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INFLUENCE OF NF-κB ON THE DEVELOPMENT OF OXIDATIVE-NITROSATIVE STRESS IN THE LIVER OF RATS UNDER CONDITIONS OF CHRONIC ALCOHOL INTOXICATION

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Alcohol-related liver disease is the most common cause of liver disease worldwide. The purpose of this work is the establishment of the influence of the transcription factor κB on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication. The experiments were performed on 24 male Wistar rats weighing 180-220 g. The animals were divided into 4 groups of 6 animals: control; animals, which were administered NF-κB inhibitor, namely ammonium pyrrolidinedithiocarbamate (PDTC) at a dose of 76 mg/kg 3 times a week; animals, on which we simulated alcoholic hepatitis and group of combination of alcoholic hepatitis and NF-κB inhibitor. We determined in rat liver homogenate the following biochemical parameters: the activity of NO synthase isoforms, superoxide dismutase and catalase activity, the concentration of malonic dialdehyde, the concentration of peroxynitrite, nitrites and nitrosothiols, concentration of sulfide anion and superoxide anion radical production. Chronic alcohol intoxication led to increased production of reactive oxygen and nitrogen species on the background of decreased antioxidant activity, thus intensifying lipid peroxidation in the liver. Blockade of the transcription factor κB during chronic alcohol intoxication despite an increase in antioxidant activity and decrease of reactive oxygen and nitrogen species production did not ameliorate oxidative damage to the liver. Blockade of activation of nuclear transcription factor κB in rat liver by PDTC reduced the risk of oxidative damage to hepatocytes, but did not reduce the risk of developing nitrosative damage to hepatocytes.

K e y w o r d s: oxidative-nitrosative stress, chronic alcohol intoxication, NF-κB, PDTC, rat liver.

Alcohol-related liver disease is the most common cause of liver disease worldwide. According to the latest WHO estimates, alcohol consumption worldwide led to 3 million deaths from alcohol (5.3% of all deaths worldwide in 2016) and to 5.1% of total diseases in 2016. Cirrhosis of the liver caused by alcohol abuse caused 607,000 deaths and about 22.2 million people became disabled [1]. Chronic alcohol abuse leads to alcoholic fatty liver disease. About a third of people who continue to drink alcohol develop alcoholic steatohepatitis, and 20% suffer from alcoholic cirrhosis. Approximately 2% of patients with cirrhosis develop primary hepatocellular carcinoma [1].

Oxidative-nitrosative damage to hepatocytes occupies a prominent place in the development of alcohol-dependent liver lesions. Metabolism of alcohol through the hepatocyte microsomal oxidation system leads to excessive formation of reactive oxygen species such as superoxide anion radical and hydrogen peroxide. At the same time, chronic alcohol consumption leads to depletion of the liver's antioxidant systems [2]. However, in the scientific literature there is evidence that enhancing the antioxidant potential of the liver through the introduction of resveratrol improves the course of alcoholic hepatitis and reduces the intensity of oxidative stress [3].

Transcription factor κB (NF-κB) is one of the nuclear transcription factors that are actively involved in the regulation of pro-/antioxidant balance in the cell. Nuclear transcription factor κB controls the transcription of a number of prooxidant cytokines (interleukin 1-β, tumor necrosis factor-α, interleukin-6, etc.) and antioxidant enzymes (super-

oxide dismutase, enzymes of glutathione cycle, catalase, etc.), which allows this transcription factor to both increase and decrease the intensity of oxidative-nitrosative stress [4].

However, the role of activation of the transcription factor κB in the development of oxidative-nitrosative stress in the liver under conditions of chronic excess ethanol intake is controversial and not fully established.

The purpose of this work is the establishment of the influence of the transcription factor κB on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication.

Materials and Methods

The experiments were performed on 24 white adult male Wistar rats weighing 180-220 g. The animals were divided into 4 groups: I group – control (n = 6); II group – animals (n = 6), which were administered NF-κB inhibitor, namely ammonium pyrrolidinedithiocarbamate (PDTC) at a dose of 76 mg/kg 3 times a week throughout the experiment [5]; III group – animals, on which we simulated alcoholic hepatitis (n = 6) by forced intermittent alcoholization for 5 days by intraperitoneal administration of 16.5% ethanol solution in 5% glucose solution, at the rate of 4 ml/kg body weight once a day. Afterward, there was a break for two days. Then animals again received intraperitoneal administration of 16.5% ethanol solution in 5% glucose solution, at the rate of 4 ml/kg body weight a day for 5 days. Then they were converted to 10% ethanol as the only source of drink for 51 days, the total experimental procedure lasted 63 days [6]. IV group – animals (n = 6), on which we simulated chronic alcohol intoxication as in group III and administered PDTC according to the scheme of group II.

The control group included animals that were subjected to similar manipulations throughout the study, but injected with saline. Conditions for keeping animals in the vivarium were standard. Average temperature was 20-22°C (68-72 0F). Relative humidity was 40-45%. Air change ratio was 12-14 fresh-air changes per hour. Animals received nutrition according to the following standards [7].

