publications [39, 40]. As in the case of data on DNA fragmentation, these differences in the expression rates of CYP2E1 and CYP3A2 with AAI and chronic alcoholism might be due to the inclusion of adaptive mechanisms of the body during prolonged alcohol exposure – up to the stage of metabolic adaptation [3].

Depending on cellular conditions, CYPs can play a major role in either the detoxification or the bioactivation of toxic substances. Thus, if a specific xenobiotic toxicant is metabolized toward bioactivation within a cell, it could damage cellular components and subsequently alter the cells' fate toward either carcinogenesis or apoptosis [41]. Induction of Cytochrome P450 2E1 noted in our experiments can aggravate toxic damage of organisms' tissues (including testes) during AAI development [27]. Even more significant for the further fate of the cell may be an increase in the level of expression of cytochrome P450 3A2 (ortholog of human CYP 450 3A4), since with its participation, the biotransformation of most drugs in the organism occurs [42]. The intensification of its expression may be one of the reasons for the insufficient effectiveness of steroid therapy for acute alcohol intoxication [8].

The pool of free amino acids is one of the most sensitive indicators for the early detection of possible deviations of metabolic transformations in the organism [24, 43, 44]. Free amino acids are involved in the integration of regulatory and adaptive systems of the body [25]. The study of their contents changes characterizes the state of the body's internal reserves, the degree of disintegration of metabolism as a whole, and the adaptive capabilities of the body and its individual organs [25].

It is supposed that the character of amino acid alterations in alcohol-treated animals could be evidence of considerable general disturbances in the metabolism of proteins [45]. This assumption is in good agreement with the previous investigations [46], which demonstrated impaired protein synthesis with ethanol consumption. Free aldehyde groups of the ethanol metabolite acetaldehyde can interfere

with amino acids and change protein structure to form new epitopes that activate autoimmune processes [47].

Amino acids, such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, arginine, and histidine are essential for white rats, whereas alanine, asparagine, aspartate, cysteine, glutamine, gluta mate, glycine, proline, serine, and tyrosine represent the group of nonessential amino acids. The changes in the ratio of nonessential/essential amino acids in the serum free amino could be caused by disturbances in metabolism of proteins and amino acids and their transport with ethanol administration [48]. A comparative analysis of our results with previously obtained data [15] showed that in both cases, under the influence of alcohol, there is a decrease in the ratio of essential amino acids to non-essential ones. With AAI, it is still slightly expressed (control – 1.073; AAI – 1.046), while in chronic alcoholism it manifests itself quite clearly (control – 1.29; chronic alcoholism – 1.15).

A decrease in valine concentrations may affect the catabolism of proteins and cause disturbances in the glucoso-alanine cycle [48]. Similar changes in the content of valine in the serum pool occurred in chronic alcoholism [15].

The character of some changes in amino acid pools could primarily evidence considerable disturbances in carbohydrate metabolism at the stage of the Krebs cycle (changes in histidine and valine) [48]. At the same time, a comparison of our results with previous data on the model of chronic alcoholism [15] showed a similar pattern of changes in the content of these amino acids, which indicates that in this case, the effect of ethanol manifests itself already with a short-term intake into the organism and then does not change with time in severity.

Along with the increase in glutamine contents, NH^+-transport disturbances could be evidenced [48]. This supposition is in accordance with the results of other authors who observed disturbances in the metabolism of nitrogenous compounds and their transport after administration of ethanol [30].

Decreased content of phenylalanine and the phenylalanine/tyrosine ratio (parameter of the intensity of phenylalanine hydroxylation) in serum free amino acids contents (from 0.71 – in the control group to 0.55 in the AAI group) could evidence of considerable inhibition of phenylalanine hydroxylases activity by ethanol [48]. In our previous experiments on the model of chronic alcoholism, we registered the reverse changes: the content of phenylalanine and the phenylalanine/tyrosine ratio increased [15], which might be due to the inclusion of adaptive mechanisms of the body during prolonged exposure to alcohol (as in cases of DNA fragmentation and CYP P450 3A2 gene expression [30].

Changes in histidine and glutamine might indicate disturbances in the metabolism of nucleic acids and nucleotides [48] and are consistent with our data on DNA fragmentation in acute alcohol intoxication. Our data are in accordance with the results on ethanol's effect on NAD metabolism and nucleic acids biosynthesis in cell nuclei reported by other authors [30].

