UDC 616-006:615.211-085:547.569

Effect of exogenous hydrogen sulfide on the efficiency of anti-inflammatory therapy

A. Mykytenko, R. Chumakova, O. Akimov

Poltava State Medical University; e-mail: mykytenkoandrej18@gmail.com

Exogenous H_2S has proven to be a potent antioxidant, not only because of its direct effect on ROS scavenging but also due to its capacity to activate the endogenous antioxidant defense system. Therefore, exogenous H_2S could potentially influence the course of inflammation by reducing secondary alterations. The study aimed to determine the combined effect of NaHS and nimesulide on the development of oxidative-nitrosative stress in the myocardium of rats subjected to lipopolysaccharide-induced systemic inflammatory response. The experiments were performed on 30 Wistar rats. The animals were divided into 5 groups: control (received saline); LPS group, rats that were injected intraperitoneally with 0.4 μg/kg of bacterial LPS S. typhi (first week thrice a week, then 1 time a week for 30 days); group of rats treated with nimesulide and LPS, received same treatment as in LPS group but in last seven days of experiment additionally were administered orally 18.15 mg/kg of nimesulide; group with administration NaHS and LPS, rats received the same treatment as in LPS group, but in the last seven days of the experiment, were additionally administered intraperitoneally 5 mg/kg of NaHS; group with administration nimesulide, NaHS and LPS, rats received same treatment as in LPS group but in last seven days of experiment additionally were administered orally 18 mg/kg of nimesulide and intraperitoneally 5 mg/kg NaHS. In the rat blood, we studied C-reactive protein content, and in 10% rat myocardium homogenate, we studied parameters of oxidative stress, functioning of nitric oxide cycle and hydrogen sulfide content. Lipopolysaccharide loading of the body is accompanied by hyperproduction of active nitrogen forms and the development of oxidative damage to myocardial lipids and proteins, with a compensatory increase in the activity of antioxidant enzymes. Simultaneous administration of nimesulide and NaHS during LPS-inflammation decreased cNOS activity by 1.22 times, nitrosothiols concentration by 3.41 times, peroxynitrite by 1.61 times, superoxide production by 4.85 times, and nitrites increased by 1.98 times, catalase activity by 2.31 times, superoxide dismutase by 3.75 times, malondialdehyde decreased by 3.47 times, sulfides by 2.98 times and oxidatively modified proteins by 2.43 times compared to the control. Thus, the use of nimesulide, the introduction of a hydrogen sulfide donor, and their combined use under conditions of lipopolysaccharide-induced systemic inflammation lead to a decrease in the excessive production of nitric oxide and its toxic metabolites in the myocardium of rats. At the same time, a synergistic effect of nimesulide and exogenous hydrogen sulfide is observed only in reducing the intensity of oxidative damage to lipid structures of the myocardium under conditions of lipopolysaccharide-induced systemic inflammatory response.

Key words: oxidative stress; hydrogen sulfide; heart; bacterial lipopolysaccharide; systemic inflammation; nimesulide.

INTRODUCTION

In recent years, an increase in the incidence of myocarditis has been noted, which is due to changes in the body's reactivity, incorrect and untimely use of medications, unjustified immunization and frequent re-immunization of the population, global environmental changes, psycho-emotional stress, and weakened immunity [1]. Bacterial myocarditis is the leading cause of sudden death in young patients [2]. Treatment of myocarditis remains difficult and complex due to the proliferation of inflammatory lesions in the myocardium [3]. Oxidative stress, which occurs when lipopolysaccharides act on cardiomyocytes, is characterized by an imbalance of antioxidant systems and the

production of free radicals in the body, which leads to long-term cell damage.

Increased reactive oxygen species (ROS) have been recognized as a key factor during vascular or cardiac injury associated with various heart diseases, such as acute myocardial infarction and ischemic heart disease [4]. In various cell types, including cardiomyocytes, several factors, such as bacterial lipopolysaccharide (LPS) or tumor necrosis factor alpha (TNF-α), can induce the release of ROS and a significant increase in cyclooxygenase 2 (COX-2) expression, which is attenuated by antioxidants [5]. Thus, experimental studies in vitro and in vivo suggest activation of COX-2 by various stimuli, but ROS may be a common factor mediating this process. According to the results of Lv PP et al., the COX-2 inhibitor nimesulide can improve myocardial function in isolated rat hearts suffering from oxidative stress. The mechanisms may be through an improvement in endothelium-dependent arterial relaxation and an increase in NO content in the heart [6].

Exogenous H_2S has proven itself as a potent antioxidant not only due to its direct effect on ROS scavenging, but also due to its ability to activate the endogenous antioxidant defense system [7]. H_2S, through sulfhydration of Kelch-like ECH-associated protein 1 and subsequent increase in nuclear translocation of erythroid-related factor-2 (Nrf-2), increases the amount of antioxidant enzymes such as catalase and superoxide dismutase, thereby reducing oxidative stress [8]. H_2S also inhibits peroxynitrite-mediated processes in vivo by scavenging peroxynitrite [9]. As we know from the literature we reviewed regarding endogenous H_2S, it is beneficial at physiological concentrations but harmful at supraphysiological concentrations, which is similar to the situation with nitric oxide (NO), another gaseous transmitter that shares many biological effects with H_2S [10]. However, in this article, we will focus on the anti-inflammatory effects of H_2S and the concept that these effects can be used to develop more effective and safer anti-inflammatory drugs. There is preclinical evidence suggesting that H_2S-releasing derivatives of several nonsteroidal anti-inflammatory drugs, including diclofenac, ketoprofen, and indomethacin, have significant advantages over the parent drugs [11].

