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ORIGINAL PAPER

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The impact of ademetionine and ipidacrine/phenibut on the NCAM distribution and behavior in the rat model of drug-induced liver injury

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ABSTRACT

Introduction. Recently, more attention is being paid to the drug-induced liver injury (DILI) as a consequence of the tuberculos is treatment and the need for new medicine is emphasized. The use of isoniazid and rifampicin has a potentiating effect, which increases the risk of substancial liver damage. In turn, systemic accumulation of toxic metabolites leads to negative changes in various organs, including the brain. It causes an imbalance in biochemical and neurophysiological processes in the brain, ultimately giving the onset to the development of hepatic encephalopathy.

Aim. The effects of rifampicin and isoniazid on the central nervous system have not been studied before and we aimed to evaluate the impact these two substances have on the neuronal cell adhesion molecules (NCAM) distribution and animal behavior in the rat model of DILI.

Material and methods. The 24 male Wistar rats, weighing 180-220 g were used for the experiment and divided to the groups (n=6): 1 – control; 2 – rats with experimental DILI; 3 – rats with DILI plus the intravenous infusion of S-adenosyl-L-methionine at a dose of 35 mg/kg; 4 – rats with DILI plus a fixed combination of ipidacrine hydrochloride at a dose 1 mg/kg body weight and phenibut at a dose 60 mg/kg body weight daily for the last 14 days of the experiment. All experimental procedures were carried out in the accordance with the principles outlined in the current Guide to the Care and Use of Experimental Animals. The locomotor and research activities were studied in the open field test. The activity of aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) in the serum of rats were tested to confirm the liver damage. The quantitative analyses of soluble and membrane forms of NCAM were performed with ELISA. The ANOVA followed by a Tukey post-hoc test was used to assess statistical differences between groups.

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Results. Our investigation in the open field test revealed a significant decrease in the locomotor and research activity of rats after 28 days of rifampicin and isoniazid administration. The recovery of investigated parameters was observed in groups of animals treated with ademetionine (AD group) or combination of ipidacrine and phenibut (IP/PB group). We also observed that changes in rats' behavior were consistent with alterations of the NCAM levels in the thalamus and hippocampus. Thus, the level of membrane NCAM was significantly decreased under DILI in both investigated brain regions (thalamus and hippocampus), while both AD and IP/PB treatments restored membrane NCAM levels towards those observed in the control group at least in the hippocampus.
Conclusion. Obtained data suggests that both ademetionine and combinated drug containing ipidacrine and phenibut possesses neuroprotective properties and could prevent the decline in synaptic plasticity under antitubercular therapy.
Keywords. ademetionine, brain, ipidacrine/phenibut, isoniazid, liver disease, NCAM, rifampicin

Introduction

Drug-induced liver injury (DILI) accounts for up to 10 % of all adverse reactions associated with the use of drugs. According to World Health Organization (WHO), 50 out of 1000 patients are hospitalized due to the drug-induced complications.[1,2] Information provided by WEB-platform LiverTox (http://livertox.nlm.nih.gov) indicates that 353 (53 %) of the 671 drugs available for analysis provoke the hepatotoxicity. Moreover, isoniazid, pyrazinamide, and rifampicin are the most toxic liver agents. In particular, in studies published in the United States in 2015, based on the analysis of more than 600 cases in 12 clinical trials, 46 % of DILI cases were associated with antimicrobial drugs, such as antibiotics and antitubercular drugs.[2]

The simultaneous use of isoniazid and rifampicin causes a potentiating effect, which increases the risk of substancial liver damage.[3] The asymptomatic «subclinical» course of drug-induced hepatitis is dangerous, and further administration of these agents leads to the development of severe hepatitis, accompanied by jaundice and hepatic encephalopathy, which manifests as acognitive impairment.[4,5]

The main role in the development of these disorders be longs to the diminished detox function of the liver. Non-ionized ammonia easily penetrates the blood-brain barrier (BBB) and enters the astrocytes, where itismetabolized in the mitochondria in the presence of α-ketoglutarate to form of glutamine, a key component in the development of the astrocytic edema.[6] The active forms of oxygen and nitrogen are highly reactive molecules and are redox-active compounds, which, depending on the concentration, have both a positive (proliferation of cells) and negative effects (cell growth arrest, cell death) on nerve cells.[7,8] Oxidative and nitrosating stresses initiate neuro transmission disorders, mitochondrial dysfunction, and energy metabolism disorders in central nervous system (CNS).[9,10] The cognitive deficits observed in hepatic encephalopathy are also the result of the synaptic plasticity violations and changes in mediator transmission.[11] In particular, it is reported that hepatic encephalopathy is associated with highactivity of the GABA-ergic system of the brain due to increased γ-aminobutyric acid (GABA) concentration, expression of GABA receptors, and production of neurotrophic steroids, specifically aloprengonone.[12] At the same time, there is a decrease in glutamatergic neurotransmission characteristic for the chronic liver disorders, and observed violations in learning and memory may be associated with the inhibition of the glutamate nitrogen oxide-cGMP regulatory effect in the hippocampus which appearsas a response to hyperammonemia or increased levels of dopamine which result form the impaired liver function.[13]

