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THE INFLUENCE OF NOVEL 4-THIAZOLIDINONE DERIVATIES IN CYTOPROTECTIVE MECHANISMS OF SMALL INTESTINE UNDER NSAID-INDUCED DAMAGE

I. I. ILKIV, R. B. LESYK, O. Ya. SKLYAROV

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine;
e-mail: ira9ilkiv@gmail.com

The aim of investigation was to compare the action of novel 4-thiazolidinone derivaties (compounds Les-5054 and Les-5055) toward parameters of nitroso-oxidative processes in mucous membrane of small intestine (MMSI) in rats on the background of indomethacin induced injury. The activity of nitric oxide synthases, myelopeoxidase, content of NO, and parameters of lipoperoxidation processes were measured in MMSI and the level of H2S and L-arginine in blood serum. Administration of indomethacin caused significant destructive damages in distal part of small intestine and increase in activity of inducible nitric oxide synthase (iNOS) and intensity of lipoperoxidation processes in comparison to control were observed. Also indomethacin injection was accompanied by decrease of H_2S and L-arginine level in blood serum. Administration of 4-thiazolidinone derivaties on the background of indomethacin induced injury reduce the activity of iNOS, myeloperoxidase, intensity of lipid peroxidation and increase generation of H_2S, that may be linked with the structure of this compounds. However compound Les-5054 showed more significant cytoprotective effect and antioxidant properties than compound Les-5055. Thus, the novel 4-thiazolidinone derivaties led to reduce of nitroso-oxidative processes caused by administration of NSAIDs.

K e y w o r d s: hydrogen sulfide, 4-thiazolidinones, small intestine, indomethacin-induced injury.

Hydrogen sulfide (H_2S) is now recognized as an important gasotransmitter together with nitric oxide (NO) and carbon monoxide (CO). H_2S has been implicated in the induction of such processes as inhibition of leukocytes adherence to blood vessels, increase of endogeneous prostaglandins production in small intestine, induction of vasodilatation, increases cyclic AMP (cAMP) production in neural retina, modulates epithelial secretion and promotes resolution of colitis[1]. The deficiency of hydrogen sulfide could lead to various pathological changes in digestive tract, such as gastric mucosal injury, liver cirrhosis etc [2].

Nowadays nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used class of drugs for treating inflammatory conditions such as: osteochondrosis, polyarthritis, headaches. However, they cause significant adverse reaction in the mucous membranes of the digestive system in the form of erosions, ulcers, impaired motor skills [3]. Owing to the inhibition of cyclooxygenase, NSAID suppress synthesis of prostaglandins which leads to leukocyte adherence to the vascular endothelium in the gastrointestinal microcirculation, reduce bicarbonate and mucus secretion by the epithelium, decrease blood flow, that play key role in the process of gastrointestinal injury caused by NSAIDs [4]. Also these drugs reduce H_2S generation by modulating the expression of activity of cystathionine-γ-lyase, which is mainly expressed in smooth muscle cells in the cardiovascular system and in the gastrointestinal tract [5].

Thus, 4-thiazolidinones are one of important source of organic sulfurcontaining compounds and have been widely investigated regarding their therapeutic applications. Thiazolidinone-based molecules are attaractive targets in rational design of “drug-like” compounds which possess anti-inflammatory, antioxidant, antitumor, choleretic, diuretic and other activities [6-8].

In the present study the effects of a novel 4-thiazolidinones (compounds Les-5054 and Les-5055), as potential H2S donors or mediators of its signaling pathways were investigated. The effects of these compounds in terms of nitroso-oxidative and cytoprotective effects, ability to decrease small intestinal injury, and acute anti-inflammatory effects were compared.

Materials and Methods

Animals. The experimental protocols were approved by the Ethical Committee of Lviv National Medical University (Ukraine). Male albino rats weighing 200-250 g were used. The rats were fed standart chow and water ad libitum, and were housed in room with controlled temperature (22 ± 1 °C), humidity (65-70%) and light cycle (12 h light/dark).