The study aimed to determine the combined effect of NaHS and nimesulide on the development of oxidative-nitrosative stress in the myocardium of rats under conditions of lipopolysaccharide-induced systemic inflammatory response.

METHODS

The experiments were performed on 30 white, sexually mature male Wistar rats, weighing 190-220 g. Throughout the experiment, the animals were kept in a vivarium under the rules of animal hygiene, maintaining a 12/12-hour daily cycle of "light-darkness" with constant aeration, an air temperature of 26°C, and a humidity of 43 ± 2%. Rats received ad libitum a mixed grain-vegetable diet and water.

The animals were divided into five groups, each containing six animals. Group I – control. Rats from this group were injected intraperitoneally with 0.1 ml of 0.9% sodium chloride solution in the first week 3 times, then once a week for 30, from the 24th day of the experiment, 1 ml of 0.9% sodium chloride solution was administered orally once a day until the end of the experiment (last 7 days). In group II (LPS), rats were intraperitoneally injected with 0.4 μg/kg of bacterial LPS of *S. typhi* (pyrogenal) in the first week, 3 times, then once a week for 30 [12]. In group III (LPS and NSAIDs), rats were intraperitoneally injected with 0.4 μg/kg of bacterial LPS of *S. typhi* (pyrogenal) in the first week 3 times, then once a week for 30 days, from the 24th day of the experiment, 18.15 mg/kg of nimesulide was administered orally once a day until the end of the experiment (last 7 days). Dose of nimesulide was calculated using the species endurance constant (R), according to the formula: $R = \sqrt{Q \times V/Kc}$, where Q is the basal metabolic rate, kcal/kg · h; V is the cardiac output, l/kg · h; Kc (brain mass, g/body mass,

kg) is the cerebration coefficient. R_1 for humans is 0.57, R_2 for rats is 3.62. Dose of nimesulide was calculated as D1(human)/R1 = D2 (rat)/R2. According to the drug instruction therapeutic dose of nimesulide for a human weighing 70 kg (D1) is 200 mg/day (100 mg twice a day). So D2 = ((200 mg/70kg) · 3.62)/0.57 = 18.15 mg/kg [13]. In group IV (LPS and NaHS), rats were intraperitoneally injected with 0.4 μg/kg of bacterial LPS of *S. typhi* (pyrogenal) in the first week, 3 times, then once a week for 30 days, from the 24th day of the experiment, 5 mg/kg of hydrogen sulfide donor NaHS was injected intraperitoneally once a day until the end of the experiment (last 7 days) [14]. In group V (LPS, NSAIDs and NaHS), rats were intraperitoneally injected with 0.4 μg/kg of bacterial LPS of *S. typhi* (pyrogenal) in the first week 3 times, then once a week for 30 days, from the 24th day of the experiment, 18.15 mg/kg of nimesulide were administered orally once a day and 5 mg/kg of the hydrogen sulfide donor NaHS intraperitoneally once a day until the end of the experiment (last 7 days).

The conditions for keeping animals in the vivarium were standard. Euthanasia of experimental animals was performed under thiopental anesthesia 100 mg/kg on the 30th day by taking blood from the right ventricle of the heart. The object of the study was the blood serum and myocardium of rats. For biochemical studies, 10% myocardial homogenate was prepared. After removing the rat heart from the thoracic cavity, the myocardium was separated and placed in a Petri dish and minced. A sample of myocardium weighing 500 mg was taken for analysis. The tissue was homogenized in 10 mmol/l tris (2-amino-2-hydroxymethyl-propane-1,3-diol)-HCl buffer, pH 7.4 (1 g of tissue per 9 ml of medium) for 30-40 s. The supernatant was used for biochemical studies. All manipulations were carried out at a temperature of 0° to +4°C (in an ice bath). During the experiments, the recommendations of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) were followed following the "General Principles of Animal Experiments" approved by the 1st National Congress on Bioethics, and the requirements of the "Procedure for Conducting Experiments and Experiments on Animals by Scientific Institutions" (2012). All manipulations with laboratory animals were approved by the Bioethical Committee of Poltava State Medical University (Record No. 228 from 20.06.2024)

Biochemical research methods. The concentration of C-reactive protein was determined in the blood plasma of rats using diagnostic kits produced by NPP "Filisit-Diagnostics". All spectrophotometric studies were performed on a Ulab 101 spectrophotometer at respective wavelengths.

Total NO synthase (gNOS) activity was determined by the increase in nitrite after incubation of 10% myocardial homogenate (0.2 ml) for 30 min in incubation solution (2.5 ml of 0.1 M tris buffer, 0.3 ml of 320 m aqueous L-arginine solution, and 0.1 ml of 1 mmol/l NADPH+H+ solution). To determine the activity of constitutive isoforms of NO synthase (cNOS), a 1% solution of aminoguanidine hydrochloride was used, and the incubation time was extended to 60 min. The activity of the inducible isoform of NO synthase (iNOS) was calculated using the formula: iNOS = gNOS - cNOS. The concentration of nitrites was determined by determining the diazo compounds formed in the reaction with sulfanilic acid, and then the reaction with α-naphthylamine (Griss-Ilosvay reagent) was carried out. As a result, red derivatives (azo dyes) are formed. The intensity of the color is proportional to the concentration of nitrites. Arginase activity was determined by the increase in L-ornithine concentration after incubation of 0.2 ml of 10% myocardial homogenate with 0.3 ml of 24 mmol/l L-arginine solution [15].

The concentration of nitrosothiols was determined by the difference in the concentration of nitrites (NO_2^-) using the Griess reagent (with Ilosvay modification) before and after the oxidation of nitrosothiol complexes (S-NO) to nitrites

with a solution of mercuric chloride ($HgCl_2$). The concentration of alkali and alkaline earth metal peroxynitrite was measured by its reaction with potassium iodide at pH 7.0 in 0.2 M phosphate buffer at the same pH, yielding the product I_3 with a maximum absorption at a wavelength of 355 nm [12].