Our study aimed to investigate neuronal cell adhesion molecules (NCAM) in various areas of the brain after the long-term administration of rifampicin and isoniazidin rats. NCAMs play an important roles in the regulation of neuronal differentiation and migration by interacting with growth factors and their receptors, as well as in the mechanisms of membrane potential regulation, determining the excitability of neurons.[14-16] Also, NCAMs in fluence the synaptic plasticity and cognitive processes of the mature brain, the short-term plasticity of existing synapses, and long-lasting plasticity associated with the elimination of old synapses and the formation of new ones. According to the contemporary notions about the functional role of NCAM, the blocked function of these proteins can lead to cognitive and emotional declines, such as changes in the perception of odor, memory, hearing, anxiety and space orientation.[17]

A lot of information on the effects of antimicrobials on the CNS has been collected over the last decade. However, the effect of rifampicin and isoniazid on the CNS could be felt no tonly by their expressive hepatotoxicity after prolonged use bu talso by the ability to cause a disturbance in the balance of the intestinal microflora and the development of dysbiosis, which potentially have negative impactas on the CNS it self as on the progression of toxic liver injury induced encephalopathy.[18] Moreover, the membrane-bound proteins, including NCAM are recognized as the primar target for the endotoxins'negative effects on CNS.[19] Therefore, the study of neuronal plasticity in the DILI model, as well as the possible ways of their pharmacological correction, is very relevant and could reveal new findings on the pathogenesis of cognitive impairmentin DILI.

As a correction of pathological conditions, when using antitubercular therapy, various substances and preparations are tested. They have various mechanisms of action (direct and indirect) that are scantily studied. In our study,

the drug Heptral was used, the active substance of which was S-adenosyl-L-methionine and the drug Kognifen, which is a combination of ipidacrine and phenibut.

Ademetionine (S-adenosyl-L-methionine) is a naturalaminoacid – a derivative of methionine and ispresent in all tissues. Ademetionine can penetrate through the BBB and its transmethylation process is key in the formation of such CNS neurotransmitters as catecholamines (dopamine, norepinephrine, adrenaline), serotonin, melatonin and histamine.[20] Neuroprotective effects of ademetionine may be mediated via the inhibition of oxidative stress and neuroinflammation by increasing the levels of endogenous glutathione and enhancing the activity of enzymes of the antioxidant defense system (superoxidismutease and glutathionetransferase), as well as improving energy metabolism in cells.[21-23]

Kognifen is a balanced combination of ipidacrine and phenibut. One of the two constitutive substances is phenibut (hydrochloride β-phenyl-γ-aminobutyric acid) – a phenyl analog of GABA. The nootropic activity of the drug is based on anti-hypoxic effects, an increasein energy metabolism, and synthetic processes in neurons.[24]

Phenibut has a direct effect on GABAergic receptors and facilitates GABA-mediated transmission of nerve impulses to the CNS.[25] It easily penetrates into all body tissues and through the BBB. Considering that GABA is the major inhibitory neurotransmitter in the CNS and as many as one-third of CNS neurons in the brain use GABA as their primary neurotransmitter, phenibut has a significant effect on the state of the central nervous system.[26]

Aim

The purpose of this work was to study the effects of ademetionine and combined drug ipidacrine/phenibut on the level of NCAMs in different brain areas in the rats with DILI provoked by the prolonged administration of rifampicin and isoniazid.

Material and methods

Animals

There search was carried out on 24 male Wistar rats, weighing 180-220 g which were housed in standard cages (48×27×20 cm) with free access to food and tap water (2–6 rats per cage). Animals were kept in a temperature-controlled room (22±2 °C), under a constant 12:12-h light/dark cycle (lights on at 06:00 hours). The experiment was conducted in the animal house of the State Establishment «Dnipropetrovsk Medical Academy of Health of the Ministry of Ukraine».All experimental procedures were carried out in the accordance with the principles outlined in the current Guide to the Care and Use of Experimental Animals and were approved by local Ethics Committee on Animal Experimentation of the State Institution "Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine" (Approval No.7, 2019).