The experiments followed the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) in accordance with the General Principles of Animal Experiments approved by the First National Congress of Bioethics, and the requirements of the Procedure for scientific research, animal experiments (2012). All manipulations with laboratory animals were approved by bioethical committee of Poltava State Medical University (Record № 197 from 23.09.2021).

Removal of animals from the experiment occurred on day 63 by taking blood from the right ventricle of the heart under thiopental anesthesia. Serum and liver were studied.

In the serum of rats, the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutaminyltranspeptidase (γ-GTP) was determined using diagnostic kits, produced by NPP "Filisit-Diahnostyka". We also calculated the de Ritis coefficient (AST/ALT).

In rat liver homogenate following biochemical parameters were determined: the activity of inducible NO synthase (iNOS) and constitutive isoforms of NO synthase (cNOS) [8], superoxide dismutase (SOD) activity [9] and catalase [10], concentration of malonic dialdehyde (MDA) [11], concentration of peroxynitrite of alkali and alkaline earth metals ($ONOO^-$) [12], concentration of nitrites, concentration of low molecular weight nitrosothiols (S-NO) [13], concentration of sulfide anion [14] and superoxide anion radical production [15].

Total NO-synthase activity was evaluated by the increase of nitrites after incubation of 10% liver homogenate (0.2 ml) for 30 min in the incubation solution (2.5 ml of 0.1 M Tris buffer, 0.3 ml of 320 mM aqueous solution of L-arginine and 0.1 ml of 1 mM NADPH+H+ solution). To determine the activity of constitutive NOS (cNOS) 1% solution of aminoguanidine hydrochloride was used and the incubation time was extended to 60 min [8]. The activity of inducible NOS (iNOS) was calculated by the formula: iNOS = gNOS-cNOS.

Adrenaline auto-oxidation reaction in an alkaline environment with the generation of superoxide was used to determine SOD activity. SOD activity was calculated in conventional units (c.u., 1 unit indicates a 50% inhibition of the reaction rate) by comparison of speed of adrenaline auto-oxidation in presence of tissue homogenate and without it [9].

The method of catalase activity estimation was based on the determination of colored products formed by the reaction of hydrogen peroxide with ammonium molybdate. The amount of hydrogen peroxide decomposed in the presence of a sample containing catalase can help us to make a conclusion about the activity of catalase [10].

Free malonic dialdehyde specifically reacts with 1-methyl-2-phenyl-indole in a mixture of methanol and acetonitrile to form chromogen (carbocyanine dye) with maximum light absorption at a wavelength of 586 nm [11].

Peroxynitrite concentration was measured by using its reaction with potassium iodide under pH 7.0 in 0.2 M phosphate buffer with the same pH, which yields I_3 with maximum absorbance at 355 nm wavelength [12].

The method for the determination of nitrosothiols was based on the determination of the difference in the concentration of nitrites (NO_2^-) using Griess reagent (modified by Ilosvay) before and after oxidation of nitrosothiol complexes (SNO) to nitrites with a solution of mercuric chloride ($HgCl_2$) [13].

Sulfides specifically react with N-N-dimethyl-para-phenylenediamine in the presence of Fe^{3+} ions and excess of hydrochloric acid to form a red-pink chromogen with maximum light absorption at a wavelength of 667 nm [14].

The method for estimation of superoxide anion radical production was based on Nitroblue tetrazolium (NBT) reduction by superoxide with the formation of diformazan, a dark blue insoluble precipitate [15].

Statistical processing of biochemical research results was performed using Kruskal–Wallis one-way analysis of variance with following pairwise comparison using the Mann-Whitney U-test. In order to avoid multiple comparison errors we used correction by Bonferroni method. All statistical calculations were performed in Microsoft Office Excel and its extension Real Statistics 2019. The difference was considered statistically significant at $P < 0.05$.

Results

Biochemical markers of cytolytic liver syndrome in blood serum under the conditions of inhibition of the transcription factor κB against the background of chronic alcohol intoxication. Biochemical analysis of blood serum of animals under the conditions of inhibition of the transcription factor κB in rats with alcohol intoxication revealed the following changes: the activity of AST in blood serum under the conditions of the introduction of the inhibitor of transcription factor κB increased by 1.87 times, under the conditions of alcohol intoxication by 2.32 times and under the conditions of their combined action it increased by 1.64 times in relation to the control group of rats (Table 1). Inhibition of the transcription factor κB against the background of alcohol intoxication reduces the activity of AST by 1.41 times in relation to the group of alcohol intoxication.

ALT activity in blood serum increased by 1.73 times under the conditions of the κB transcription factor inhibitor, by 3.01 times under the conditions of alcohol intoxication, and by 1.24 times under the conditions of their combined effect in relation to the control group of rats. Inhibition of the transcription factor κB against the background of alcohol intoxication reduces the activity of ALT by 2.43 times in relation to the group of rats with alcohol intoxication.