The production of superoxide anion radical was determined by the reaction of the latter with nitroblue tetrazolium dihydrochloride (NBT), which leads to the conversion of NBT, yellow in color, to diformazan, blue. The maximum absorption of diformazan in chloroform is at a wavelength of 500-570 nm. Catalase activity was determined by the color of the products formed as a result of the reaction of hydrogen peroxide with ammonium molybdate. The calculation was made by the amount of hydrogen peroxide that was catabolized in the presence of 0.1 ml of 10% myocardial homogenate containing catalase per unit time. To determine the activity of superoxide dismutase (SOD), the reaction of adrenaline autoxidation in an alkaline medium with the formation of superoxide was used, by comparing the rate of adrenaline autoxidation in the presence of 0.1 ml of 10% myocardial homogenate and without it. The activity of SOD was calculated in conventional units (c.u., 1 unit indicates a decrease in the reaction rate by 50%). Free malondialdehyde was estimated by a specific reaction with 1-methyl-2-phenyl-indole in a mixture of methanol and acetonitrile to form a chromogen (carbocyanine dye) with maximum light absorption at a wavelength of 586 nm [15].

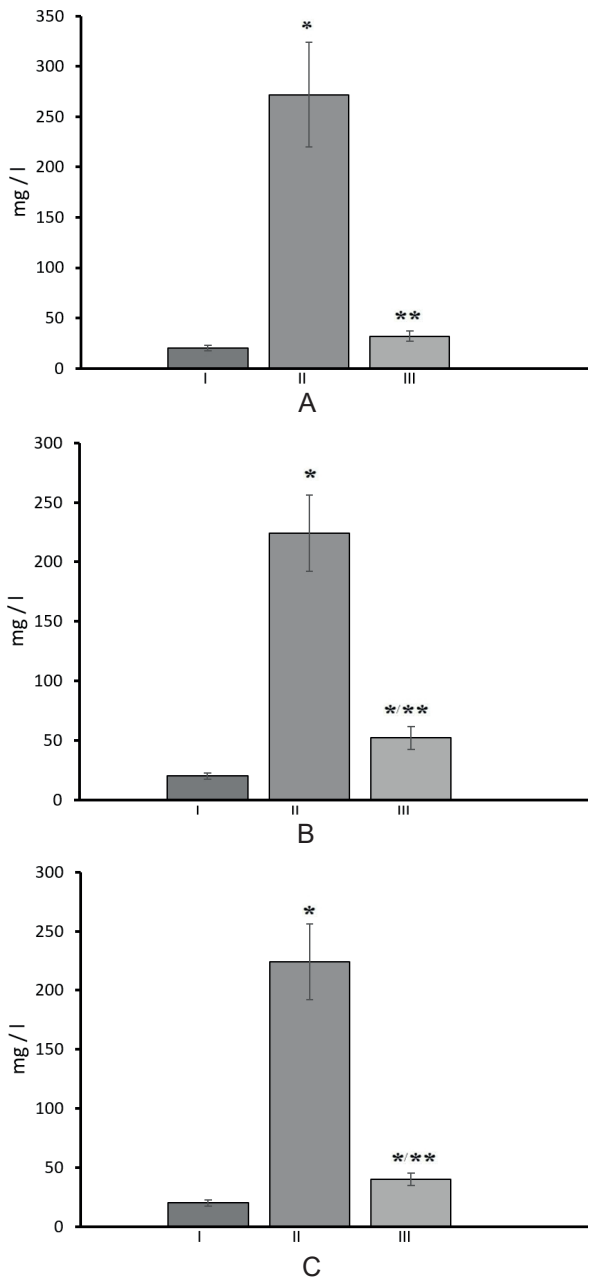
The concentration of oxidatively modified proteins (OMP) was determined spectrophotometrically; the principle of the method is that 2,4-dinitrophenylhydrazine reacts with the carbonyl groups of oxidized proteins to form dinitrophenylhydrazones. [12].

The concentration of sulfide anion was calculated as the concentration of H_2S. H_2S specifically reacts with N-N-dimethyl-paraphenylenediamine in the presence of Fe^{3+} ions and excess hydrochloric acid to form a red-pink chromogen with maximum light absorption at a wavelength of 667 nm [16].

Mathematical and statistical research methods. Statistical processing of the biochemical study parameters was performed using nonparametric analysis of variance according to the Kruskal–Wallis method. This was followed by a subsequent pairwise comparison of the groups according to the Mann–Whitney method. To avoid the phenomenon of multiple comparison error, the Bonferroni correction was used. Statistical analysis of the associated parameters of biochemical studies was carried out using the Wilcoxon test. All statistical calculations were performed in Microsoft Office Excel and its Real Statistics 2019 extension. The difference was considered statistically significant at $P < 0.05$. Data in figures and tables is presented as mean ± standard error of mean (M ± SEM).

