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ORIGINAL PAPER

Role of sulfide anion in the development of chronic alcoholic hepatitis under the conditions of modulation of adenosine monophosphate kinase – a correlational study

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ABSTRACT

Introduction and aim. Hydrogen sulfide (H_2S) has attracted the attention of researchers as a novel signaling molecule that affects vascular metabolism, immune function, stress and inflammation. It plays an important role in pathophysiological disorders under the conditions of the development of obesity, diabetes, non-alcoholic fatty liver disease and cardiovascular diseases. The purpose of this work is to establish correlation ratios of H_2S concentration with markers of oxidative-nitrosative stress and extracellular matrix metabolism of the liver during chronic alcoholic hepatitis modeling and AMPK modulation by phenformin and doxorubicin.

Material and methods. The experiments were performed on 36 white, sexually mature male Wistar rats, weighing 180-220 g. Alcoholic hepatitis was modelled by alcohol administration, on the background of alcoholic hepatitis animals received phenformin orally at a dose of 10 mg/kg or doxorubicin at a dose of 1.25 mg/kg intraperitoneally. Statistical processing of the results of biochemical studies was carried out using the non-parametric method of Spearman to determine correlations.

Results. H_2S during alcoholic hepatitis inversely proportionally strongly correlates with the concentration of nitrites, oxyproline and arginase activity. Phenformin administration during alcoholic hepatitis leads to formation of inversely proportionally strongly correlation of H_2S with the production of superoxide anion radical, the concentration of malondialdehyde, activities of constitutive NO-synthases, nitrite reductases, nitrate reductases, and arginase. Doxorubicin administration during alcoholic hepatitis leads to formation of directly proportional strongly correlation of H_2S with the activity of constitutive NO-synthases, nitrite reductases, nitrate reductases.

Conclusion. Administration of phenformin or doxorubicin expands correlations between H_2S and indicators of oxidative-nitrosative stress.

Keywords. AMPK, chronic alcohol hepatitis, doxorubicin, liver, phenformin, sulfide anion

Introduction

In recent years, hydrogen sulfide (H_2S) has attracted the attention of researchers as a novel signaling molecule that affects vascular metabolism, has influence on immune function, changes stress and inflammation progression. It plays an important role in pathophysiological disorders under the conditions of the development of obesity, diabetes, non-alcoholic fatty liver disease and cardiovascular diseases.[1] H_2S exerts physiological functions by targeting proteins, enzymes, and transcription factors through a post-translational mod-

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ification known as persulfidation.[2] The main effects of H_2S are neuromodulation, regulation of vascular tone, cytoprotection, anti-inflammatory action, sensing (reception) of oxygen, angiogenesis and energy generation.[3-4]

Enzymatic formation of H_2S is catalyzed by cystathionine γ-lyase (EC 4.4.1.1, CSE), cystathionine β-synthase (EC 4.2.1.22, CBS) and 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2, MST).[5] All these three enzymes are present in the liver and through the synthesis of H_2S regulate its functions. A small part of endogenous H_2S is formed by the non-enzymatic reduction of sulfur contained in certain metabolites (persulphides, thiosulphates and polysulphides).[2] Hepatic H_2S metabolism affects glucose metabolism, insulin sensitivity, lipoprotein synthesis, mitochondrial biogenetics, and biogenesis. H_2S can be involved in many liver diseases such as fibrosis, cirrhosis and liver cancer.[5-6]

Several pathways are involved in the pathogenesis of ethanol-induced liver disease. One of the central pathways involves the induction of cytochrome P450 2E1 by ethanol, which leads to the induction of lipid peroxidation in hepatocytes. The second pathway involves ethanol regulation of transcription factors associated with lipid metabolism. Ethanol also affects the activity of enzymes involved in energy metabolism, including AMP-activated protein kinase (AMPK) and sirtuin-1 (SIRT1) [7]. Ethanol-mediated dysregulation of hepatic AMPK, a master regulator of lipid metabolism, is one of the main mechanisms in the pathogenesis of alcoholic fatty liver disease, because impaired AMPK signaling accelerates lipid accumulation and inhibits lipid catabolism, ultimately leading to the development of alcoholic fatty liver disease in animals.[8]