The De Ritis coefficient under conditions of alcohol intoxication decreased by 1.31 times and it increased by 1.31 times under the conditions of administration of an inhibitor of the transcription factor κB against the background of alcohol intoxication in relation to the control group of rats. Inhibition of the transcription factor κB against the background of alcohol intoxication increased the De Ritis coefficient by 1.71 times in relation to the group of rats with alcohol intoxication.

The activity of γ-GTP in blood serum under the conditions of the introduction of the transcription factor κB inhibitor increased by 1.33 times, under the conditions of alcohol intoxication, it decreased by 5.58 times, and under the conditions of combined action, it increased by 1.7 times in relation to the control group of rats. Inhibition of the transcription factor κB against the background of alcohol intoxication increases the activity of γ-GTP by 9.5 times in relation to the group of rats that were simulated only alcohol intoxication.

Influence of transcription factor κB on biochemical parameters of rat liver. We found that under the conditions of administration of the NF-κB inhibitor, the activity of cNOS in the liver of rats increased by 1.44 compared with the control group ($P = 0.00477$) (Table 2). Under these conditions, the concentration of $ONOO^-$ in the liver of rats increased 4.18 times ($P = 0.00477$), and the concentration of nitrites and S-NO decreased by 3.2 times ($P = 0.00413$) and 2.4 times ($P = 0.00485$), respectively, under conditions of inhibition of NF-κB compared with the control group (Table 2).

Analyzing the antiradical protection and production of reactive oxygen species under conditions of NF-κB inhibition, we found that the activity of superoxide dismutase decreased by 1.25 times ($P = 0.00455$), the activity of catalase decreased by 1.36 times ($P = 0.00462$). The production of

Table 1. Biochemical indicators of blood serum of rats under the conditions of inhibition of the transcription factor κB and alcohol intoxication, M ± m

Biochemical parameters	Group			
	Control	NF-κB inhibition	Alcohol intoxication	NF-κB inhibition on the background of alcohol intoxication
AST activity, mmol/h per l	1.71 ± 0.07	3.19 ± 0.02*	3.96 ± 0.13*^	2.80 ± 0.02*^#
ALT activity, mmol/h per l	1.22 ± 0.06	2.11 ± 0.02*	3.67 ± 0.06*^	1.51 ± 0.04*^#
De Ritis coefficient	1.41 ± 0.06	1.51 ± 0.02	1.08 ± 0.04*^	1.85 ± 0.04*^#
γ-GTP activity, μcat/l	0.67 ± 0.04	0.89 ± 0.01*	0.12 ± 0.02*^	1.14 ± 0.01*^#

*$P < 0.05$ compared to the control group; ^$P < 0.05$ compared to the NF-κB inhibition group; #$P < 0.05$ compared to alcohol intoxication group

superoxide-anion radical decreased by 1.56 times ($P = 0.0047$), and the concentration of malonic dialdehyde increased by 2.35 times ($P = 0.00492$) in the liver of rats compared with these indicators in the control group. The concentration of sulfide anion in the liver of rats under conditions of inhibition of NF-κB increased 1.43 times ($P = 0.00485$) compared with the control group (Table 2).

The effect of prolonged alcohol intoxication on the biochemical parameters of the liver of rats.

We described the effect of long-term alcohol intoxication on the biochemical parameters of the liver of rats in detail in our previous publications. The received experimental data indicated that the development of oxidative-nitrosative stress played a leading role in liver damage under conditions of chronic alcohol intoxication. H_2S-mediated mechanism contributed to the preservation of hepatocyte integrity under ethanol-induced hepatocyte injury [16].

The effect of inhibition of the transcription factor κB under conditions of prolonged alcohol intoxication on the biochemical parameters of the liver of rats.

Under the conditions of NF-κB inhibition against the background of prolonged alcohol intoxication, we found that the activity of iNOS in the liver of rats increased by 3.63 times ($P = 0.005$) compared with the control group and by 4.14 times ($P = 0.00477$) compared with the NF-κB inhibitor group. The activity of cNOS in the liver of rats increased 4.04 times ($P = 0.00492$) under conditions of inhibition of NF-κB on the background of prolonged alcohol intoxication compared with the control group and by 2.79 times ($P = 0.00477$) compared with the group of animals which were administered the NF-κB inhibitor and by 2.48 times ($P = 0.005$) compared with the group of rats with prolonged alcohol intoxication (Table 2).