RESULTS AND DISCUSSION

The results of C-reactive protein concentration in the blood plasma of rats before and after correction of LPS-induced systemic inflammation with nimesulide, inorganic hydrogen sulfide donor (NaHS), and their combination are presented in Figure. In the group of rats, where correction of changes in the myocardium caused by systemic inflammation was performed using nimesulide, the concentration of C-reactive protein increased by 13.6 times on day 23 compared to the indicator on the first day before LPS administration, on day 30 of the experiment the concentration of C-reactive protein decreased by 8.5 times compared to the indicator on day 23 ($P < 0.05$). In the group of rats, where correction of changes in the myocardium caused by systemic inflammation was performed using NaHS, the concentration of C-reactive protein increased by 11.2 times on day 23 compared to the indicator on the first day before LPS administration, on day 30 of the experiment the concentration of C-reactive protein decreased by 4.31 times compared to the indicator on day 23 ($P < 0.05$). In the group of rats, where correction of changes in the



Concentration of C-reactive protein in the blood plasma of rats under the conditions of administration of nimesulide (A), inorganic hydrogen sulfide donor (NaHS) (B), and their combination (C) during the last 7 days of modeling LPS-induced systemic inflammation, which was simulated for 30 days. I, data before the administration of LPS; II, data before the administration of nimesulide and NaHS; III, data after the administration of nimesulide and NaHS. Notes: Data are represented as mean (M) ± SEM. *P < 0.05 compared to the indicators before the start of LPS administration (1 day); **P < 0.05 compared to the indicators before the introduction of the studied corrector (23 days)

myocardium caused by systemic inflammation was performed using nimesulide and NaHS, the concentration of C-reactive protein increased by 11.2 times on day 23 compared to the indicator on the first day before LPS administration, on day 30 of the experiment the concentration of C-reactive protein decreased by 5.6 times compared to the indicator on day 23 ($P < 0.05$).

We found that under the conditions of LPS administration, gNOS activity in the rat myocardium increased by 2.61 times, iNOS by 2.71 times, cNOS by 2.09 times, the concentration of nitrosothiols by 1.18 times, peroxynitrite by 1.57 times, and nitrites by 8.83 times catalase activity by 3.07 times, SOD by 1.23 times, MDA concentration by 7.73 times, sulfide anions by 3.91 times and OMP content by 2.82 times compared to the control ($P < 0.05$) (Table). Meanwhile, arginase activity decreased by 3.04 times, compared to the control ($P < 0.05$).

Systemic inflammation, which is formed in the body under conditions of regular administration of LPS, is accompanied by excessive production of pro-inflammatory cytokines (IL-6, IL-1, TNF-α, etc.), which is caused by activation of the transcription factor NF-κB. In addition to pro-inflammatory cytokines, iNOS gene expression is also under transcriptional control of NF-κB, which explains its increased activity observed in our study [17]. Given the dominant role of iNOS in nitric oxide production, the increase in the concentration of peroxynitrite in the myocardium may be associated with an increase in the activity of this enzyme. Excessive formation of peroxynitrites and increased activity of antioxidant enzymes (mainly SOD) may be the reason for the absence of statistically significant changes in the formation of superoxide anion radical in the myocardium of rats under the conditions of administration of bacterial lipopolysaccharide to rats. At the same time, the formation of peroxynitrite excludes nitric oxide from the physiological cycle of its transformations and creates a certain deficit of its effects, which may explain the accumulation of nitrites and the activation of constitutive

Biochemical parameters in the myocardium of rats under conditions of administration of hydrogen sulfide donor (NaHS) and nimesulide under conditions of lipopolysaccharide-induced systemic inflammatory response (M±SEM)

Parameters	Control	LPS 30 days	LPS and Nimesulide	LPS and NaHS	LPS, Nimesulide and NaHS
Total NO-synthase activity, μmol/min per g of protein	0.64±0.08	1.67±0.32*	0.41±0.02*/**	0.71±0.02**/***	0.87±0.04 **/***/****
Inducible NO-synthase, μmol / min per g of protein	0.56±0.08	1.52±0.32*	0.35±0.02*/**	0.66±0.02**/***	0.81±0.04 **/***/****
Constitutive NO-synthase, μmol/min per g of protein	0.0736±0.0004	0.1541±0.0034*	0.0611±0.0007*/**	0.0579±0.0002 */**/***	0.0604±0.0004 */**/****
Arginase activity, μmol/min per g of protein	1.49±0.01	0.49±0.01*	2.01±0.02*/**	1.77±0.04 */**/***	1.41±0.03 **/***/****
Nitrosothiols concentration, μmol/g	0.239±0.003	0.281±0.008*	0.137±0.003*/**	0.086±0.006 */**/***	0.07±0.004 */**/***/****
Peroxynitrite concentration, μmol/g	1.91±0.01	3±0.04*	1.55±0.03*/**	0.9±0.03 */**/***	1.19±0.05 */**/***/****
Nitrite concentration, nmol/g	3.04±0.11	26.85±1.74*	2.18±0.15*/**	4.21±0.55**	6.03±0.43 */**/***/****
Superoxide anion radical production, nmol/s per g	1.31±0.01	1.25±0.08	0.23±0.01*/**	0.27±0.01 */**/***	0.27±0.01*/**/***
Catalase activity, μkat/g	0.494±0.002	1.518±0.074*	1.285±0.024*/**	0.695±0.016 */**/***	1.14±0.005 */**/***/****
Superoxide dismutase activity, c.u.	1.87±0.1	2.3±0.15*	9.29±0.25*/**	6.75±0.55 */**/***	7.01±0.21*/**/***
Malondialdehyde concentration, μmol/g	5.16±0.06	39.91±4.63*	20.44±0.58*/**	29.48±0.61 */***	17.88±0.18 */**/***/****
Oxidatively modified proteins concentration, c.u.	0.044±0.0004	0.124±0.004*	0.146±0.001*/**	0.2±0.002 */**/***	0.107±0.001 */**/***/****
Sulfide anion concentration, μmol/g	3.2±0.14	12.52±0.7*	9.58±0.11*/**	8.48±0.13 */**/***	9.52±0.08 */**/****

*P< 0,05 compared to control;** P< 0,05 compared to LPS group; ***P < 0,05 compared to group LPS and nimesulide; ****P< 0,05 compared to group LPS and NaHS.