Among the chemicals and pharmacological preparations that can enhance the activity of AMPK we should note the effect of biguanides (phenformin, buformin, metformin, etc.).[9-10] Among the biguanides, it should be noted that phenformin has a higher ability to phosphorylate AMPK (50 times more active than metformin) and thereby activate AMPK-dependent transcription cascades.[11-12] Doxorubicin has a powerful inhibitory effect on AMPK activity. The use of doxorubicin at a dose of 2.5 mg/kg has a persistent inhibitory effect on AMPK activity in the heart.[13] A single intraperitoneal injection of doxorubicin at a dose of 20 mg/kg also causes a persistent decrease in AMPK expression and leads to development of oxidative stress due to a decrease in the expression of antioxidant enzymes (superoxide dismutase and catalase).[14] Thus, most of the negative effects of doxorubicin (cytotoxicity, damage to mitochondria, development of oxidative stress) are associated with its ability to inhibit AMPK activity.[15-17]

It has been reported, that diallyl disulfide (DADS) has hepatoprotective effects against alcoholic liver disease (ALD), while the underlying mechanisms of action of H_2S remain largely unknown. Research by Shi-Xuan Liu et al. (2022) reported that DADS ameliorated ethanol-induced downregulation of peroxisome proliferator-activated receptor α (PPARα), of carnitine palmitoyltransferase 1 (CPT1) and phosphorylated AMP-activated protein kinase in mouse liver and AML12 cells. These results demonstrate that DADS can prevent ethanol-induced hepatic steatosis and early inflammation by regulating the gut-liver axis and supporting fatty acid catabolism.[18]

The search for ways to reduce oxidative-nitrosative damage to the liver under the conditions of the development of chronic alcoholic hepatitis led us to believe that the modulation of the AMPK cascade plays an important role in the pathogenesis of this disease. Considering the antioxidant and regulatory potential of hydrogen sulfide, which undoubtedly changes its metabolism under conditions of chronic alcoholic hepatitis, the question arises as to what is the role of hydrogen sulfide in changing metabolism of the hepatocyte. Establishing correlations between indicators of oxidative-nitrosative stress and indicators of metabolism of the extracellular matrix of the liver will bring us closer to establishing the role of hydrogen sulfide in the pathogenesis of chronic alcoholic hepatitis.

Aim

We aimed to establish correlation ratios of H_2S concentration with markers of oxidative-nitrosative stress (total NO-synthase activity, activity of constitutive and inducible isoforms of NO-synthase, concentration of nitrosothiols and nitrites, concentration of peroxynitrites of alkali and alkaline earth metals, the activity of nitrite- and nitrate reductases, arginases, superoxide dismutase and catalase, concentration of malondialdehyde, oxidation-modified proteins and production of superoxide anion) and parameters of extracellular matrix metabolism of the liver (total concentration of glycosaminoglycans, concentrations of heparin-heparan, keratan-dermatan and chondroitin fractions, concentration of free oxyproline and sialic acids) during AMPK modulation by phenformin and doxorubicin under conditions of chronic alcoholic hepatitis modeling.

Materials and methods

Ethical approval

Research was conducted in accordance with the standards of the Council of Europe Convention on Bioethics "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1997), general ethical principles of experiments on animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national leg-

islation in this area. The rats were kept in a vivarium accredited in accordance with the "Standard rules of order, equipment and maintenance of experimental biological clinics (vivarium)". All experimental procedures were approved by Bioethical Committee of Poltava State Medical University (Record № 197 from 23.09.2021).

Sample and experimental groups

The experiments were performed on 36 white, sexually mature male Wistar rats, weighing 180-220 g. The animals were divided into 6 groups:

I – control (n=6). The control group included animals that were subjected to similar manipulations throughout the study period, but were injected with a physiological solution.

II – phenformin group (n=6), received phenformin hydrochloride according to phenformin injection protocol;

III – alcoholic hepatitis group (n=6), received alcohol according to chronic alcoholic hepatitis protocol.

IV – alcoholic hepatitis + phenformin group (n=6), was subjected to chronic alcoholic hepatitis and phenformin injection protocols.

V – doxorubicin group (n=6) which received doxorubicin hydrochloride according to doxorubicin injection protocol.

VI – alcoholic hepatitis + doxorubicin group (n=6), was subjected to chronic alcoholic hepatitis and doxorubicin injection protocols.

The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on the 63rd day by blood sampling from the right ventricle of the heart under thiopental anesthesia. Devices used for research have passed metrological control.