We found that the concentration of $ONOO^-$ in the liver of rats increased by 4.78 times ($P = 0.0047$) under conditions of inhibition of NF-κB on the background of prolonged alcohol intoxication compared with the control group and by 1.14 times ($P = 0.0047$) compared with the group of animals injected with NF-κB inhibitor, but it decreased by 1.98 times ($P = 0.00485$) compared with the group of rats, on which we simulated prolonged alcohol intoxication (Table 1). The concentration of nitrites in the liver of rats decreased by 2.07 times ($P = 0.0047$) under conditions of inhibition of NF-κB on the background of prolonged alcohol intoxication compared with the control group and by 1.64 times ($P = 0.00477$) compared to alcohol intoxication group and increased by 1.55 times ($P = 0.00406$) compared with the group of animals treated with NF-κB inhibitor. The concentration of S-NO, which acts as a buffer of nitric oxide, decreased by 2.4 times ($P = 0.00508$) under conditions of inhibition of NF-κB on the background of prolonged alcohol intoxication compared with the control group (Table 2).

Analyzing the development of oxidative stress in the liver of animals injected with NF-κB inhibitor on the background of prolonged alcohol intoxication, we found that the activity of superoxide dismutase in the liver of rats increased by 1.21 times ($P = 0.0109$) compared to controls and by 1.51 times

Table 2. Biochemical changes in rat liver under conditions of NF-κB inhibition and alcohol intoxication (M ± m)

Biochemical parameters	Group			
	Control	NF-κB inhibition	Alcohol intoxication	NF-κB inhibition on the background of alcohol intoxication
Inducible NO synthase, μmol/min per g of protein	0.16 ± 0.02	0.14 ± 0.02	0.72 ± 0.07*^	0.58 ± 0.18*^
Constitutive NO synthases, μmol/min per g of protein	0.027 ± 0.0003	0.039 ± 0.0007*	0.044 ± 0.0009*^	0.109 ± 0.0005*^#
Superoxide dismutase, c.u.	12.34 ± 0.55	9.87 ± 0.08*	12.23 ± 1.03	14.87 ± 0.31*^
Catalase, μkat/g	0.38 ± 0.008	0.28 ± 0.022*	0.23 ± 0.01*	0.32 ± 0.002*#
Malonic dialdehyde, μmol/g	12.32 ± 0.11	29.01 ± 1.26*	15.91 ± 0.32*^	17.1 ± 0.2*^#
Superoxide anion radical, nmol s per g	1.84 ± 0.004	1.18 ± 0.008*	2.71 ± 0.03*^	1.78 ± 0.01*^#
$ONOO^-$, μmol/g	0.45 ± 0.01	1.88 ± 0.03*	4.26 ± 0.03*^	2.15 ± 0.02*^#
S-NO, μmol/g	0.36 ± 0.019	0.15 ± 0.008*	0.18 ± 0.034*	0.12 ± 0.026*
NO_2 concentration, nmol/g	7.14 ± 0.17	2.23 ± 0.06*	5.67 ± 0.34*^	3.45 ± 0.17*^#
Sulfide anion, μmol/g	7.23 ± 0.17	10.32 ± 0.24*	15.01 ± 0.32*^	2.25 ± 0.35*^#

*$P < 0.05$ compared to the control group; ^$P < 0.05$ compared to the NF-κB inhibition group; #$P < 0.05$ compared to alcohol intoxication group

($P = 0.00455$) compared to animals injected with NF-κB inhibitor. The activity of catalase in the liver of rats in the group of animals injected with NF-κB inhibitor on the background of prolonged alcohol intoxication decreased by 1.19 times ($P = 0.00462$) compared with the control group and increased 1.39 times ($P = 0.00462$) compared to prolonged alcohol intoxication group. The production of superoxide anion radical in the liver of rats in the group administered the NF-κB inhibitor on the background of prolonged alcohol intoxication decreased by 1.03 times ($P = 0.00462$) compared to the control and by 1.52 ($P = 0.0047$) times compared with prolonged alcohol intoxication group and increased by 1.51 times ($P = 0.0047$) compared to animals injected only with NF-κB inhibitor. The concentration of malonic dialdehyde in the liver of rats in the group administered the NF-κB inhibitor on the background of prolonged alcohol intoxication increased by 1.39 times ($P = 0.00477$) compared to the control and by 1.07 times ($P = 0.0298$) compared with prolonged alcohol intoxication group and decreased 1.7 times ($P = 0.00477$) compared to the group of animals injected with NF-κB inhibitor. The concentration of sulfide anion in the liver of rats injected with NF-κB inhibitor on the background of prolonged alcohol intoxication decreased by 3.21 times ($P = 0.0047$) compared with controls, by 4.59 times ($P = 0.00477$) compared to the group of animals injected with NF-κB inhibitor and by 6.67 times ($P = 0.00477$) compared to prolonged alcohol intoxication group.

Discussion

The decrease in the activity of superoxide dismutase and catalase during the blockade of NF-κB activation is due to the fact, that superoxide dismutase and catalase are under the direct transcriptional control of NF-κB [17]. Decrease in production of superoxide anion radical is connected with its "interception" by nitric oxide. The reaction rate between nitric oxide and superoxide anion radical with peroxynitrite formation is higher than the reac-

tion rate of superoxide anion radical with superoxide dismutase, however, under physiological conditions, low concentrations of nitric oxide contribute to the predominance of superoxide dismutase-dependent pathway superoxide anion radical metabolism [18]. Under conditions of NF-κB activation blockade, superoxide dismutase activity decreases, which directs the superoxide anion radical to react with nitric oxide, the production of which increases from the constitutive isoforms of NOS, with the formation of an excess of ONOO-. The predominance of this pathway of superoxide anion radical metabolism is also evidenced by the decrease in the concentration of nitrites and S-NO.