isoforms of NO synthase under the influence of bacterial lipopolysaccharide. It is obviously that cNOS activation is unable to restore the physiological pool of nitric oxide, which is used for the formation of peroxynitrites; therefore, activation of the nitrate, nitrite reductase pathway of nitric oxide formation is an adaptive response to the deficiency of its effects, as evidenced by the accumulation of nitrites in the myocardium. At the same time, the increase in the concentration of MDA and the content of OMP against the background of unchanged production of superoxide anion radical indicates an increase in the intensity of oxidative stress due to other oxygen radicals (hydroxyl, oxygen radicals of higher fatty acids, etc.). The decrease in arginase activity may be due to a deficiency of the substrate for this enzyme, which is aimed at maintaining high iNOS activity. Stimulation of the body with bacterial lipopolysaccharide, according to scientific literature, reduces the activity of Cystathionine-γ-lyase, which should reduce H_2S production and its concentration in tissues [18]. Considering that Cystathionine-γ-lyase is the dominant enzyme that produces H_2S in the cardiovascular system, the increase in its concentration observed in our study may be associated with non-enzymatic mechanisms of its formation (reduction from sulfites or release from polysulfide groups of proteins) and is an adaptive response to LPS-induced damage. An increase in C-reactive protein concentration in rat blood during stimulation of the organism with bacterial LPS can be attributed to activation of transcriptional factor NF-κB [19].

Under the conditions of nimesulide administration against the background of modeling of LPS-induced systemic inflammation, the activity of gNOS in the myocardium of rats decreased by 1.56 times, iNOS by 1.6 times, cNOS by 1.2 times, the concentration of nitrosothiols by 1.74 times, peroxynitrite by 1.23 times and nitrites by 1.39 times, production of superoxide anion radical in the rat myocardium decreased by 5.7 times compared to the control ($P < 0.05$). Arginase activity increased by 1.35 times, catalase activity by 2.6 times, SOD by 4.97 times, MDA concentration by 3.96 times, sulfide anion by 2.99 times, and OMP content by 3.32 times compared to the control ($P < 0.05$).

Compared with the group of rats that received LPS, the activity of gNOS in the myocardium of rats administered nimesulide against the background of LPS-induced systemic inflammation decreased by 4.07 times, iNOS by 4.34 times, cNOS by 2.52 times, the concentration of nitrosothiols by 2.05 times, peroxynitrite by 1.94 times and nitrites by 12.32 times, production of superoxide anion radical decreased by 5.43 times, catalase activity by 1.18 times, MDA concentration by 1.95 times and sulfide anion concentration by 1.31 times. Meanwhile, arginase activity increased by 4.1 times, SOD activity by 4.04 times and OMP content by 1.18 times ($P < 0.05$).

The evidence of nimesulide's effectiveness during the treatment of systemic inflammation can be attributed to its ability to inhibit COX-2, which is one of the leading mechanisms in pathological changes during systemic inflammation [20]. COX-2-dependent mechanism may also underlie the effectiveness of nimesulide in decreasing CRP levels observed in our study. The ability of exogenous hydrogen sulfide administration to influence systemic inflammation was also shown in scientific literature, which is realized by COX-2 inhibition and sulfhydration of human anigens [21]. Abdelrahman R.S. et al. showed in their study that hydrogen sulfide administration decreases CRP content during cardiovascular dysfunction [22].

The main mechanism of action of nimesulide on the body is selective blockade of COX-2 [23]. Although blocking COX-2 does not directly impact the activity of NF-κB transcription factors, it can minimise secondary alterations, which are caused by excessive production of prostaglandins (COX-2 products), which indirectly reduce the activity of the transcription factor NF-κB. The scientific literature provides data on the presence of antioxidant properties in nimesulide, which explains the increase in SOD and catalase

activity in our study, since the administration of nimesulide reduces the functional load on these enzymes [24]. The scientific literature provides limited data on the effect of nimesulide on the activity of enzymes that produce endogenous H_2S, therefore, the decrease in its concentration in the nimesulide group may be associated with a lower level of heart tissue damage.

Under the conditions of the introduction of a hydrogen sulfide donor (NaHS) against the background of modeling LPS-induced systemic inflammation, the activity of cNOS in the myocardium of rats decreased by 1.27 times, the concentration of nitrosothiols by 2.78 times, peroxynitrite by 2.12 times, the production of superoxide anion radical by 4.85 times compared to the control ($P < 0.05$). The activity of arginase increased by 1.19 times, catalase by 1.41 times, SOD by 3.61 times, the concentration of MDA by 5.71 times, sulfide anions by 2.65 times and the content of OMP by 4.55 times compared to the control ($P < 0.05$).

Compared with the group of rats that received LPS, the activity of gNOS in the myocardium of rats under the conditions of NaHS administration against the background of systemic inflammation decreased by 2.35 times, iNOS by 2.3 times, cNOS by 2.66 times, the concentration of nitrosothiols by 3.27 times, peroxynitrite by 3.33 times, nitrite by 6.38 times, the production of superoxide anion radical by 4.63 times, catalase activity by 2.18 times and the concentration of sulfide anions by 1.48 times ($P < 0.05$). The activity of arginase increased by 3.61 times, SOD by 2.93 times and the content of OMP by 1.61 times ($P<0.05$).

Comparing two correctors, nimesulide and hydrogen sulfide donor (NaHS), which were administered against the background of systemic inflammation, an increase in gNOS in the myocardium of rats in the LPS and NaHS group was found by 1.73 times, iNOS by 1.89 times, superoxide anion radical production by 1.17 times, MDA concentration by 1.44 times, and OMP content by 1.37 times. Meanwhile, cNOS activity decreased by 1.06 times, arginase activity by 1.14 times, nitrosothiol concentration by 1.59 times, peroxynitrite by 1.72 times, catalase activity by 1.85 times, SOD by 1.38 times and sulfide anion concentration by 1.13 times compared to the LPS and nimesulide group ($P < 0.05$).

