Vascular mechanism in the formation of diclophenac induced gastrotoxicity: the association with the level of hydrogen sulfide

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Abstract

Background: Non-steroid anti-inflammatory drugs (NSAIDs)-induced gastrotoxicity arises as a result of imbalance between vasodilator and vasoconstrictor bioregulators. The influence of deficiency and excess of hydrogen sulfide on vascular mechanisms in the formation of NSAIDs-induced gastrotoxicity was investigated.

Material and methods: Male nonlinear rats underwent preconditioning with donor of H2S (NaHS) and inhibitor of its synthesis (propargilglycine). Diclophenac sodium was introduced orally (8 mg/kg). In homogenates of rats' gastric mucosa was evaluated the activity of prostaglandin-H-synthase (PgH-synthase), NO-synthase, content of nitrites and nitrates, H_2S and the activity of cystathionine-γ-lyase. In vitro H_2S-induced relaxation of mesenteric arteries was measured.

Results: Diclophenac sodium decreased cystathionine-γ-lyase enzyme activity, NO-synthase and PGH-synthase (by 17-24%), content of their H_2S metabolites and nitrites/nitrates (by 20-22%) in gastric mucosa, and accompanied with the decrease of mesenteric artery sensitivity to vasodilatory action of H_2S (EC_{50} increased to 27.5%). H_2S deficiency – increases and excess of H_2S – inhibits the negative influence of diclophenac on the production of vasoactive molecules and H_2S-induced relaxation of mesenteric arteries.

Conclusions: Excess of H_2S in organism increases the content of vasoligating molecules and thus can prevent vascular disturbances caused by NSAIDs in rat stomach mucosa.

Key words: hydrogen sulfide, diclophenac, gastrotoxicity, prostaglandin-H-synthase, NO-synthase, cystathionine-γ-lyase.

Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) – a group of drugs with the unique combination of anti-inflammatory, analgesic and antipyretic effects responsible for their widespread use by patients of all ages. However, even short-term intake of these drugs can cause serious side effects that threaten the health and lives of patients. One of the dangerous side effects of NSAIDs is the involvement of gastric

mucosa [1, 2]. It is present approximately in 70% of patients taking NSAIDs for 1.5 months or more. The leading place in the pathogenesis of NSAIDs-induced microcirculatory disorders belongs to gastric toxicity that arises as a result of imbalance between vasodilator and vasoconstrictor bioregulators. It is shown that intake of NSAIDs increases the production of vasoconstrictors – free oxygen radicals, endothelin-1 and decreases the synthesis of vasodilating molecules – nitrogen oxide and prostaglandins [1, 2].

In recent years, increasing attention of scientists attracts the new gas-transmitting molecule – hydrogen sulfide. H_2S is produced by cystathionine-β-synthase, cystathionine-γ-lyase and 3-mercapto-pyruvate-sulfur-transferase in mammalian cells. Two-thirds of H_2S molecules dissociate into hydrogen ions (H^+) and bisulfide ions (HS^-) under physiological conditions. Therefore, sodium hydrosulfide (NaHS) can be administered as a water-soluble H_2S donor. H_2S is involved in the regulation of vascular tone, inflammation, and also has cytoprotective properties [3, 4]. Recently it has been shown that the injection of NSAIDs has inhibiting influence on the formation of hydrogen sulfide in rat's gastric mucosa and causes a decrease of H_2S-induced vasorelaxation, which is one of the possible factors of gastric toxicity of this group of drugs [5]. However, the influence of different saturation effects in rats with hydrogen sulfide on NSAIDs-induced changes in the balance of vasodilators/vasoconstrictors remains unclear. Therefore, the aim of our study was to evaluate the effect of diclofenac sodium on the production of prostaglandins, nitrogen monoxide, hydrogen sulfide in gastric mucosa and H_2S-stimulated ring fragments relaxation of rats' mesenteric arteries on the background of deficiency and excess of hydrogen sulfide.