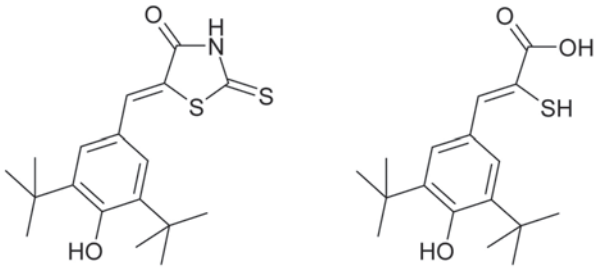
The study comprised of the following series of experiments: 1 – intact animals were used as controls (n = 10); 2 – small intestine lesions in rats were induced by indomethacin (Sigma, USA) in dose of 35 mg/kg subcutaneously (n = 10) as previously described (n = 10) [9]; 3,4 – experimental groups, animals received compounds Les-5054, Les-5055 (10 mg/kg) per os once daily per 72 h on the background of indomethacin-induced injury (n = 10). The compounds Les-5054, Les-5055 were synthesized by prof. Lesyk R. in the Department of Pharmaceutical, Organic and Bioorganic Chemistry Danylo Halytsky Lviv National Medical University (Fig. 1) [8, 10].

Under general anesthesia, rats were sacrificed by decapitation and 10 sм of distal part of small intestine was then blindly evaluated for hemorrhagic damage. This involved measuring the lengths (mm) of all hemorrhagic lesions. The intestinal damage scores were then calculated by summing the lengths of all lesions for each rats. The mucous membrane of small intestine (MMSI) samples were homogenized in phosphate buffer pH 6.0 1:4 and centrifuged at 3000 rpm, supernatant was used to determine values of biochemical parameters.

Determination of NO-system in mucous membrane of small intestine. The content of NO in homogenate was determined as nitrites by the method of Green et al. [11]. The absorbance was read in a Stat fax at 550 nm. NO concentration was expressed as mmol/g. NO-synthases (general NOS, iNOS, and eNOS) activity was measured by the method described in detail [12]. NOS activity was expressed in nmol L-citrylline/min×mg of protein.

Measurement of L-arginine and H_2S in blood serum. The level of L-arginine in plasma samples was measured by Sakaguchi reaction [13]. Plasma L-arginine level was expressed as mmol/l. H_2S concentration was determined by reaction with N,N-dimethyl-para-phenylenediamine in the presence of $FeCl_3$ and expressed as mmol/g [14].

Lipid peroxidation determination. Lipid peroxidation level was expressed as MDA (malonic dialdehyde) concentration in homogenates of MMSI. It was measured according to the procedure of Timirbulatow et al. [15]. MDA levels were expressed as mmol/l.



Les-5054 Les-5055

Fig. 1. Structures of 4-thiazolidinone derivatives

Intracellular myeloperoxidase activity. Myeloperoxidase (MPO) content in homogenates of MMSI measured at 460 nm according to the procedure of Bradley et al. [16]. MPO level were expressed as U/mg.

Antioxidant enzymes defence determination. Activity of superoxide dismutase (SOD) was determined by the reaction of reduction of nitrotetrazoliume blue to nitroformazan [17]. SOD activity was expressed in mmol/min×mg of protein. Catalase (CAT) activity was determined by measuring of the decrease in hydrogen peroxide concentration at 410 nm by the Korolyuk method [18]. Colon mucosal catalase activity was expressed in mmol H_2O_2/min×mg of protein.

Statistics. The statistical processing of the data was done by conventional methods for analysis of variance using MS Excel software for Student's t-test. The difference was considered to be significant at $P < 0.05$.