Experimental Design

Animals were divided into four groups. The first group (C, n=6) of healthy rats, served as a control group. Control rats received administered with LAUROPAN T/80 Polysorbate (Industria Chimica Panzeri, Orio al Serio, Italy) and distilled water as a vehicle of in comparable volumes with experimental groups according to body weight daily during 28 days. The second group (DILI, n=6) included rats with DILI, an experimental which was reproduced by chronic intragastric administration of rifampicin at a dose of 86 mg/kg (PJSC SIC «Borshchahivskiy CPP», Kiev, Ukraine) and isoniazid in a dose of 50 mg/kg (PJSC «LUGAL», Kiev, Ukraine) using a standard solvent LAUROPAN T/80 Polysorbate (Industria Chimica Panzeri, Orio al Serio, Italy) and distilled water as a vehicle daily during 28 days[27]. Third group (AD, n=6) consisted of rats with DILI and received the intravenous infusion ofS-adenosyl-L-methionine (ademetionine) ("Geptral", Abbott Lababoratories GmbH, Hannover, Germany) at a dose of 35 mg/kg daily during the last 14 days of the experiment. Fourth group (IP/PB, n=6) consisted of rats with DILI which received a fixed combination of ipidacrine hydrochloride at a dose 1 mg/kg body weight and phenibut at a dose 60 mg/kg body weight (Kognifen®, Olainfarm, Olaineatvia). Rats were euthanized by cervical dislocation 24 hours after the last administration of drugs.

Open field test

For the assessment of locomotor and research activity, an open field test was used.[27,28] On the last (28th) day of the study, a 3 min open field session in the testing arena (100x100 cm, 30cm high walls, made of white plast) was performed for each animal. The testing arena contained 16 holes, each with a diameter of 6 cm that were located equidistantly from each other and the walls on the floor plane of its installation. For the test, each animal was placed at the center of the arena and its behavior was observed for 3 min, after that the animal was returned to its home cage, and the device was washed and sterilized with 70% ethanol to remove any trace of odor that could cause distractions. An assessment was made on the number of crossed squares (horizontal activity), vertical elevations (vertical activity) and holesvisits (research activity).

Protein extraction and determination

The hippocampus and thalamus were isolated from the brain and used subsequently for extraction of cytosolic and membrane protein fractions by differential ultracentrifugation. Homogenization of the brain was carried out in buffer A, which contained 25 mM tris - HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), 0.01 % sodium azide (NaN_3), 0.2 mM phenylmethylsulfonyl fluoride (FMSF). During successive centrifugation

stages (stage I – centrifugation for 60 minutes at 20,000 g, stage II – incubation of the precipitate 24 hours after the first centrifugation in buffer A, which additionally contained Triton X-100 – 2%, and centrifugation for 60 minutes at 20 000 g) fractions containing soluble NCAM (soluble – sNCAM isolated in stage I centrifugation) and membrane NCAM proteins (membrane – mNCAM isolated in stage II centrifugation) were isolated. All reagents were purchased from Sigma, St. Louis, Missouri, USA.

The quantitative determination of NCAM was performed using an ELISA, with primary rabbit monospecific polyclonal antibodies against NCAM (Abcam, Cambridge, UK), secondary anti-rabbit antibodies labeled with horseradish peroxidase (Abcam, Cambridge, UK), and purified NCAM proteinas a standard (R&D Systems, USA, Minneapolis, Canada) using a competitive solid-phase immunoassay analysis. Results obtained were printed outusing an Anthos-2010 absorbance reader (Anthos Labtec Instruments GmbH, Wals-Siezenheim, Austria).

Blood collection and analysis

Blood samples were taken immediately after midline thoracotomy from the right ventricle of heart of rats in terminal anesthesia using a 5 ml syringe with 25-gauge needle on the last day of the study period. Serum was obtained by placing whole blood in an empty tube and allowed the blood to clot. After that, the samples were centrifuged for 15 min at 3000 rpm. Serum was removed and the samples were stored at -20°C until further analysis.

The activity of aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2) in the serum of rats to confirm liver damage using reagent kits for clinical biochemistry manufactured by High Technology Inc. (North Attleborough, MA, USA) on HTI BioChem SA biochemical analyzer manufactured by High Technology Inc. (North Attleborough, MA, USA), according to the recommended methodology.[29] The De Ritis ratio was determined standardly using the ratio of serum activity of AST and ALT (AST/ALT ratio).[30]

Statistical alanalysis

An ANOVA, followed by a Tukey post-hoc test was used to assess statistical differences between groups. To assess data distribution, Shapiro-Wilk normality test was performed. Statistical processing of data was carried out using GraphPad Prism, v 8.1.0 software (GraphPad Software, Inc, San Diego, CA, USA). Data are represented as mean value (M) and the standard error of the mean (SEM). In all statistical analyses $p<0.05$ was considered significant.

Results

The results of the current study indicate that under rifampicin and isoniazid administration, the activity of both AlAT and AsAT in rat serum significantly ($p<0.001$) increased (1.5-fold increase for AlAT (from 44.45±2.72 to 64.60±2.81 U/l) and 3-fold increase for AsAT (from 36.64±2.13 to 104.52±5.86 U/l)), the De Ritis ratio was 1.61.

Results obtained from the open field test revealed that levels of horizontal and vertical activity in rats from the DILI group were decreased by 27.8% and 35.43% ($p<0.05$), respectively, when compared to those observed in C group rats (Fig. 1 A). Also, a significant ($p<0.05$) 52.16% decrease in the level of research activity when compared to the control animals from group C, was found in the DILI group (Fig. 1 A).