The growth of free MDA concentration indicates the development of oxidative damage of hepatocytes under conditions of NF-κB activation blockade. During the development of oxidative stress, NF-κB and activator protein-1 (AP-1) are activated as an adaptive response to oxidative stress [19, 20]. AP-1 can, independently of NF-κB, activate iNOS gene expression, but the intensity of AP-1-dependent iNOS gene expression will be significantly lower than NF-κB-controlled expression [21]. This explains the lack of statistically significant changes in iNOS activity in the group of animals with inhibited NF-κB activation.

Therefore, inhibition of NF-κB in control animals may lead to the development of oxidative-nitrosative damage to hepatocytes. Increasing the concentration of sulfide anion can be considered as an adaptive response to the development of oxidative-nitrosative damage to hepatocytes, because H_2S has pronounced antioxidant properties and can serve as a "scavenger" of reactive oxygen and nitrogen species [22].

Alcohol (C_2H_5OH) has several possible pathways of degradation in organism. First and foremost one is its transformation by alcohol dehydrogenase (EC 1.1.1.1) to acetaldehyde with subsequent conversion to acetate via acetaldehyde-dehydrogenase (EC 1.2.1.10). Acetaldehyde can stimulate mitophagy due to impairment of mitochondrial functions and increased reactive oxygen species (ROS) production [23]. This may be the result of decreased activation of AMPK and sirtuin-3 expression in mitochondria [24]. Acetaldehyde-induced mitophagy may be omitted if mitochondrial acetaldehyde-dehydrogenase 2 (ALDH-2) function is not impaired. ALDH-2 protects mitochondria by decreasing ROS generation and regulating Beclin-1 pathway, thus decreasing autophagy processes in cell [25]. In case of increased expression of ALDH-2 during alcohol intoxication, it can be translocated to the endoplasmic reticulum. Such translocation promotes the formation of GP78/Insig1/ HMG-CoA reductase complex, which leads to increased HMG-CoA reductase degradation through ubiquitination. This results in decreased cholesterol formation [26]. However, increased ALDH-2 activity may cause elevation of acetate concentration in mitochondria. Acetate accumulation in the liver may force substrate induction of acetyl-CoA synthetase, which will transform excessive acetate to acetyl-CoA. Increase of acetyl-CoA will lead to excessive free fatty acid formation [27]. Together with alcohol-induced stimulation of diacylglycerol acyltransferase 2, these events will provide necessary conditions for neutral fat accumulation in liver during chronic alcohol intoxication and alcoholic steatohepatitis development [28].

Another pathway of ethanol degradation in organism involves microsomal ethanol oxidation system (MEOS) [29]. MEOS involves a cruder approach to alcohol destruction, which summarizes to ethanol destruction by oxidation with usage of reactive oxygen species generated by cytochromes of P450 family [29]. The most prominent cytochromes from P450 family involved in alcohol destruction are CYP2E1 and CYP2A6 (CYP2A3 for rats). CYP2E1 uses molecular oxygen (O2) and consumes NADPH provided by the NADPH-cytochrome P450 reductase, which results in the production of superoxide anion radical used for alcohol degradation [30]. The general assumption states that CYP2E1 only accounts for a small amount (about 10%) of total ethanol metabolism during one-time excessive alcohol intake, but it becomes more important during chronic alcohol consumption [30]. This statement can be explained by the transfer of electrons from NADH, formed in the reaction of alcohol dehydrogenase-dependent alcohol degradation, to NADPH via nicotinamide nucleotide transhydrogenase [31]. Increased NADPH concentration during chronic ethanol consumption may cause substrate induction of CYP2E1 with subsequent increase in ROS production and oxidative stress development. Increased activity of CYP2E1 may cause activation of nuclear factor erythroid 2-related factor 2 (Nrf-2), which in turn stimulates transcription of CYP2A5. CYP2A5, in turn, further aggravates oxidative stress by increase in ROS production [32]. Bacterial lipopolysaccharide translocation from gut to bloodstream during chronic alco-

hol intake can also increase CYP2E1 activity [30]. Therefore, activation of MEOS system may be the main contributor to increased superoxide anion production observed in our study.

Increased MDA concentration in the chronic alcohol intoxication group indicates the development of oxidative damage to the liver tissues. In response to this and to alcohol-dependent lipopolysaccharide translocation from gut to bloodstream, NF-κB may be activated [33]. NF-κB cascade activation explains increased iNOS activity in this group of animals. Increased iNOS activity and superoxide anion production create conditions for peroxynitrite formation, which is greatly increased in this group. Decreased catalase activity may result from increased workload on the enzyme. Since it has to both metabolize ethanol and hydrogen peroxide produced by superoxide dismutase during chronic alcohol intoxication [29].