As already noted above, exogenous H_2S is a stimulator of activation of the transcription factor Nrf-2, which has an antagonistic nature towards NF-κB, which is manifested in competition for DNA binding sites, which explains the NaHS-induced decrease in iNOS activity and peroxynitrite concentration in the rat heart [8]. In addition to its ability to influence redox processes by activating the expression of antioxidant genes, H_2S has a direct antiradical effect on peroxynitrites and superoxide anion radical, which explains the decrease in the intensity of oxidative damage to lipid and protein structures of the heart observed in our study [9]. The introduction of exogenous H_2S leads to reciprocal inhibition of its formation from other compounds (sulfites, disulfide groups), since exogenous H_2S does not affect the activity of enzymes of its synthesis in healthy rats [25].

Under the conditions of simultaneous administration of nimesulide and hydrogen sulfide donor (NaHS) against the background of modeling of systemic inflammation, cNOS activity in the myocardium of rats decreased by 1.22 times, the concentration of nitrosothiols by 3.41 times, peroxynitrite by 1.61 times, the production of superoxide anion radical by 4.85 times compared to the control ($P<0.05$). The concentration of nitrites increased by 1.98 times, catalase activity by 2.31 times, SOD by 3.75 times, MDA concentration by 3.47 times, sulfide anion by 2.98 times and OMP content by 2.43 times compared to the control ($P < 0.05$).

Compared with the group of rats that received LPS, the activity of gNOS in the myocardium of rats under the conditions of simultaneous administration of nimesulide and NaHS against the background of systemic inflammation decreased by 1.92 times, iNOS by 1.88 times, cNOS by 2.55 times, the concentration of nitrosothiols by 4.01 times, peroxynitrite by 2.52 times, nitrites

by 4.45 times, the production of superoxide anion radical by 4.63 times, catalase activity by 1.33 times and MDA concentration by 2.23 times, sulfide anions by 1.32 times and OMP content by 1.16 times ($P < 0.05$). Meanwhile, the activity of arginase increased by 2.88 times and SOD by 3.05 times ($P < 0.05$).

Under the conditions of simultaneous administration of nimesulide and hydrogen sulfide donor (NaHS) against the background of modeling of systemic inflammation, gNOS activity increased in the myocardium of rats by 2.12 times, iNOS by 2.31 times, nitrite concentration by 2.77 times, superoxide anion radical production by 1.17 times compared to the group of animals that were administered nimesulide against the background of modeling of systemic inflammation ($P < 0.05$). Arginase activity decreased by 1.43 times, nitrosothiol concentration by 1.96 times, peroxynitrite by 1.3 times, catalase activity by 1.13 times, SOD by 1.33 times, MDA concentration by 1.14 times and OMP content by 1.36 times compared to the group of animals that were administered nimesulide against the background of modeling of systemic inflammation ($P < 0.05$).

Under the conditions of simultaneous administration of nimesulide and hydrogen sulfide donor (NaHS) against the background of modeling of LPS-induced systemic inflammation, the activity of gNOS increased in the myocardium of rats by 1.23 times, iNOS by 1.23 times, cNOS by 1.04 times, the concentration of peroxynitrite by 1.32 times, nitrite by 1.43 times, catalase activity by 1.64 times and the concentration of sulfide anions by 1.12 times compared to the group of animals that were administered NaHS against the background of modeling of systemic inflammation ($P < 0.05$). The activity of arginase decreased by 1.26 times, the concentration of nitrosothiols by 1.23 times, MDA by 1.65 times, and the content of OMP by 1.87 times compared to the group of animals that were administered NaHS against the background of modeling of systemic inflammation ($P < 0.05$).

The same direction of changes in all groups of correction of the effect of bacterial lipopolysaccharide indicates the activation of similar genetic mechanisms by nimesulide and exogenous hydrogen sulfide, which probably include modulation of the activities of such redox-sensitive transcription factors as Nrf-2 and NF-κB, which requires further study. The synergistic effect of nimesulide and exogenous hydrogen sulfide is manifested only in the effect on the intensity of damage to lipid structures of the myocardium and may be associated with the direct antioxidant effects of nimesulide and hydrogen sulfide.

The increase in arginase activity in all groups of correction of the systemic inflammatory response is likely a consequence of a decrease in the activity of the inducible isoform of NO synthase, which releases a substrate for arginase.

The limitations of our study are: a small number of experimental animals in study groups, the absence of control groups for nimesulide, hydrogen sulfide, and their combination (administration of studied drugs to intact animals).

CONCLUSIONS

Lipopolysaccharide loading of the body is accompanied by hyperproduction of active nitrogen forms and the development of oxidative damage to myocardial lipids and proteins, with a compensatory increase in the activity of antioxidant enzymes. Using nimesulide, a hydrogen sulfide donor, and combining the two under conditions of lipopolysaccharide-induced systemic inflammation all lead to a decrease in excessive nitric oxide production and its toxic metabolites in the rat myocardium. At the same time, a synergistic effect of nimesulide and exogenous hydrogen sulfide is observed only concerning reducing the intensity of oxidative damage to lipid structures of the myocardium under conditions of lipopolysaccharide-induced systemic inflammatory response.