Horizontal activity values noted in AD group didn't differ significantly from those observed in the group C animals, however, the AD group animals demonstrated significantly ($p<0.05$) higher horizontal activity (by 31%) when compared to DILI rats (Figure 1 A). Horizontal activity of animals from the IP/PB group didn't differ significantly either from the such one observed in the C or DILI groups (Figure 1 A). The vertical activity of rats in both AD and IP/PB groups remained at the level of the control group (Figure 1 B).

The significant ($p<0.05$) decrease in research activity of 36% and 16.7% was noted for the animals from the AD and IP/PB groups, respectively, when compared to the control animals from group C (Figure 1 C). In the same time, the values of research activity in both AD and IP/PB groups, were significantly ($p<0.05$) higher, by 25% and 42.6%, respectively, than in DILI group. IP/PB group animals demonstrated significantly ($p<0.05$) higher research activity than the AD group rats (Figure 1 C).

C – the control group of healthy rats, administered with the vehicle, n=6; DILI – group of rats with drug-induced liver injury (DILI), n=6; AD – group of rats with DILI receiving ademetionine, n=6; IP/PB – group of rats with DILI receiving a fixed combination of ipidacrine hydrochloride and phenibut, n=6. Small letters given with result bars mean significant differences when $p<0.05$.

The content of the total protein in the soluble fraction obtained from the thalamus of rats from the DILI group was significantly ($p<0.05$) decreased by 17% compared to the group C (Figure 2 A). Values observed in the both AD and IP/PB groups were significantly ($p<0.05$) higher (by 38.6% and 63.5%, respectively) than those noted in the DILI group, and even higher than values seen in the group C (14.6% and 35% difference, respectively, $p<0.05$). The level of cytosolic protein in the IP/PB group was 18% higher than in the AD group ($p<0.05$) (Figure 2A).

The levels of total cytosolic and water-soluble protein in the hippocampus of DILI group rats didn't sig-

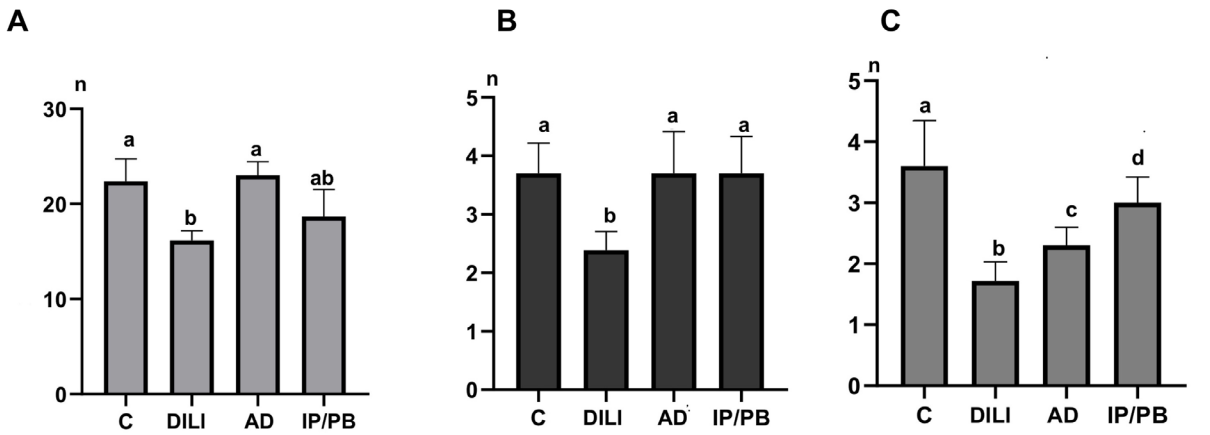

Fig. 1. A-C. The behavior of rats in the open field test on the last study day. A – Horizontal activity; B – Vertical activity; C – Research activity