Results and Discussion

In our study, injection of indomethacin (35 mg/kg) manifested by erosions and hemorrhages, with a total area of 74 ± 5,94 mm^2 (Fig. 2, 3). Indometacin induced injury in the MMSI was associated with change of the activity of NO-synthases: activity of general NOS decreased (from 815.5 ± 49.8 to 595.54 ± 73.7 nmol/min×mg) ($P < 0.05$), activity of eNOS was decreased by 55% ($P < 0.01$), and activity of iNOS increased more than threefold ($P < 0.01$) as compared with indeces of control group. In MMSI concentration of NO was markedly elevated in two times and, concomitantly ($P < 0.01$), content of L-ar-

ginine in serum blood decreased for 33% ($P < 0.05$). It was found that H_2S formation from L-cysteine was dependently inhibited by addition of indomethacin by 10% ($P < 0.05$) (Table 1).

MMSI, affected with indometacin induced injury, was subjected to the following changes: enhanced activity of lipoperoxidation processes manifested by a steep rise of MDA concentration – for 56% ($P < 0.01$) at that, MPO activity enhanced more than 4-fold ($P < 0.01$), and catalase activity – for 32% ($P < 0.01$). The activity of SOD was not statistically significant (Table 2).

In the presence study the development of NSAID-induced small intestinal damage via indomethacin injection was acompanied by enhanced processes of lipid peroxidation, increase activity of iNOS and myeloperoxidase that led to development of hemorrhagic lesions in distal part of small intestine. This destructive changes caused by inflammation processes and acomplished throught decrease contents of H_2S which enhance gastrointestinal mucosal resistance to injury [19].

On another hand, the injection of NSAIDs such as diclofenac, naproxen, indomethacin caused the non selective inhibition of both COX-1 and COX-2 and, as result, a significant reduction in production of prostaglandins that is the key factor in development of enteropathies.

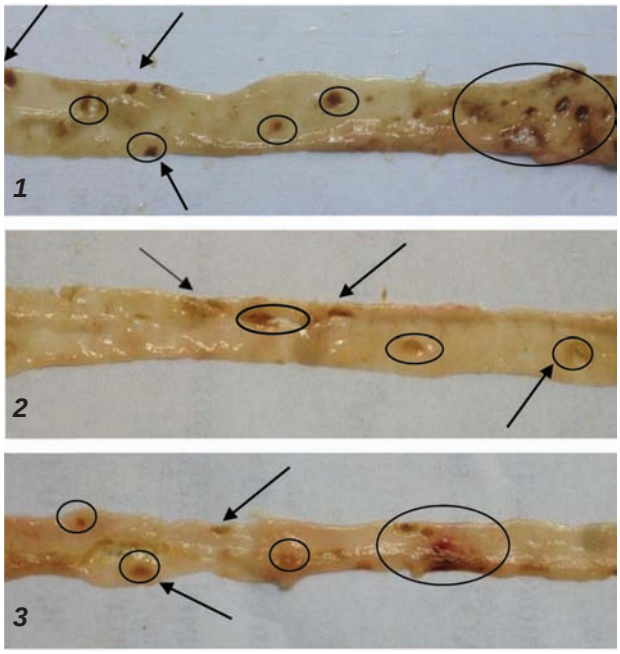


Fig. 2. Representative photographs of the small intestinal injury: 1 – indomethacin; 2 – Les-5054 + indomethacin; 3 – Les-5055 + indomethacin

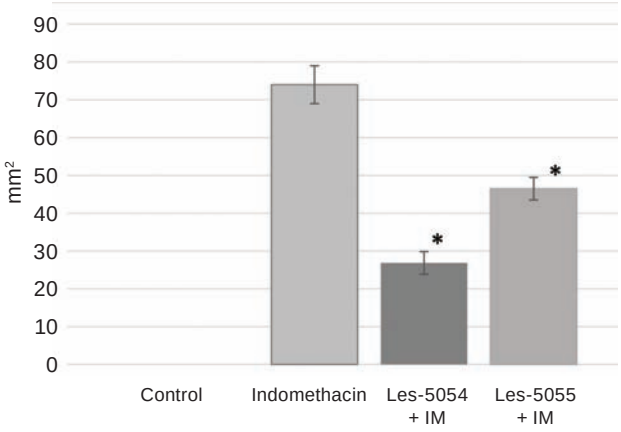


*Fig. 3. The area of the structure-hemorrhagic damage of the MMSI. *P < 0.05 versus the indexes in indomethacin (IM)*