The authors of this study confirm that the research and publication of the results were not associated

with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

А. Микитенко, Р. Чумакова, О. Акімов

ВПЛИВ ЕКЗОГЕННОГО СІРКОВОДНЮ НА ЕФЕКТИВНІСТЬ ПРОТИЗАПАЛЬНОЇ ТЕРАПІЇ

Полтавський медичний університет;
e-mail: mykytenkoandrej18@gmail.com

Екзогенний H_2S зарекомендував себе як потужний антиоксидант не тільки завдяки прямому впливу на поглинання активних форм кисню, але й своїй здатності активувати ендогенну антиоксидантну захисну систему. Тому потенційно він може впливати на перебіг запалення через зменшення вторинної альтерації. Мета нашого дослідження — визначити поєднаний вплив NaHS та німесуліду на розвиток оксидативно-нітрозативного стресу в міокарді щурів за умов ліпополісахаридіндукованої системної запальної відповіді. Дослідження проводили на 30 щурах лінії Вістар, яких поділили на 5 груп. Тварини I контрольної групи отримували фізіологічний розчин. Щурам II групи вводили внутрішньоочеревинно 0,4 мкг/кг бактеріальний ліпополісахарид *S. typhi* (ЛПС) у перший тиждень тричі на тиждень, потім раз на тиждень упродовж 30 днів; тварин III групи отримували ЛПС, але в останні 7 днів експерименту їм додатково вводили перорально18 мг/кг німесуліду. Щурам IV групи вводили ЛПС, але в останні 7 днів додатково ще 5 мг/кг NaHS внутрішньоочеревинно. Щури V групи отримували ЛПС, але в останні 7 днів їм додатково вводили 18,15 мг/кг німесуліду та 5 мг/кг NaHS. У крові щурів досліджували вміст С-реактивного білка, а в 10%-му гомогенаті міокарда щурів – показники оксидативного стресу, функціонування циклу оксиду азоту та вміст сірководню. Ліпополісахаридне навантаження організму супроводжується гіперпродукцією активних форм азоту та розвитком оксидативного пошкодження ліпідів і білків міокарда з компенсаторним підвищенням активності антиоксидантних ферментів. Одночасний прийом німесуліду та NaHS при ЛПС-запаленні знижував активність конститутивної NOS у 1,22 раза, концентрацію нітрозотіолів у 3,41 раза, пероксинітриту в 1,61 раза, продукцію супероксиду в 4,85 раза, а вміст нітритів збільшувався в 1,98 раза, активність каталази в 2,31 раза, супероксиддисмутази – в 3,75 раза, вміст малонового діальдегіду знижувався у 3,47 раза, сульфідів у 2,98 раза та окисномодифікований білок у 2,43 раза порівняно з контролем. Використання німесуліду, введення донора сірководню та їх поєднане застосування за умов ЛПС-індукованого системного запалення призводить до зниження надлишкової продукції оксиду азоту та його токсичних метаболітів у міокарді щурів. При цьому синергічна дія німесуліду та екзогенного сірководню спостерігається лише щодо зниження інтенсивності оксидативного пошкодження ліпідних структур міокарда в умовах ЛПС-індукованої системної запальної відповіді.
Ключові слова: оксидативний стрес, сірководень, серце, бактеріальний ліпополісахарид, системне запалення, німесулід.

REFERENCES

1. Li M, Gou Y, Yu H, Ji T, Li Y, Qin L, Sun W. Mechanism of metformin on LPS-induced bacterial myocarditis. Dose Response. 2019;17(2):1559325819847409. doi: 10.1177/1559325819847409.
2. Shen L, Lu K, Chen Z, Zhu Y, Zhang C, Zhang L. Pretreatment with galectin-1 attenuates lipopolysaccharide-induced myocarditis by regulating the Nrf2 pathway. Eur J Histochem. 2023;67(4):3816. doi: 10.4081/ejh.2023.3816.
3. Fan S, Hu K, Zhang D, Liu F. Interference of circRNA HIPK3 alleviates cardiac dysfunction in lipopolysaccharide-induced mice models and apoptosis in H_9C_2 cardiomyocytes. Ann Transl Med. 2020;8(18):1147. doi: 10.21037/atm-20-5306.
4. Pozo-Navas B, Stessel H, Wölkart G, Brunner F. Role of myocardial nitric oxide in diabetic ischemia-reperfusion dysfunction: studies in mice with myocyte-specific overexpression of endothelial nitric-oxide synthase. J Pharmacol Exp Ther. 2006;319(2):729-38. doi: 10.1124/jpet.106.107854.
5. Adderley SR, Fitzgerald DJ. Oxidative damage of cardiomyocytes is limited by extracellular regulated kinases 1/2-mediated induction of cyclooxygenase-2. J Biol Chem. 1999;274(8):5038-46. doi: 10.1074/jbc.274.8.5038.
6. Lv PP, Fan Y, Chen WL, Shen YL, Zhu L, Wang LL, Chen YY. COX-2 inhibitor nimesulide protects rat heart against oxidative stress by improving endothelial function and enhancing NO production. Sheng Li Xue Bao. 2007;59(5):674-80.
7. Huerta de la Cruz S, Santiago-Castañeda CL, Rodríguez-Palma EJ, Medina-Terol GJ, López-Preza FI, Rocha L, Sánchez-López A, Freeman K, Centurión D. Targeting hydrogen sulfide and nitric oxide to repair cardiovascular injury after trauma. Nitric Oxide. 2022;129:82-101. doi: 10.1016/j.niox.2022.10.003.
8. Corsello T, Komaravelli N, Casola A. Role of hydrogen sulfide in NRF2- and sirtuin-dependent maintenance of cellular redox balance. Antioxidants (Basel). 2018;7(10):129. doi: 10.3390/antiox7100129.
9. Whiteman M, Armstrong JS, Chu SH, Jia-Ling S, Wong BS, Cheung NS, Halliwell B, Moore PK. The novel neuromodulator hydrogen sulfide: an endogenous peroxynitrite 'scavenger'? J Neurochem. 2004;90(3):765-8. doi: 10.1111/j.1471-4159.2004.02617.x.