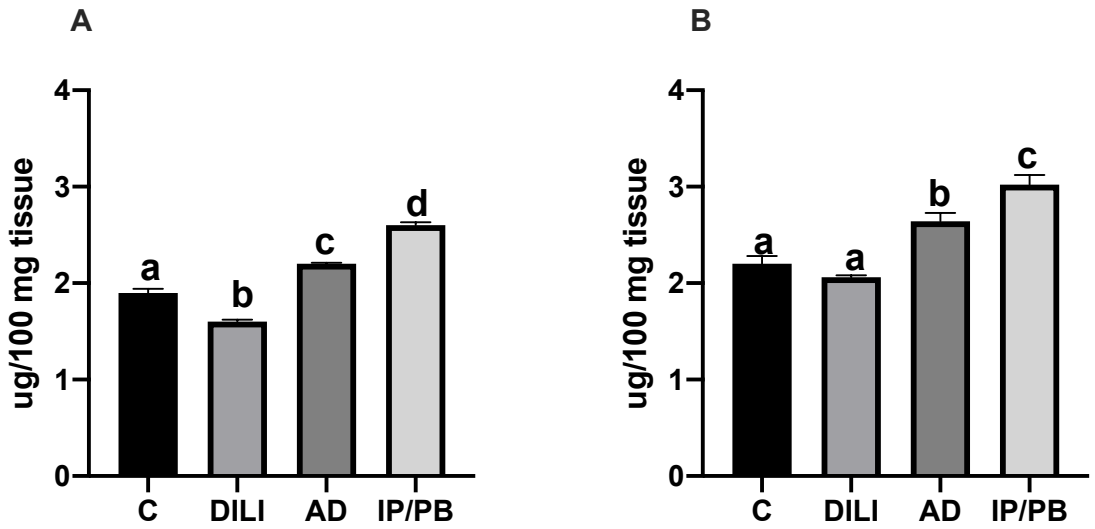

Fig. 2. A, B. The content of the total cytosolic and water-soluble protein extracted from the thalamus (A) and hippocampus (B) of rats. C – the control group of healthy rats, administered with the vehicle, n=6; DILI – group of rats with drug-induced liver injury (DILI), n=6; AD – group of rats with DILI receiving ademetionine, n=6; IP/PB – group of rats with DILI receiving a fixed combination of ipidacrine hydrochloride and phenibut, n=6. Small letters given with result bars mean significant differences when $p<0.05$

nificantly differ from those observed in the group C, while a significant ($p<0.05$) increase (by 20% and 37.3%, respectively), was seen in the AD and IP/PB groups (Figure 2 B). Total protein levels observed in the AD and IP/PB groups were significantly ($p<0.05$) higher (by 28% and 46%, respectively) when compared to the DILI group. The level of cytosolic protein in the IP/PB group was 14.4% higher than in the AD group, $p<0.05$ (Figure 2 B).

The content of the membrane fraction of total protein in the rats' thalamus significantly ($p<0.05$) decreased by 23.5%, 24% and 14% in the DILI, AD and IP/PB groups, respectively, when compared to the group C (Figure 3 A).

The content of the membrane fraction of total protein in the rats' hippocampus significantly ($p<0.05$) decreased by 10.7% in the DILI group compared to the group C (Figure 3 B). The levels of the membrane fraction of the total protein in the AD group didn't differ significantly from the values noted in the DILI group, however, values in the IP/BP group significantly ($p<0.05$) increased by 16.5% and 30.4% compared with C and DILI groups respectively.

The content of the soluble NCAM (sNCAM) fraction in the thalamus of animals from the DILI and IP/PB groups didn't differ significantly from that one observed in the group C. In the same time, values noted in the AD group, were significantly ($p<0.05$) lower (by ca 20%) when compared with those seen in the groups C and IB/PB (Figure 4 A).

The level of the sNCAM in the hippocampus of rats from the both AD and DILI groups didn't differ significantly from that one seen in the group C, while IP/PB treatmeant led to the significant ($p<0.05$) increase

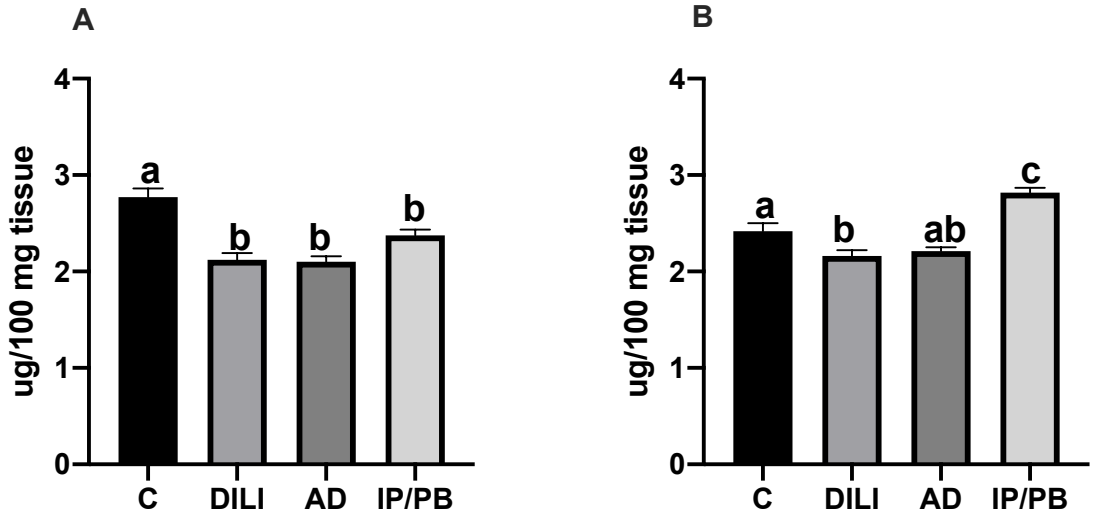

Fig. 3. A, B. The content of the total membrane protein in the thalamus (A) and hippocampus (B) of rats. C – the control group of healthy rats, administered with the vehicle, n=6; DILI – group of rats with drug-induced liver injury (DILI), n=6; AD – group of rats with DILI receiving ademetionine, n=6; IP/PB – group of rats with DILI receiving a fixed combination of ipidacrine hydrochloride and phenibut, n=6. Small letters given with result bars mean significant differences when $p<0.05$