Phenformin injection protocol

Phenformin hydrochloride (phenformin, Sigma-Aldrich), as activator of AMP-activated protein kinase was introduced orally at a dose 10 mg/kg daily for 63 days.[19]

Chronic alcoholic hepatitis modelling protocol

Chronic alcoholic hepatitis in rats was modeled by the method of forced intermittent alcoholization for 5 days, with a repeat after two days by intraperitoneal injection of 16.5% ethanol solution in 5% glucose solution, at the rate of 4 ml/kg of body weight. After that, they were transferred to 10% ethanol as the only source of drinking.[20] Modelling lasted for 63 days.

Doxorubicin injection protocol

Doxorubicin hydrochloride (doxorubicin, S.C. Sindan-Pharma S.R.L.), as inhibitor of AMP-activated protein kinase, was injected intraperitoneally at a dose 1,25 mg/kg four times a week for 63 days.[21]

Biochemical analysis

For biochemical analysis we used 10% liver tissue homogenate and blood serum. Liver tissue homogenate was obtained after homogenization of 1 g of rat liver with 9 ml of 0.2 M Tris-buffer solution (Trisaminomethane-hydrochloric acid buffer, pH=7.4). Then it was centrifugated at 3000 g for 10 minutes. Upper layer (supernantant) was used for further biochemical analysis. Blood plasma was obtained after addition of 0,109 M sodium citrate at ratio 9:1 and subsequent centrifugation at 3000 g for 10 minutes.

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

Concentration of sulfide anion (calculated as H_2S concentration) specifically reacts 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 a maximum light absorption at a wavelength of 667 nm.[22]

Total NO-synthase activity (gNOS) was evaluated by the increase of nitrites after incubation of 10% tissue homogenate (0.2 ml) for 30 min in the incubation solution (2.5 ml of 0.1 M trisbuffer, 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 cNOS 1% solution of aminoguanidine hydrochloride was used and the incubation time was extended to 60 min.[23-24] The activity of iNOS was calculated by the formula: iNOS= gNOS-cNOS.

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 (S-NO) to nitrites with a solution of mercuric chloride ($HgCl_2$).[25]

The concentration of nitrite and peroxynitrite of alkali and alkaline earth metals, the activity of nitrite- and nitrate reductases, arginases, superoxide dismutase (SOD) and catalase, concentration of malondialdehyde (MDA), oxidation-modified proteins and production of superoxide anion, GAG fractions (heparin-heparan, keratan- dermatan and chondroitin), the concentration of free oxyproline and sialic acids were studied in rat liver 10% homogenate.[23,26-33]

Statistical analysis

Statistical processing of the results of biochemical studies was carried out using the non-parametric method of Spearman to determine correlations (with the exception of groups where the studied parameters corresponded to a normal distribution with very small values of the standard deviation, where the Pearson method was

used). All statistical calculations were performed in the Microsoft Office Excel program and its extension Real Statistics 2019. Correlation was considered statistically significant at $p<0.05$.

Results

The role of the AMPK cascade in the development of chronic hepatitis remains unclear. But it is known that modulation of AMPK activity leads to changes in the pathogenesis of chronic alcoholic hepatitis. Establishing correlational and pathogenetic relationships between the concentration of hydrogen sulfide and biochemical indicators of oxidative-nitrosative stress and markers of the metabolism of the extracellular matrix of the liver under the conditions of chronic alcoholic hepatitis will allow the use of donors and scavengers of hydrogen sulfide in the pathogenetic therapy of alcoholic liver disease.

Blood biochemical markers of chronic alcoholic hepatitis (AST, ALT activity and de Ritis coefficient) under the conditions of modulation of AMPK cascade are shown in Fig. 1. Activity of AST, ALT and de Ritis coefficient proved a presence of cytolytic process in rat liver in chronic alcohol hepatitis group.

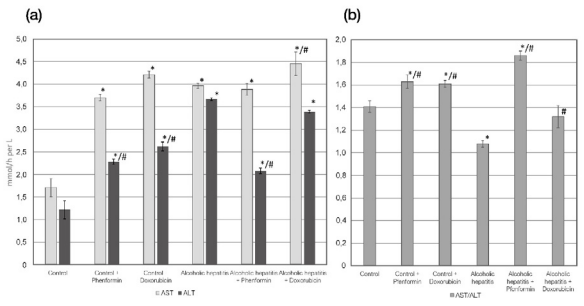

Fig. 1. Biochemical indicators of blood plasma of rats under the conditions of modeling chronic alcoholic hepatitis and modulation of AMPK cascade: (a) activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT); (b) the de Ritis coefficient (AST/ALT); * – indicates that difference is statistically significant compared to control group; # – indicates that difference is statistically significant compared to alcoholic hepatitis group

In the control group of animals, no statistically significant correlations were found between the concentration of sulfide anion and other biochemical parameters. In the group of animals injected with phenformin, it was found that the concentration of sulfide anion is inversely proportionally strongly correlated with the activity of superoxide dismutase and inversely proportionally strongly correlated with the concentration of the keratan-dermatan fraction of glycosaminoglycans (Table 1).