Decreased S-NO concentration in the chronic alcohol intoxication group may be the result of alcohol-induced increase in glutaredoxin-1 activity [34]. Glutaredoxins can destroy low molecular weight nitrosothyols and may be viewed as a protective response to nitrosative stress caused by excessive alcohol intake [35].

The increase in the concentration of sulfide anion during chronic alcohol intoxication can be explained by the compensatory antioxidant effect associated with the ability of H_2S to interact with the superoxide anion radical to form thiosulfate and sulfate, which results in the hepatoprotective effect of H_2S [36, 37].

Increased iNOS activity in combination with chronic alcohol intoxication and NF-κB blockade may be associated with activation of the transcription factor AP-1 in response to alcoholic hepatocyte damage [19]. Under conditions of blockade of NF-κB activation, excessive activation of STAT-3 is possible due to alcohol-induced oxidative damage to liver cells [38]. Activated STAT-3 has the ability to reduce the production of superoxide anion radicals and increase the expression of antioxidant enzymes (superoxide dismutase and catalase) by activating nrf-2 [39]. Elevated concentrations of MDA may be associated with increased, relative to the control group, concentration of $ONOO^-$ and decreased concentrations of H_2S.

Limitations of the study. The limitation of our study is that the exact amount of expression of NF-κB-related proteins was not measured.

Conclusions. Blockade of activation of nuclear transcription factor κB in rat liver by PDTC reduces the risk of oxidative damage to hepatocytes, as evidenced by a decrease in the production of superoxide anion radical and a high activity of antioxidant enzymes. However, this does not reduce the risk of developing nitrosative damage to hepatocytes, as evidenced by the absence of changes in NO-synthase activity and a high concentration of peroxynitrites relative to control. Thus, blockade of the activation of nuclear transcription factor κB in the liver cannot be considered a full-fledged means of pathogenetic therapy for alcohol-induced liver damage.

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

ВПЛИВ NF-κB НА РОЗВИТОК ОКСИДАТИВНО-НІТРОЗАТИВНОГО СТРЕСУ В ПЕЧІНЦІ ЩУРІВ ЗА УМОВ ХРОНІЧНОЇ АЛКОГОЛЬНОЇ ІНТОКСИКАЦІЇ

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Вживання алкоголю є найпоширенішою причиною захворювань печінки у всьому світі. Метою даної роботи є встановлення впливу транскрипційного фактора κB на розвиток оксидативно-нітрозативного стресу в печінці щурів за умов хронічної алкогольної інтоксикації. Досліди проводили на 24 щурах-самцях лінії Wistar, вагою 180-220 г. Щурів розділили на 4 групи по 6 тварин: контрольна; тварини, яким вводили інгібітор NF-κB – амоній піролідиндитіокарбамат (PDTC) у дозі 76 мг/кг 3 рази на тиждень; тварини з алкогольним гепатитом та група тварин, яким вводили інгібітор NF-κB на фоні алкогольного гепатиту. У гомогенаті печінки щурів визначали такі біохімічні показники: активність ізоформ NO-синтаз, суперок-

сиддисмутази та каталази, концентрацію малонового діальдегіду, пероксинітриту, нітритів та нітрозотіолів, концентрацію сульфід-аніону та продукцію супероксид-аніону. Встановлено, що хронічна алкогольна інтоксикація призводила до збільшення продукції активних форм кисню та азоту на фоні зниження антиоксидантної активності і посилення пероксидного окислення ліпідів у печінці. Блокада транскрипційного фактора κB під час хронічної алкогольної інтоксикації, незважаючи на підвищення антиоксидантної активності та зниження продукції активних форм кисню та азоту, не запобігала оксидативному пошкодженню печінки. Зроблено висновок, що блокада активації ядерного транскрипційного фактора κB у печінці щурів за допомогою PDTC знижує ризик окисного, але не зменшує ризик розвитку нітрозативного пошкодження гепатоцитів.

Ключові слова: оксидативно-нітрозативний стрес, хронічна алкогольна інтоксикація, NF-κB, PDTC, печінка щурів.