Conclusions

In conclusion, the investigation of the effects ofshort-term alcohol administration in rats permitted us to evaluate the complex metabolomic changes at the different levels. The main outcome at that conditions seems to be to some extent similar to changes described for rats with chronic alcohol consumption. However, based on our findings, the processes of DNA fragmentation in testes are more intensive early in ethanol toxicity, potentially indicating a lack of adaptive defense mechanisms present in chronic alcoholism. Heightened expression in cytochrome CYP2E1 and CYP3A2 gene in short-term ethanol administration was demonstrated. According to our previous results, the discrepancies in CYP2E1 and CYP3A2 expression between acute and chronic alcohol exposure are observed and may stem from the inclusion of adaptive mechanisms during prolonged ethanol exposure, indicating metabolic adaptation. The above-mentioned changes are accompanied by notable alterations in the serum pool of free amino acids.

Thus, our results demonstrated profound changes in testes affecting the state of the genome, transcription processes and the metabolism of amino acids and proteins. We suggest that the revealed testicular metabolic disorders could have negative implications on cellular regulation of spermatogenesis even under short-term ethanol exposure.

Interests disclosure

Larysa Bondarenko is the member of the Editorial Council of *Innovative Biosystems and Bioengineering* and was not involved in the editorial evaluation or decision to accept this article for publication. The other authors have no conflicts of interest to declare.

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ВПЛИВ ГОСТРОЇ АЛКОГОЛЬНОЇ ІНТОКСИКАЦІЇ НА СТАБІЛЬНІСТЬ ТЕСТИКУЛЯРНОЇ ДНК, ЕКСПРЕСІЮ ГЕНІВ ЦИТОХРОМІВ CYP3A ТА CYP2E1 І ПУЛ ВІЛЬНИХ АМІНОКИСЛОТ СИРОВАТКИ КРОВІ ЩУРІВ

Проблематика. Токсична дія алкоголю на організм – давно відома медична проблема. Ушкоджуючий вплив алкоголю є кінцевим результатом складної взаємодії процесів метаболізму етанолу, запаленням і реакцій вродженого імунітету. В попередніх дослідженнях нами було вивчено віддалені наслідки хронічного алкоголізму і показано, що особливо глибокі зміни на рівні протеома і генома відбуваються в сім'яниках.
Мета. Дослідити вплив короткочасної гострої алкогольної інтоксикації (ГАІ) на фрагментацію ДНК сім'яників, експресію генів цитохромів CYP3A і CYP2E1 та пул вільних амінокислот у сироватці крові щурів.
Методика реалізації. Щурів-альбіносів лінії Вістар розділили на 2 групи (по 8 тварин у кожній): 1 – контрольна (інтактні щури), 2 – модель ГАІ (щури з відтворенням моделі короткотермінової ГАІ). ГАІ індукували повторним пероральним введенням 40 %-ного розчину етанолу в дозі 7 мл/кг маси тіла протягом 7-ми днів. Оцінювали вміст вільних амінокислот у сироватці крові, експресію мРНК CYP2E1 і CYP3A2 та фрагментацію ДНК у сім'яниках тварин.
Результати. У наших експериментах розвиток гострої алкогольної інтоксикації призводив до посилення процесів фрагментації ДНК у сім'яниках дорослих щурів порівняно з контрольною групою. Крім того, в сироватці крові щурів, які отримували етанол, рівень гістидину підвищувався в 1,67 разу, а глутаміну – в 1,13 разу, що корелювало з цією патологією. З іншого боку, рівні валіну, фенілаланіну, а також замінних і незамінних амінокислот знижувалися. Крім того, спостерігалося статистично значуще посилення експресії генів CYP2E1 і CYP3A2 у сім'яниках щурів в умовах ГАІ.
Висновки. Дослідження ефектів короткочасного прийому алкоголю щурами дало змогу отримати комплексну картину складних метаболічних змін в організмі на різних рівнях. Загалом наслідки короткочасного введення етанолу щурам у наших експериментах, очевидно, до певної міри схожі на зміни, описані для щурів із хронічним споживанням алкоголю. Наші результати продемонстрували наявність глибоких змін у сім'яниках, що впливають на стан геному, процеси транскрипції та обмін амінокислот і білків. Ми можемо припустити, що навіть виявлені тестикулярні метаболічні порушення за короткочасного впливу етанолу здатні спричиняти негативні наслідки для клітинної регуляції сперматогенезу.

Ключові слова: гостра алкогольна інтоксикація; фрагментація ДНК; експресія генів; CYP450; пул вільних амінокислот; сім'яники.