Table 1. Correlation analysis of biochemical indicators of the liver of rats under the conditions of modeling chronic alcoholic hepatitis and stimulation of AMPK cascade activation[a]

Correlation relationships of biochemical parameters	Group							
	Control		Control+ Phenformin		Alcoholic hepatitis		Alcoholic hepatitis + Phenformin	
	rho	p	rho	p	rho	p	rho	p
Sulfide anion (μmol/g) / Catalase (μkat/g)	0.739	0.09	-0.029	0.96	-0.243	0.64	-0.319	0.54
Sulfide anion (μmol/g) / Superoxide dismutase (c.u.)	-0.739	0.09	-0.828	0.04	-0.176	0.74	-0.478	0.34
Sulfide anion (μmol/g) / Superoxide anion radical (nmol/s per g)	0	1	0.478	0.34	-0.179	0.73	-0.956	0.003
Sulfide anion (μmol/g) / MDA (μmol/g)	-0.134	0.8	-0.429	0.4	-0.7	0.12	-0.886	0.02
Sulfide anion (μmol/g) / Oxidation-modified proteins (c.u.)	0.618	0.19	0.696	0.12	-0.582	0.23	0.6	0.21
Sulfide anion (μmol/g) / Inducible NO synthase (μmol/min per g of protein)	0.088	0.87	0.486	0.33	-0.203	0.7	-0.543	0.27
Sulfide anion (μmol/g) / Constitutive NO synthases (μmol/min per g of protein)	0.045	0.93	0.486	0.33	0.029	0.96	-0.947*	0.004
Sulfide anion (μmol/g) / Nitrite reductase activity (μmol/min per g of protein)	-0.265	0.61	0.6	0.21	0.348	0.5	-0.996*	p<0.001
Sulfide anion (μmol/g) / Nitrate reductase activity (μmol/min per g of protein)	-0.618	0.19	0.486	0.33	0.406	0.42	-0.947*	0.004
Sulfide anion (μmol/g) / $ONOO^-$ (μmol/g)	0.091	0.86	-0.714	0.11	-0.696	0.12	0.943	0.005
Sulfide anion (μmol/g) / S-NO (μmol/g)	-0.088	0.87	-0.116	0.83	0.667	0.15	0.754	0.08
Sulfide anion (μmol/g) / NO_2 concentration (nmol/g)	0.091	0.86	0	1	-0.882	0.02	-0.478	0.34
Sulfide anion (μmol/g) / Arginase activity (μmol/min per g of protein)	0,177	0.74	0.486	0.33	-0.812	0.0499	-0.83	0.04
Sulfide anion (μmol/g) / Concentration of heparin-heparan fraction (μmol/L)	-0.739	0.09	0.66	0.16	-0.294	0.57	0.543	0.27
Sulfide anion (μmol/g) / Concentration of keratin-dermatan fraction (μmol/L)	-0.739	0.09	-0.986	0.0003	-0.176	0.74	0.94	0.005
Sulfide anion (μmol/g) / Concentration of chondroitin fraction (μmol / l)	-0.739	0.09	0.66	0.16	-0.35	0.49	-0.94	0.004
Sulfide anion (μmol/g) / Concentration of free oxyproline (μmol / g)	0.739	0.09	0.657	0.16	-0.912	0.01	-0.486	0.33
Sulfide anion (μmol/g) / Concentration of sialic acids (mg / g)	-0.582	0.23	0.086	0.87	-0.09	0.87	-0.412	0.42

[a] * – the correlation coefficient was calculated by Pearson's method (r^2)

In a group of rats with chronic alcoholic hepatitis, it was found that sulfide anion is inversely proportionally strongly correlated with the concentration of nitrites, oxyproline and arginase activity.