References

1. Avila MA, Dufour JF, Gerbes AL, Zoulim F, Bataller R, Burra P, Cortez-Pinto H, Gao B, Gilmore I, Mathurin P, Moreno C, Poznyak V, Schnabl B, Szabo G, Thiele M, Thursz MR. Recent advances in alcohol-related liver disease (ALD): summary of a Gut round table meeting. *Gut*. 2020; 69(4): 764-780.
2. Kim HG, Huang M, Xin Y, Zhang Y, Zhang X, Wang G, Liu S, Wan J, Ahmadi AR, Sun Z, Liangpunsakul S, Xiong X, Dong XC. The epigenetic regulator SIRT6 protects the liver from alcohol-induced tissue injury by reducing oxidative stress in mice. *J Hepatol*. 2019; 71(5): 960-969.
3. Petrella C, Carito V, Carere C, Ferraguti G, Ciafrè S, Natella F, Bello C, Greco A, Ralli M, Mancinelli R, Messina MP, Fiore M, Ceccanti M. Oxidative stress inhibition by resveratrol in alcohol-dependent mice. *Nutrition*. 2020; 79-80: 110783.
4. Wang Y, Hu H, Yin J, Shi Y, Tan J, Zheng L, Wang C, Li X, Xue M, Liu J, Wang Y, Li Y, Li X, Liu F, Liu Q, Yan S. TLR4 participates in sympathetic hyperactivity Post-MI in the PVN by regulating NF-κB pathway and ROS production. *Redox Biol.* 2019; 24: 101186.
5. Qin JD, Cao ZH, Li XF, Kang XL, Xue Y, Li YL, Zhang D, Liu XY, Xue YZ. Effect of ammonium pyrrolidine dithiocarbamate (PDTC) on NF-κB activation and CYP2E1 content of rats with immunological liver injury. *Pharm Biol*. 2014; 52(11): 1460-1466.
6. Stepanov YuM, Didenko VI, Dynnik OB, Konenko IS, Oshmianskaia NYu, Galinsky AA. Association of morphological changes in the liver parenchyma and its rigidity under the conditions of the experimental modeling of alcoholic and toxic hepatitis. *Journal of the NAMSU*. 2017; 23(3-4): 196-204.
7. National Research Council (US) Subcommittee on Laboratory Animal Nutrition. Nutrient Requirements of Laboratory Animals: Fourth Revised Edition, 1995. Washington (DC): National Academies Press (US); 1995.
8. Mykytenko AO, Yeroshenko GA. Reaction of hemomicrocirculatory bed of rat liver and changes in the functional state of the nitric oxide cycle under the conditions of modeling alcoholic hepatitis. *World Med Biol*. 2020; 16(73): 194-200.
9. Brusov OS, Gerasimov AM, Panchenko LF. The influence of natural inhibitors of radical reactions on autooxidation of adrenaline. *Bull Exp Biol Med.* 1976; 81(1): 33-35. (In Russian).
10. Korolyuk MA, Ivanova LI, Mayorova IG, Tokarev VE. A method of determining catalase activity. *Lab Delo.* 1988; (1): 16-19. (In Russian).
11. Gérard-Monnier D, Erdelmeier I, Régnard K, Moze-Henry N, Yadan JC, Chaudière J. Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Analytical applications to a colorimetric assay of lipid peroxidation. *Chem Res Toxicol.* 1998; 11(10): 1176-1183.
12. Yelins'ka AM, Akimov OYe, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. *Ukr Biochem J*. 2019; 91(1): 80-85.
13. Gaston B, Reilly J, Drazen JM, Fackler J, Ramdev P, Arnelle D, Mullins ME, Sugarbaker DJ, Chee C, Singel DJ, Loscalzo J, Stamler J. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci USA*. 1993; 90(23): 10957-10961.