Material and methods

Investigations were carried out on male albino nonlinear rats weighing 180-210 gr, the animals were kept on a standard diet with access to water ad libitum at the temperature of 22°C ± 5°C with 12-hour lighting in the vivarium of Vinnitsa Pirogov National Medical University. All experiments were conducted in accordance with the «Regulations on the use of animals in biomedical research». Excess and deficiency of hydrogen sulfide were created in the animals by intraperitoneal administration of hydrogen sulfide donor – NaHS (Sigma, USA) at a dose of 1.5 mg/kg in phosphate buffer (pH = 7.4) and a specific inhibitor of the synthesis of this gastransmitter, propargilglycine (Sigma, USA) at a dose of 50 mg/kg for 5 days, respectively. The experimental animals were divided into several groups: group I – intact control (received equivalent amounts of solvents). The animals of group II were administered diclophenac sodium ("Voltaren"), Novartis, 8 mg/kg of 1% starch gel for 5 days intra-gastric. Rats of III and IV groups were administered the NSAIDs on the background of excess and deficiency of hydrogen sulfide, respectively. Test substances were used in conventionally therapeutic doses (1/20 LD50), borrowed from the literature or calculated previously [6]. Euthanasia was performed by cervical dislocation according to the requirements of bioethics. For biochemical studies gastric mucosa was isolated. It was perfused by 1.15% cold potassium chloride solution and homogenized at 3000 rev/min (teflon-to-glass) in the medium of 1.15% potassium chloride (the ratio was 1:3). Homogenates were centrifuged for 30 min at 600 g, the aliquotes of post-nuclear supernatant were chosen into microtubes Erpendorf and before research were stored at 20°C. Prostaglandins production in homogenates of rats' gastric mucosa was evaluated spectrophotometrically by the determining of total activity of prostaglandin-H-synthase (PgH-synthase) with the accumulation of oxidized electron donor adrenaline [7]. The total activity of NO-synthase (eNOS and iNOS) adjusted by the number of formed nitrite anion (NO2-) after incubation in 1 ml of medium which contained 50 mM KH2PO4-NaOH-buffer (pH 7.0), 1 mM MgCl2, 2 mM CaCl2, 1 mM NADPH, 2,2 mM L-arginine. 0.2 ml of the sample was added to the medium which contained 8 mg of protein post-nuclear homogenate. The incubation time was 60 min [8]. The content of nitric oxide metabolites – nitrites and nitrates were determined by the reaction with a reagent Gris – 0.2% and 12% solution of acetic acid [9] after the preceding deposition of proteins with acetonitrile. Previously nitrates were renewed to nitrites by the mixture which contained zinc powder and solution of ammonia. H2S content in the serum was determined by spectrophotometry based on the formation of thionine in the reaction between the sulfide anion, and p-phenylenediamine hydrochloride in acidic medium in the presence of iron (III) [10]. The activity of cystathionine-γ-lyase (EC 4.4.1.1) was measured by the number of hydrogen sulfide formed after the incubation in the medium containing 0.67 mM of pyridoxal phosphate, L-cysteine, 3.3 mM tris-0.083 M buffer (pH 8.5). Registration of contractile activity of isolated vascular preparations was carried out in a mode that approached isometric by tenzometric setting created at "Institute of physiology of Academy of Medical Sciences of Ukraine named after A. A. Bogomolets "by generally established method" [11]. Statistic analyses of received data were performed by software «STATISTIK 5.5» (which belongs to the Center of scientific information technologies of N. I. Pirogov National Medical University of Vinnitsa №AXXR910A374605FA). Mean (M) and standard errors (m) were calculated, percentile analyses were carried out, probability of differences (P) was evaluated using Student's t-Criteria. The difference $p < 0.05$ was reliable.

Results and discussion

Introduction of NSAIDs had inhibiting influence on the formation of hydrogen sulfide in rats' gastric mucosa (table 1). Thus, in the group of animals that received diclophenac sodium there was a reliable decrease ($p < 0.05$) of H_2S-producing enzyme of cystathionine-γ-lyase action and the content of H_2S by 23.6% and 21.7%, respectively, compared with the control group. Administration of diclophenac sodium on the background of application of NaHS didn't sig-

nificantly influence the production on hydrogen sulfide. At the same time using of NSAIDs together with propargilglycine increases the magnitude of modified H2S synthesis in gastric mucosa: cystathionine-γ-lyase activity and H_2S level were 20.9% and 22.7%, respectively, less than in the group of animals that received only diclophenac sodium.

Table 1

Effect of NaHS and propargilglycine on diclophenac sodium-induced changes in H_2S content and activity of cystathionine-γ-lyase in gastric mucosa (M ± m, n = 10)

Number	Groups	H_2S, nmol/mg protein	Cystathionine-γ-lyase, nmol/min on 1 mg of protein
1	Control	1.52 ± 0.04	0.144 ± 0.012
2	Diclofenac (D)	1.19 ± 0.06*	0.110 ± 0.009*
2	D + NaHS	1.46 ± 0.07#	0.133 ± 0.004#
3	D + propargilglycine	1.01 ± 0.03*#	0.085 ± 0.003*#

Notes: 1. * – statistically reliable differences ($p < 0.05$) about the control group;

2. # – statistically reliable differences ($p < 0.05$) about the group that received only diclophenac sodium.