Table 2. Correlation analysis of biochemical indicators of the liver of rats under the conditions of modeling chronic alcoholic hepatitis and blockade of AMPK-cascade activation

Correlation relationships of biochemical parameters	Group							
	Control		Control+ doxorubicin		Alcoholic hepatitis		Alcoholic hepatitis + doxorubicin	
	rho	p	rho	p	rho	p	rho	p
Sulfide anion (μmol/g) / Catalase (μkat/g)	0.739	0.09	0.377	0.46	-0.243	0.64	0.543	0.27
Sulfide anion (μmol/g) / Superoxide dismutase (c.u.)	-0.739	0.09	-0.478	0.34	-0.176	0.74	-0.478	0.34
Sulfide anion (μmol/g) / Superoxide anion radical (nmol/s per g)	0	1	0.478	0.34	-0.179	0.73	0.478	0.34
Sulfide anion (μmol/g) / MDA (μmol/g)	-0.134	0.8	0.377	0.46	-0.7	0.12	-0.714	0.11
Sulfide anion (μmol/g) / Oxidation-modified proteins (c.u.)	0.618	0.19	-0.429	0.4	-0.582	0.23	0.406	0.42
Sulfide anion (μmol/g) / Inducible NO synthase (μmol/min per g of protein)	0.088	0.87	-0.486	0.33	-0.203	0.7	0.586	0.33
Sulfide anion (μmol/g) / Constitutive NO synthases (μmol/min per g of protein)	0.045	0.93	-0.486	0.33	0.029	0.96	0.943	0.004
Sulfide anion (μmol/g) / Nitrite reductase activity (μmol/min per g of protein)	-0.265	0.61	0.429	0.4	0.348	0.5	0.943	0.005
Sulfide anion (μmol/g) / Nitrate reductase activity (μmol/min per g of protein)	-0.618	0.19	0.429	0.4	0.406	0.42	0.943	0.005
Sulfide anion (μmol/g) / $ONOO^-$ (μmol/g)	0.091	0.86	0.429	0.4	-0.696	0.12	-0.2	0.7
Sulfide anion (μmol/g) / S-NO (μmol/g)	-0.088	0.87	0.371	0.47	0.667	0.15	-0.986	0.0003
Sulfide anion (μmol/g) / NO_2 concentration (nmol/g)	0.091	0.86	0.828	0.04	-0.882	0.02	-0.478	0.34
Sulfide anion (μmol/g) / Arginase activity (μmol/min per g of protein)	0,177	0.74	-0.429	0.4	-0.812	0.0499	0.486	0.33
Sulfide anion (μmol/g) / Concentration of heparin-heparan fraction (μmol/L)	-0.739	0.09	-0.429	0.4	-0.294	0.57	-0.886	0.02
Sulfide anion (μmol/g) / Concentration of keratin-dermatan fraction (μmol/L)	-0.739	0.09	-0.319	0.54	-0.176	0.74	0.89	0.02
Sulfide anion (μmol/g) / Concentration of chondroitin fraction (μmol/L)	-0.739	0.09	-0.43	0.4	-0.35	0.49	-0.54	0.27
Sulfide anion (μmol/g) / Concentration of free oxyproline (μmol/g)	0.739	0.09	0.429	0.4	-0.912	0.01	-0.943	0.005
Sulfide anion (μmol/g) / Concentration of sialic acids (mg/g)	-0.582	0.23	-0.088	0.87	-0.09	0.87	0.429	0.4

During phenformin correction of chronic alcoholic hepatitis in rats, it was found that sulfide anion is inversely proportionally strongly correlated with the production of superoxide anion radical, the concentration of malondialdehyde and the chondroitin fraction of glycosaminoglycans, as well as with activities of constitutive NO-synthases, nitrite reductases, nitrate reductases, and arginase. It was also found that the sulfide anion is directly proportional strongly correlated to the concentration of peroxynitrite and the keratan-dermatan fraction of glycosaminoglycans (Table 1).

Doxorubicin injection to rats on background of chronic alcoholic hepatitis led to following changes: concentration of sulfide anion directly proportional strongly correlated to the concentration of nitrites, the activity of constitutive NO-synthases, nitrite reductases, nitrate reductases and the concentration of the keratan-dermatan fraction of glycosaminoglycans. It was also found that sulfide anion is inversely proportionally strongly correlated with the concentration of nitrosothiols, free oxyproline and the heparin-heparan fraction of glycosaminoglycans (Table 2).