10. Cirino G, Szabo C, Papapetropoulos A. Physiological roles of hydrogen sulfide in mammalian cells, tissues, and organs. Physiol Rev. 2023;103(1):31-276. doi: 10.1152/physrev.00028.2021.
11. Zaorska E, Tomasova L, Koszelewski D, Ostaszewski R, Ufnal M. Hydrogen sulfide in pharmacotherapy, beyond the hydrogen sulfide-donors. Biomolecules. 2020;10(2):323. doi: 10.3390/biom10020323.
12. Pletnov V, Tkachenko O, Akimov O, Mykytenko A. Effect of lipopolysaccharide on the development of oxidative-nitrosative stress in salivary glands and soft periodontal tissues of rats under conditions of water avoidance stress. Eur J Clin Exp Med. 2024;22(2):404-16. doi: 10.15584/ejcem.2024.2.30
13. Hladkykh FV, Stepaniuk NH. Experimental substantiation of effectively administration of vinboron for analgesic activity increase of ibuprofen. Curr Issues Pharm Med: Sci Pract. 2016;3(22):41-8.
14. Gong QH, Wang Q, Pan LL, Liu XH, Huang H, Zhu YZ. Hydrogen sulfide attenuates lipopolysaccharide-induced cognitive impairment: a pro-inflammatory pathway in rats. Pharmacol Biochem Behav. 2010;96(1):52-8. doi: 10.1016/j.pbb.2010.04.006.
15. Mykytenko A, Akimov O, Yeroshenko G, Neporada K. Phenformin attenuates the oxidative-nitrosative stress in the liver of rats under long-term ethanol administration. Ukr Biochem J. 2024;96(3):22-30. doi: 10.15407/ubj96.03.022
16. Mykytenko AO, Akimov OY, Shevchenko OM, Neporada KS. Role of sulfide anion in the development of chronic alcoholic hepatitis under the conditions of modulation of adenosine monophosphate kinase – a correlational study. Eur J Clin Exp Med. 2023;21(3):567-75. doi: 10.15584/ejcem.2023.3.24.
17. You Z, Yang Z, Cao S, Deng S, Chen Y. The Novel KLF4/BIG1 Regulates LPS-mediated neuro-inflammation and migration in BV2 cells via PI3K/Akt/NF-κB signaling pathway. Neuroscience. 2022;488:102-11. doi: 10.1016/j.neuroscience.2022.01.014.
18. Lin T, Bai X, Gao Y, Zhang B, Shi J, Yuan B, Chen W, Li J, Zhang Y, Zhang Q, Zhao X. CTH/H_2S regulates LPS-induced inflammation through IL-8 signaling in MAC-T cells. Int J Mol Sci. 2022;23(19):11822. doi: 10.3390/ijms231911822.
19. Quagliariello V, Canale ML, Bisceglia I, Iovine M, Paccone A, Maurea C, Scherillo M, Merola A, Giordano V, Palma G, Luciano A, Bruzzese F, Zito Marino F, Montella M, Franco R, Berretta M, Gabrielli D, Gallucci G, Maurea N. Sodium-glucose cotransporter 2 inhibitor dapagliflozin prevents ejection fraction reduction, reduces myocardial and renal NF-κB expression and systemic pro-inflammatory biomarkers in models of short-term doxorubicin cardiotoxicity. Front Cardiovascul Med. 2024;11:1289663. doi: 10.3389/fcvm.2024.1289663.
20. Choudhary R, Kumar P, Shukla SK, Bhagat A, Anal JMH, Kour G, Ahmed Z. Synthesis and potential anti-inflammatory response of indole and amide derivatives of ursolic acid in LPS-induced RAW 264.7 cells and systemic inflammation mice model: Insights into iNOS, COX2 and NF-κB. Bioorg Chem. 2025;155:108091. doi: 10.1016/j.bioorg.2024.108091.
21. Guo S, Huang Z, Zhu J, Yue T, Wang X, Pan Y, Bu D, Liu Y, Wang P, Chen S. CBS-H_2S axis preserves the intestinal barrier function by inhibiting COX-2 through sulfhydrating human antigen R in colitis. J Adv Res. 2023;44:201-12. doi: 10.1016/j.jare.2022.03.010.
22. Abdelrahman RS, El-Awady MS, Nader MA, Ammar EM. Hydrogen sulfide ameliorates cardiovascular dysfunction induced by cecal ligation and puncture in rats. Hum Exp Toxicol. 2015;34(10):953-64. doi: 10.1177/0960327114564794.
23. Vunnam N, Young MC, Liao EE, Lo CH, Huber E, Been M, Thomas DD, Sachs JN. Nimesulide, a COX-2 inhibitor, sensitizes pancreatic cancer cells to TRAIL-induced apoptosis by promoting DR5 clustering. Cancer Biol Ther. 2023;24(1):2176692. doi: 10.1080/15384047.2023.2176692.
24. Luo W, Luo Y, Yang J. Proteomics-based screening of the target proteins associated with antidepressant-like effect and mechanism of nimesulide. Sci Rep. 2020;10(1):11052. doi: 10.1038/s41598-020-66420-z.
25. Huerta de la Cruz S, Rodríguez-Palma EJ, Santiago-Castañeda CL, Beltrán-Ornelas JH, Sánchez-López A, Rocha L, Centurión D. Exogenous hydrogen sulfide restores CSE and CBS but no 3-MST protein expression in the hypothalamus and brainstem after severe traumatic brain injury. Metab Brain Dis. 2022;37(6):1863-74. doi: 10.1007/s11011-022-01033-1.

Received 20.03.2025