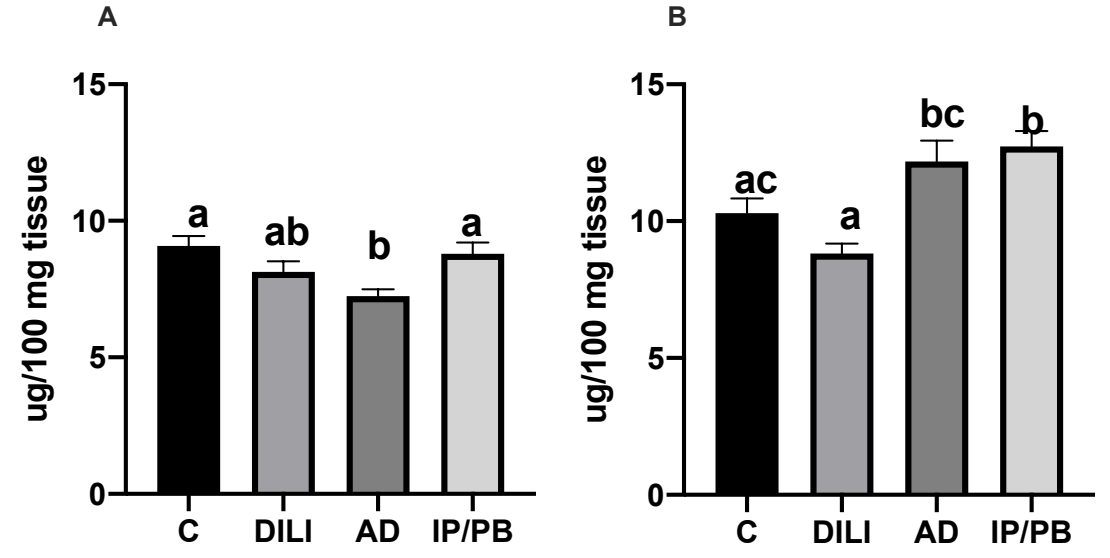

Fig. 4. A, B. The content of the soluble NCAM in the thalamus (A) and hippocampus (B) of rats C – the control group of healthy rats, administered with the vehicle, n=6; DILI – group of rats with drug-induced liver injury (DILI), n=6; AD – group of rats with DILI receiving ademetionine, n=6; IP/PB – group of rats with DILI receiving a fixed combination of ipidacrine hydrochloride and phenibut, n=6. Small letters given with result bars mean significant differences when $p<0.05$

of sNCAM content when compared to the C group values. The sNCAM levels observed in animals from both the AD and IP/PB groups, were significantly ($p<0.05$) higher (40.8% and 30%, respectively), when compared to those noted in the group DILI (Figure 4 B).

With regards to the membrane NCAM (mNCAM) fraction, in the thalamus of the DILI group rats it was significantly ($p<0.05$) decreased by 11% when compared to the group C. The values obtained in the AD and IP/PB groups were significantly ($p<0.05$) decreased by 19.2% and 19.3%, respectively, when compared to the C group. Values, observed in the AD group were significantly ($p<0.05$) decreased in comparison with those observed in the DILI group, while in IP/PB group mNCAM content didn't differ from that one, observed in DILI group (Figure 5 A).

The contents of the membrane NCAM fraction in the hippocampus in the DILI group was significantly ($p<0.05$) decreased by 9.5% when compared to the C group. In the same time, mNCAM content in AD and