Discussion

The summary of correlation ratios between H_2S and biochemical parameters of rat liver are presented in Fig. 2. In brief, during excessive alcohol intake sulfide anion receives strong negative correlation bonds with nitrite content, concentration of free L-oxyproline and arginase activity (ARG), which were absent under normal conditions. Stimulation of AMPK cascade by phenformin leaves correlation of sulfide anion with arginase intact, but removes its correlation with nitrites and L-oxyproline, while creating new correlations with nitrite reductases (NiR), nitrate reductases (NaR), MDA, cNOS, peroxynitrite ($ONOO^-$) and superoxide.

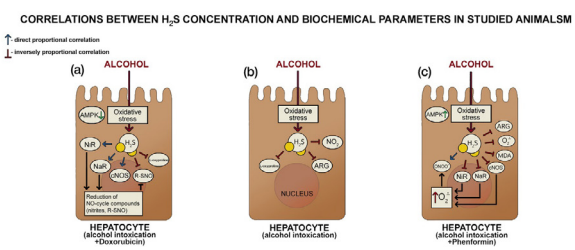

Fig. 2. Correlation of H_2S concentration with markers of oxidative-nitrosative stress and extracellular matrix metabolism of the liver during chronic alcoholic hepatitis modeling and AMPK modulation: (a) influence of alcohol intoxication and doxorubicin on H_2S correlation with biochemical parameters, (b) influence of alcohol intoxication on H_2S correlation with biochemical parameters, (c) influence of alcohol intoxication and phenformin on H_2S correlation with biochemical parameters

Despite its inversed correlation with superoxide production, sulfide anion can potentially cause increased production of superoxide from NiR and NaR (as parts of xanthine oxidoreductase complex), thus leading to in-

crease of peroxynitrite formation, which may explain appearance of direct correlation between sulfide anion and peroxynitrite during combined influence of phenformin and alcohol. Inhibition of AMPK cascade by doxorubicin leaves correlation of sulfide anion with L-oxyproline intact, but removes its correlation with nitrites and arginase, while creating new correlations with NiR, NaR, S-NO, and cNOS. It is worth mentioning, that correlations between sulfide anion and NiR, NaR, and cNOS in doxorubicin+alcoholic hepatitis group have different vector compared to phenformin+alcoholic hepatitis group. In doxorubicin+ alcoholic hepatitis group sulfide anion can potentially create conditions under which main source of NO production will shift towards predominance of L-arginine-independent pathway.

The absence of statistically significant correlations in the control group of animals may indicate the non-linearity of the relationship between the sulfide anion content and the investigated biochemical parameters. At the same time, modulation of AMPK activity leads to the appearance of statistically significant correlations. The appearance of an inversely proportional strong relationship between SOD activity and H_2S concentration under the conditions of stimulation of AMPK activation by phenformin may be associated with a decrease in the production of reactive oxygen species (ROS) by mitochondria under the influence of AMPK.[34] The appearance of a correlation relationship similar in direction and strength between H_2S and the concentration of the keratan-dermatan fraction of GAG is related to the ability of AMPK to directly affect the concentration of different fractions of sulfated GAG.[35] Reduction of the degree of AMPK activation by doxorubicin leads to the appearance of a direct strong relationship between H_2S and nitrite concentration, which may indicate the ability of H_2S to enhance gene expression of constitutive and inducible NOS isoforms.[36]

Under the conditions of chronic alcoholic hepatitis, the appearance of an inversely proportional strong relationship between H_2S and nitrite concentration is noted, which may be related to the ability of ethanol to induce the expression of genes of the inducible isoform of NOS, regardless of H_2S concentration.[37] The appearance of the relationship with similar direction and strength between H_2S and arginase activity in the group of chronic alcoholic hepatitis is also related to the biological effects of alcohol, namely its ability to decrease arginase activity, while H_2S can increase its activity.[38-39] The inversely proportional strong relationship between the concentrations of H_2S and L-oxyproline under conditions of chronic alcoholic hepatitis can be explained by the origin of free L-oxyproline, which under conditions of excessive accumulation of alcohol is released from collagen fibers during oxidative stress, while H_2S is a powerful antioxidant.[40]

Stimulation of the activation of the AMPK cascade in the background of simulation of alcohol intoxication significantly enhances the effect of H_2S on the nitric oxide system. The appearance of inversely proportional strong relationships with the activities of constitutive NOS isoforms, nitrate reductases and nitrite reductases can be explained by the joint inhibitory effect of both the AMPK cascade and alcohol on the activity of the xanthine oxidoreductase complex, especially on its reductase domain.[41-43] Considering the fact that inhibition of AMPK cascade activation by doxorubicin completely reverses the relationship between H_2S and the enzymes described above to a directly proportional strong one, it can be assumed that AMPK can affect the biological function of H_2S in relation to the xanthine oxidoreductase complex.