There is one more molecule – nitrogen monoxide – which takes part in the process of regulation of mesenteric vessels tone. Therefore we evaluated summary activity of NO synthesis and general content of nitrates and nitrites in gastric mucosa due to introduction of NSAIDs on the background of different level of hydrogen sulfide in the organism (table 2). Studies have shown that the administration of diclophenac sodium was accompanied with reliable decrease of summary activity of NO-synthase and content of NO^{2-} + NO^{3-} in gastric mucosa by 23.9% and 20.7%, respectively, those facts coincided with the data of relevant literature. However, combined introduction of hydrogen sulfide donor and NSAIDs neared the investigated data of system NO-synthase/NO to control group of animals. Creating of hydrogen sulfide deficiency with the help of propargilglycine significantly increased the inhibitory effect of diclophenac on summary activity of NO-synthase: due to those conditions its activity was 40.6% less compared with the control group.

Table 2

Effect of diclophenac sodium on the content of nitrates and nitrites and the summary activity and NO-synthase in gastric mucosa due to different saturation of rats' organism with hydrogen sulfide (M ± m, n = 10)

Number	Groups	NO^{2-} + NO^{3-}, nmol/g of tissue	NO-synthase, pmol/min on 1mg of proteine
1	Control	1,07 ± 0.04	1.55 ± 0.11
2	Diclofenac (D)	0.85 ± 0.03*	1.18 ± 0.09*
2	D + NaHS	1.01 ± 0.05#	1.50 ± 0.08#
3	D + Propargilglycine	0.70 ± 0.02*#	0.92 ± 0.05*#

Notes: 1. * – statistically reliable differences ($p < 0.05$) about the control group;

2. # – statistically reliable differences ($p < 0.05$) about the group that received only diclophenac sodium.

The same tendency was noted during the investigation of prostaglandin synthase activity (fig. 1). The received data showed that the introduction of diclophenac sodium to rats led to the reduction of prostaglandin-H-synthase activity by 16.9% compared with the control group. Preconditioning with H_2S had almost renovated the activity of this enzyme to its level in the control group of rats. Application of diclophenac sodium to the propargilglycine-preconditioning rats, accompanied with more expressed fall of prostaglandin-H-synthase activity: the enzyme activity was 33.8% less than in the control group and 20.3% less compared with isolated introduction of diclophenac.

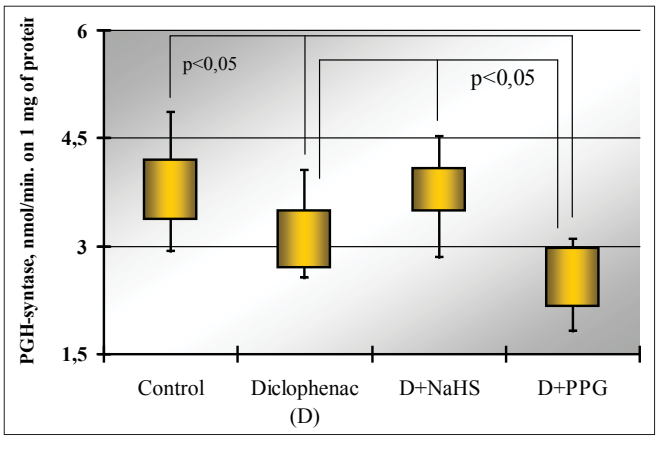

Fig. 1. Effect of diclofenac sodium on the activity of PGH-synthase due to different saturation of rats' organism with hydrogen sulfide. Cubicles include the results from 25 to 75 percentile, vertical lines outside the cubicles – minimal and maximal results.

Administration of diclophenac sodium induced the decrease of mesenteric artery sensitivity to the effect of H_2S (fig. 2, 3). Under such circumstances H_2S concentrations range 10^{-6}-10^{-2}M induced less expressed vasorelaxation compared with the control group. Displacement of curve «dose-effect» to the right side was noted. EC_{50} (H_2S) significantly increased (by 27.5%). Combined application of diclophenac sodium with hydrogen sulfide donor almost completely renovated the mesenteric artery sensitivity to the vasorelaxation effect of H_2S: the curve "dose-effect" displaced to the left side in the group of animals that received only NSAIDs; indicator of EC_{50} (H_2S) significantly didn't differ from the control group. Introduction of propargilglycine together with diclophenac resulted in more expressed decrease of H_2S-induced vasorelaxation: the curve "dose-effect" displaced to the right side in the group of animals that received only NSAIDs; indicator of

EC_{50} (H_2S) was significantly higher compared with isolated administering of diclophenac.