14. Sugahara S, Suzuki M, Kamiya H, Yamamuro M, Semura H, Senga Y, Egawa M, Seike Y. Colorimetric Determination of Sulfide in Microsamples. *Anal Sci*. 2016; 32(10): 1129-1131.
15. Kostenko VO, Tsebrzhins'kii OI. Production of superoxide anion radical and nitric oxide in renal tissues sutured with different surgical suture material. *Fiziol Zh*. 2000; 46(5): 56-62. (In Ukrainian).
16. Mykytenko AO, Akimov OY, Neporada KS. Influence of lipopolysaccharide on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication. *Fiziol Zh*. 2022; 68(2): 29-35.
17. Yang W, Yuan W, Peng X, Wang M, Xiao J, Wu C, Luo L. PPAR γ/Nnat/NF-κB Axis Involved in Promoting Effects of Adiponectin on Preadipocyte Differentiation. *Mediators Inflamm*. 2019; 2019: 5618023.
18. Radi R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proc Natl Acad Sci USA*. 2018; 115(23): 5839-5848.
19. Hayes JD, Dinkova-Kostova AT, Tew KD. Oxidative Stress in Cancer. *Cancer Cell*. 2020;38(2):167-197.
20. Matsytska YeK, Akimov OYe, Mykytenko AO. Influence of corvitin and metformin on biochemical changes in lacrimal glands of rats during water avoidance stress modeling. *J Ophthalmol*. 2022; (3): 39-44.
21. Ahmad N, Ansari MY, Bano S, Haqqi TM. Imperatorin suppresses IL-1β-induced iNOS expression via inhibiting ERK-MAPK/AP1 signaling in primary human OA chondrocytes. *Int Immunopharmacol*. 2020; 85: 106612.
22. Murphy B, Bhattacharya R, Mukherjee P. Hydrogen sulfide signaling in mitochondria and disease. *FASEB J*. 2019; 33(12): 13098-13125.
23. Srinivasan MP, Bhopale KK, Caracheo AA, Kaphalia L, Loganathan G, Balamurugan AN, Rastellini C, Kaphalia BS. Differential cytotoxicity, ER/oxidative stress, dysregulated AMPKα signaling, and mitochondrial stress by ethanol and its metabolites in human pancreatic acinar cells. *Alcohol Clin Exp Res*. 2021; 45(5): 961-978.
24. Silva J, Spatz MH, Folk C, Chang A, Cadenas E, Liang J, Davies DL. Dihydromyricetin improves mitochondrial outcomes in the liver of alcohol-fed mice via the AMPK/Sirt-1/PGC-1α signaling axis. *Alcohol*. 2021; 91: 1-9.
25. Xu T, Guo J, Wei M, Wang J, Yang K, Pan C, Pang J, Xue L, Yuan Q, Xue M, Zhang J, Sang W, Jiang T, Chen Y, Xu F. Aldehyde dehydrogenase 2 protects against acute kidney injury by regulating autophagy via the Beclin-1 pathway. *JCI Insight*. 2021; 6(15): e138183.
26. Zhong S, Li L, Liang N, Zhang L, Xu X, Chen S, Yin H. Acetaldehyde Dehydrogenase 2 regulates HMG-CoA reductase stability and cholesterol synthesis in the liver. *Redox Biol*. 2021; 41: 101919.
27. Yoshii Y, Furukawa T, Saga T, Fujibayashi Y. Acetate/acetyl-CoA metabolism associated with cancer fatty acid synthesis: overview and application. *Cancer Lett*. 2015; 356(2 Pt A): 211-216.
28. Wang M, Ma LJ, Yang Y, Xiao Z, Wan JB. n-3 Polyunsaturated fatty acids for the management of alcoholic liver disease: A critical review. *Crit Rev Food Sci Nutr*. 2019; 59(sup1): S116-S129.
29. Zima T. Alcohol Abuse. *EJIFCC*. 2018; 29(4): 285-289.
30. Lu Y, Cederbaum AI. Cytochrome P450s and Alcoholic Liver Disease. *Curr Pharm Des*. 2018; 24(14): 1502-1517.
31. Xie N, Zhang L, Gao W, Huang C, Huber PE, Zhou X, Li C, Shen G, Zou B. NAD^+ metabolism: pathophysiologic mechanisms and therapeutic potential. *Signal Transduct Target Ther*. 2020; 5(1): 227.
32. Abdelhamid AM, Elsheakh AR, Suddek GM, Abdelaziz RR. Telmisartan alleviates alcohol-induced liver injury by activation of PPAR-γ/Nrf-2 crosstalk in mice. *Int Immunopharmacol*. 2021; 99: 107963.
33. Tang J, Xu L, Zeng Y, Gong F. Effect of gut microbiota on LPS-induced acute lung injury by regulating the TLR4/NF-kB signaling pathway. *Int Immunopharmacol*. 2021; 91: 107272.
34. Sun X, Ye C, Deng Q, Chen J, Guo C. Contribution of glutaredoxin-1 to Fas s-glutathionylation and inflammation in ethanol-induced liver injury. *Life Sc*i. 2021; 264: 118678.
35. Ren X, Sengupta R, Lu J, Lundberg JO, Holmgren A. Characterization of mammalian glutaredoxin isoforms as S-denitrosylases. *FEBS Lett*. 2019; 593(14): 1799-1806.
36. Bostelaar T, Vitvitsky V, Kumutima J, Lewis BE, Yadav PK, Brunold TC, Filipovic M, Lehnert N,

Stemmler TL, Banerjee R. Hydrogen Sulfide Oxidation by Myoglobin. *J Am Chem Soc.* 2016; 138(27): 8476-8488.

37. Liu Z, Wang X, Li L, Wei G, Zhao M. Hydrogen Sulfide Protects against Paraquat-Induced Acute Liver Injury in Rats by Regulating Oxidative Stress, Mitochondrial Function, and Inflammation. *Oxid Med Cell Longev.* 2020; 2020: 6325378.
38. Grohmann M, Wiede F, Dodd GT, Gurzov EN, Ooi GJ, Butt T, Rasmiena AA, Kaur S, Gulati T, Goh PK, Treloar AE, Archer S, Brown WA, Muller M, Watt MJ, Ohara O, McLean CA, Tiganis T. Obesity Drives STAT-1-Dependent NASH and STAT-3-Dependent HCC. *Cell.* 2018; 175(5): 1289-1306.e20.
39. Zhu Q, Li H, Xie X, Chen X, Kosuru R, Li S, Lian Q, Cheung CW, Irwin MG, Ge RS, Xia Z. Adiponectin Facilitates Postconditioning Cardioprotection through Both AMPK-Dependent Nuclear and AMPK-Independent Mitochondrial STAT3 Activation. *Oxid Med Cell Longev.* 2020; 2020: 4253457.