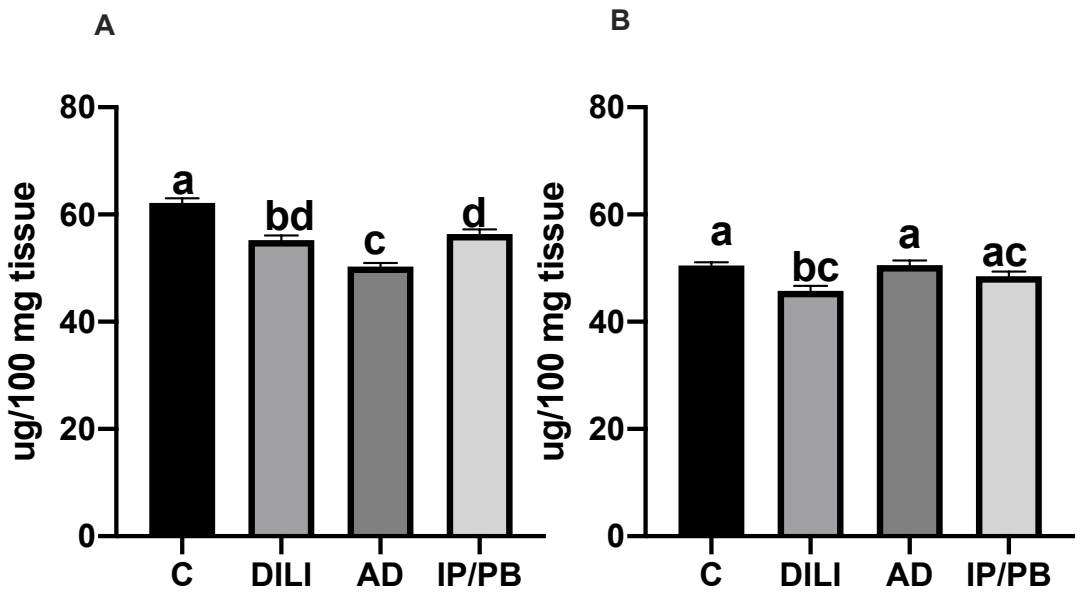

Fig. 5. A, B. The content of the membrane NCAM in the thalamus (A) and hippocampus (B) of rats. C – the control group of healthy rats, administered with the vehicle, n=6; DILI – group of rats with drug-induced liver injury (DILI), n=6; AD – group of rats with DILI receiving ademetionine, n=6; IP/PB – group of rats with DILI receiving a fixed combination of ipidacrine hydrochloride and phenibut, n=6. Small letters given with result bars mean significant differences when $p<0.05$

IP/PB groups remained at the level of the values of the C group. Compared with the DILI group, the mNCAM levels in the AD group was significantly ($p <0.05$) increased by 10.5% (Figure 5 B).

Discussion

The long-term use of antitubercular drugs leads primarily to the liver disorders, affecting the multiple organs of the experimental animals and leaving substancial influence on certain organs, including the brain. In case of liver homeostasis disruption, the increased permeability of BBB and following transfer of toxic products into the brain were observed previously.[31] In the present study we investigated the effects of ademetionine and combined drug IP/PB on the NCAM distribution in the brain in the rat model of DILI induced by the long-term administration of rifampicin and isoniazidum.

The obtained data of elevated activity of transaminases (AST and ALT) in the serum of the experimental animals was indicative for the injury caused to the liver, namely, to the mitochondria of hepatocytes, which results in the mitochondrial isoform of AST entering the bloodstream. Our results confirmed the data obtained by Awodele et al. showing the stimulating effect of rifampicin on the liver enzymes AST and ALT activity in serum.[32]

Previously it was concluded that the use of ademetionine as a drug with high protective and therapeutic effects of liver injury is promising. Lee and Ko showed the hepatoprotective effect of ademethionine in experimental liver injury.[33] Rats from the pre-treated with S-adenosylmethionine group showed a significant restoration of the ALT and AST levels in serum, compared with the experimental group.The result of the correction of hepatotoxicity by the hepatoprotective drug restored the value of biochemical parameters to the control level.

When studying the behavior of rats in the open field test, rats from the DILI group showed a significant decrease in locomotor and research activities, as well as in parameters of mnemonic functions, compared with the group C. Horizontal and vertical locomotor activity of rats from group with AD was restored to level of control animals. The partial recovery of horizontal locomotor activity and full recovery of vertical locomotor activity was observed in IP/PB group.

The main function of the soluble NCAM is to regulate extracellular signaling, intercellular adhesion andmigration of neurons.[34] Transmembrane adhesive molecules are not only mediators in the process of recognition between cells, but they can also convert signals with in the cell and, thus, cause a cellular response that regulates ontogenesis and synaptic plasticity (including learning and memory).[14,17] The redistribution of NCAM between two forms (soluble and membrane) under long-term effect of rifampicin and isoniazid was noted. The balanced content of transmembrane and soluble isoforms of this protein is of great importance for the normal development and functioning of the brain.[35,36]

The target of the negative effect of endotoxins in the brain is primarily membrane-bound proteins, including NCAM, which was manifested by a significant decrease in the concentration of these molecules ($p<0.05$)

in the PI / PB group in the fraction isolated from the hippocampus. In parallel with this, a significant increase ($p<0.05$) was observed in the hippocampus relative to the control of the content of the soluble form of NCAM due to enzymatic cleavage of membrane-bound NCAM, "cutting" of these molecules from the cell membrane. Therefore, as a result of the redistribution of NCAM between fractions during an experimental drug-induced liver injury, when an increase in the number of soluble and a decrease in membrane molecules was observed in the hippocampus, the normal functioning of both sNCAM and mNCAM is disrupted.