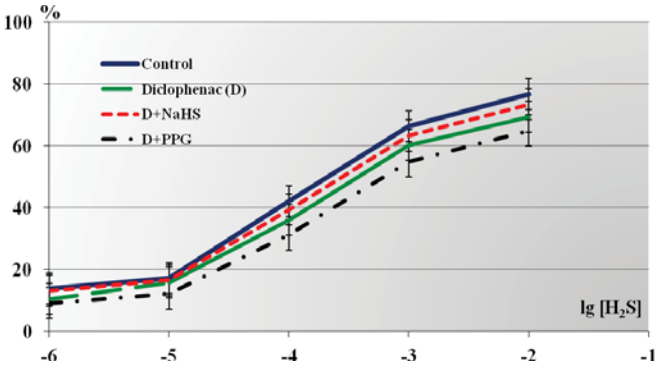

Fig. 2. Effect of different saturation of rats' organism with hydrogen sulfide on diclophenac-induced changes of H_2S-stimulated ring fragments relaxation of rats' mesenteric arteries. On the axis of abscises there is a decimal logarithm of H_2S (M) concentrations in superfusional solution. On the axis of ordinate there is a standardized intensity of ring fragments relaxation of investigated vessels under the influence of H_2S increased concentrations. The accepted level of H_2S-stimulated relaxation of investigated vessels ring fragments corresponded to the maximum meaning of phenylephrin-induced, precontraction was 100%. The mean data of 5 investigations and errors are illustrated.

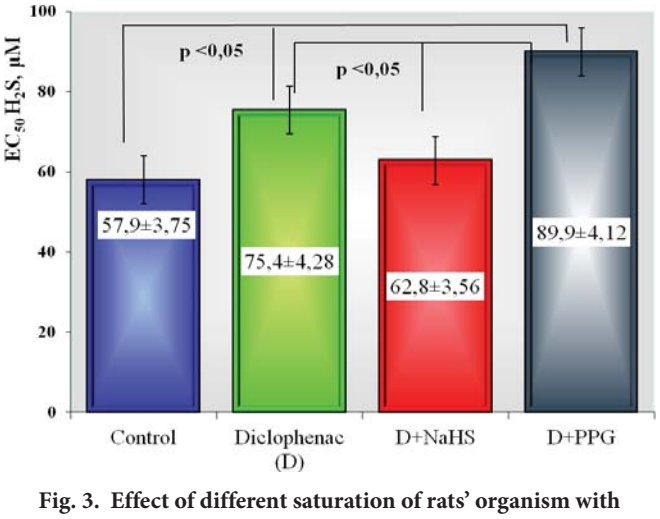

Fig. 3. Effect of different saturation of rats' organism with hydrogen sulfide on diclophenac-induced changes of EC_{50} H_2S in mesenteric arteries.

Thus, the administration of diclophenac sodium results in the decrease of vasodilator production – nitric oxide, prostaglandins, and hydrogen sulfide – and sensitivity of mesenteric artery walls to the effect of H_2S. Introduction of such NSAID for the preconditioning with the hydrogen sulfide (H_2S) donor NaHS reduced the inhibitory effect of diclophenac on the synthesis of above mentioned vasoactive mediators and normalized the regulatory effect of H_2S on the tone of mesenteric vessels. At the same time, introduction of NSAIDs simultaneously with propargilglycine (inhibitor of endogenous H_2S synthesis) significantly increased the metabolic conversions in the metabolism of vasodilating molecules. Saturation level of rats' organism with hydrogen sulfide was one of the factors, which determined the expressiveness of NSAIDs influence on the dynamic equilibrium in vasodilators/vasoconstrictors system and the state of microcirculation in gastric mucosa. The diverse physiological functions of H_2S make it capable of protecting the heart [12], brain [13], liver [14], kidney [15], and lung [16] against ischemia-reperfusion injury when given at sub-toxic doses. The mechanisms of protection appear to include suppressing oxidative stress via antioxidant activities, reducing inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10) and intercellular cell adhesion molecule-1 (ICAM-1), and reducing apoptosis. Additionally, H_2S can up-regulate B-cell lymphoma-2 (Bcl-2) expression [17]. Possible mechanisms through which the ability of H2S to prevent NSAIDs-induced vasoconstriction was realized: direct stimulating effect of H2S on the activity of NO-synthase [3, 4]; direct activating effect of H2S, excess of NO on PGH-synthase activity; decrease of oxidative modification of active centers redox-sensitive proteins – NO-synthase and PGH-synthase associated with antioxidant action of H_2S [5, 6, 18, 19].

Conclusions

1. Application of diclophenac sodium resulted in the decrease of cystathionine-γ-lyase enzyme activity in gastric mucosa, NO-synthase and PGH-synthase (by 17-24%), content of their H_2S metabolites and nitrites/nitrates (by 20-22%) and accompanied with the decrease of mesenteric artery sensitivity to vasodilatory effect of H_2S (EC_{50} increased to 27.5%).

2. Introduction of NSAIDs on the background of H_2S deficiency (induced by propargilglycine) accompanied with the increase of inhibiting influence of diclofenac on the production of investigated vasoactive molecules and H2S-induced mesenteric arteries relaxation.

3. Application of NSAIDs on the background of H_2S excess (induced by NaHS) almost completely decreased the negative effect of diclophenac sodium on NO production, vasodilatory prostaglandins, H_2S and the processes of mesenteric arteries contractility regulation with the participation of H_2S.

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