H_2S has the ability to stimulate the conversion of the xanthine oxidoreductase complex into nitrite reductase and promote the formation of nitric oxide from this source.[44] The blockade of the transition of the xanthine oxidoreductase complex, due to the activation of AMPK, to nitrite reductase can contribute to the excessive formation of ROS from the oxidase domain, which explains the directly proportional strong relationship between H_2S and $ONOO^-$ in the group of combined exposure to phenformin and chronic alcoholic hepatitis. Conversely, blockade of AMPK activation and H_2S-dependent stimulation of conversion of the xanthine oxidoreductase complex to nitrite reductase may explain the directly proportional strong relationship between H_2S and nitrosothiols in a group of animals under combined exposure to doxorubicin and chronic alcoholic hepatitis.

The disappearance of the relationship between H_2S and arginase activity in a group of animals with combined exposure to doxorubicin and chronic alcoholic hepatitis may be associated with a redistribution of the effect of H_2S on the nitrate-nitrite reductase pathway of nitric oxide formation towards the predominance of the effect on nitrosothiols, which can modulate the activity of arginase by releasing nitrous oxide.[45]

Modulation of AMPK activity does not change the direction and strength of the relationship between H_2S and the concentration of free L-oxyproline under conditions of chronic alcoholic hepatitis. Changes in the relationships between H_2S and the concentration of different GAG fractions depend to a greater extent on the influence of AMPK on the concentration of the latter and require further investigation.[35]

The limitation of this study is that we did not access the expression of AMPK in studied groups.

Perspectives of further research

Perspective of further research lies in establishing of causation between sulfide anion concentration and

changes in biochemical parameters with which it has shown strong correlations. On the studied models of modulation of AMPK cascade during alcohol intoxication we can establish dependence of abovementioned biochemical parameters, especially those, that showed significant correlations, from changes in concentration of sulfide anion in liver, which can be achieved by addition of sulfide donors and/or scavengers. Estimation of pathogenetic role of sulfide anion in development of alcoholic hepatitis and its interplay with AMPK cascade may open a path for usage of sulfide anion as a pathogenetically sound treatment of alcoholic hepatitis, free from negative impacts of direct influence on AMPK by specific modulators.

Conclusion

Modeling of chronic alcoholic hepatitis leads to the appearance of correlations between the concentration of endogenous H_2S and the activity of arginases, the concentration of nitrites and free L-oxyproline in the liver of rats.

Administration of phenformin under the conditions of chronic alcoholic hepatitis modeling expands correlations between endogenous H_2S and indicators of oxidative-nitrosative stress in the liver of rats, due to new correlations with superoxide anion-radical, peroxynitrite, nitrate-nitrite reductases, constitutive NO-synthases and malondialdehyde. However, the administration of phenformin leads to the loss of the correlation between endogenous H_2S and the concentrations of free L-oxyproline and nitrite.

Administration of doxorubicin under the conditions of chronic alcoholic hepatitis modeling expands correlations between endogenous H_2S and indicators of oxidative-nitrosative stress in the liver of rats, due to new correlations with peroxynitrite, nitrate-nitrite reductases, constitutive NO-synthases and nitrosothiols. However, administration of doxorubicin leads to a loss of correlation between endogenous H_2S and arginase activity and nitrite concentration.

Declarations

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Author contributions

Conceptualization, A.M. and K.N.; Methodology, A.M.; Software, O.A.; Validation, A.M., O.A., O.S. and K.N.; Formal Analysis, A.M.; Investigation, A.M. and O.A.; Resources, A.M.; Data Curation, A.M. and O.A.; Writing – Original Draft Preparation, A.M.; Writing – Review & Editing, O.S. and K.N.; Visualization, A.M.; Supervision, O.S. and K.N.; Project Administration, A.M.; Funding Acquisition, A.M.

Conflicts of interest

The authors declare that no conflicts exist.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

Research was conducted in accordance with the standards of the Council of Europe Convention on Bioethics "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1997), general ethical principles of experiments on animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this area. Research was approved by Ethical Committee of Poltava State Medical University.

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