A number of drugs tend to penetrate the BBB. Rifampicin is no exception.[31,37] In studies of Shobo et al., drug penetration was visualized and its exact distribution in the rat brain was shown. And even with the sligh test concentrations of rifampicin, it penetrates the BBB and enters the brain tissue.[38] In DeMarco studies, after a single intravenous injection, rifampicin is rapidly appearingin the liver, blood and brain tissues. In the liver, the absorption index is the highest and its increase was observed within 60 minutes of the test. In the blood in the first 10 minutes, the results were maximum and decreased by the 60th minute. Also, rifampicin concentrations in the brain were found to around 15% of the levels found in blood.[39] Similar results were demonstrated in the article by Awodele et al., where authors presented histopathological data showing that rifampicin is able to penetrate the BBB and cause congestion of the meninges.[32]

Our own results obtained in the present study demonstrate the decrease in total protein content both in the soluble and membrane fractions of thalamus in the DILI group, when compared to the group C. In the hippocampus the same tendency was noted for membrane proteins, however, the total level of cytosolic/water-soluble proteins was increased. Obtained data demonstrates that rifampicin may affect protein synthesis in the brain.

In the same time, the total protein content in the soluble fraction of both hippocampus and thalamus in the AD and IP/PB groups was significantly increased, and even exceeded the values observed in the group C. The level of total protein in the membrane fraction of the thalamus and hippocampus of the AD group remained at the level of DILI animals. In the IP/PB group, the total protein level in the membrane fraction of both parts of the brain exceeded the DILI and AD groups. And in the hippocampus, these indicators were significantly increased and exceeded the values observed in group C.

The results shown in Figures 4–5, demonstrate a significant decrease in membrane NCAM content in both thalamus and hippocampus of animals from the DILI group. Taking in to account the central role of the thalamus in maintaining consciousness and attention, the data of behavior in the open field test confirm the obtained results (Fig. 1) with reducing the research activity in DILI animals. Moreover, the results showed a sufficient decrease in the content of soluble NCAM in the hippocampus, which is compar able to the decrease in the concentration of attention in rats from DILI group.[17,40] A significant reduction of the NCAM content observed in thalamus under the AD treatment compared to the DILI group, indicated a decrease in NCAM adhesive properties and inhibition of NCAM synthesis.[41] The effect of the drug IP/PB, is more positive because, compared with AD animals, the results are significantly restored to the level of Control group.

In the hippocampus an increase in the NCAM levels in the soluble fraction was observed in both AD and IP/PB groups compared to the Control and DILI animals. It can be assumed that the restoration of the soluble form of NCAM occurs due to enzymatic dissolution of membrane-bound proteins.[42] After all, the cytosolic form of NCAM reacts quickly even to minor changes in the body's sustainability by releasing the NCAM pool, which restores the strength of the contacts between the cells.[43]

Both in animal experiments and clinical studies it was suggested that S-adenosylmethionine can be used for the treatment of nervous system diseases, since it can pass through the BBB. Also, recently, curative effects of S-adenosylmethionine on depression, drug addiction, and cognitive dysfunction have been reported.[20] However, there is no direct evidence of the effect of both of the used drugs (AD and IP/PB).

In studies of Vavers et al., the positive results are presented indicating the neuroprotective activity of R-phenibut. Treatment with R-Phenibut at a dose of 50 mg/kg significantly alleviated reduction of brain volume in damaged hemisphere.[44] The results obtained after the correction of toxic drug-induced liver injury with AD or IP/PB indicate a positive effect of these drugs on the content the total protein and NCAM in the soluble and membrane fraction in the thalamus and hippocampus of rats. But the administration of drugs was carried out for 14 days. During this period, combination drug of ipidacrine/phenibut, having a directed effect on the nervous system. What can be observed by the results of the IP/PB group in the soluble NCAM fraction, which were increased and exceeded the values of the control group. And the results in the NCAM membrane fraction were restored to the level of Control animals. Ademetionin demonstrated a positive effect on the content of NCAM, but in our results of the content of NCAM in the AD group in the thalamus of both fractions, results remained at the DILI group level. Based on the experimental data, we can assume that its main target was the liver, and therefore the effect on the brain is mediated and that in order to improve brain performance, a longer period of administration of ademetionin is needed.

Conclusion

The long-term effect of isoniazid and rifampicin leads to decrease oflocomotor, exploratory activity and emotional reactivity, as well as parameters of mnemonic functions, which is confirmed by changes in the membrane NCAM content both in the thalamus and hippocampus. It should be noted that correcting treatment (hepatoprotective drug ademetionine and the nootropic combinated drug ipidacrine/phenibut) had a positive effect on NCAM level in the brain, compared to the results open field tests of behavior under effect of isoniazid and rifampicin in DILI group. Our investigation revealed that the combinated drug containing IP/PB which possesses neuroprotective properties could prevent the decline in synaptic plasticity under drug-induced liver injury.

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