Compound Les-5054 displayed significant cytoprotective effect that manifested by separate hemorrhages with the absence of considerable destructive changes of the MMSI. The total area of hemorrhagic lessins decreased for 63% ($P < 0.05$) (Fig. 2, 3). The administration of Les-5054 on the background of indomethacin-induced injury decrease the activity of iNOS for 35% ($P < 0.01$), and activity of eNOS increased for 52% ($P < 0.01$) as compared with independent action of indomethacin. Intensity of lipoperoxidation processes were determined much lower than under the effect of indomethacine, MDA concentration declined for 32% ($P < 0.01$). Administration of compound Les-5054 reversed the inhibition of H_2S caused by indomethacin and increase it for 24% ($P < 0.05$) as compared with indices of the second group.

Compound Les-5054 led to 3 time decreased MPO activity as compared with indices of 2 group ($P < 0.05$). It was found the administration of compounds Les-5054 and Les-5055 as H_2S releasing compounds can inhibit peroxidase activity of MPO, key role is production of reactive oxidants (HOCl etc) which influence to a healthy tissue is the main indication of MPO's pathophysiological functions [20].

In our investigations it was shown that administration of Les-5055 on the background of indomethacin-induced injury also shows cytoprotective effect but not as much as compound Les-5054. The total area of hemorrhagic lessins decreased for 37% ($P < 0.05$) as compared with independent action of indomethacin (Fig 2, 3). Activity of eNO-synthases had a tendency to increase, whereas iNOS activity

Table 1. Effect of novel 4-thiazolidinones at the background of indomethacin-induced injury on concentration of malonic dialdehyde, nitrite anion, activity of nitric oxide synthases and arginase in MMSI and concentration of H_2S and L-arginine in serum blood

Variable	Experimental Groups			
	Control group	Indomethacin 35 mg/kg	Les-5054 + Indomethacin	Les-5055 + Indomethacin
Malonic dialdehyde, (μmol/g)	186.6 ± 8.1	291.4 ± 26.7**	199.1 ± 11.1##	242.6 ± 16.0##
Nitrite anion, (μmol/g)	1.2 ± 0.1	2.8 ± 0.2**	1.8 ± 0.2	1.9 ± 0.1
Total nitric oxide synthase – NOS, (nmol/min·g)	815.5 ± 49.8	595.5 ± 73.1	637.9 ± 42.8	578.2 ± 64.7
Inducible nitric oxide synthase – iNOS, (nmol/min·g)	66.1 ± 24.9	203.6 ± 26.8**	132.9 ± 27.5##	162.3 ± 27.7#
Constitutive nitric oxide synthase – cNOS, (nmol/min·g)	728.6 ± 66.1	331.9 ± 62.5**	506.3 ± 45.3##	415.9 ± 44.0#
Arginase, (μmol/ min·g)	0.20 ± 0.03	0.05 ± 0.02**	0.15 ± 0.04#	0.09 ± 0.006
L-Arginine, (μg/ml)	46.7 ± 3.6	31.2 ± 2.8	42.6 ± 4.1##	40.7 ± 4.2
H_2S (μmol/g×min)	88.4 ± 2.7	79.5 ± 1.0*	98.5 ± 2.6#	93.0 ± 2.5#

Here and for table 2 results are expressed as mean ± SD for 10 rats per group; *$P < 0.05$, **$P < 0.01$ in comparison of control group; #$P < 0.05$, ##$P < 0.01$ versus the indices of indomethacin action.

Table 2. The activity of myeloperoxidase and antioxidant enzymes in MMSI of rats injected indomethacin with investigated compounds

Variable	Experimental Groups			
	Control group	Indomethacin 35 mg/kg	Les-5054 + Indomethacin	Les-5055 + Indomethacin
SOD, (mmol/min×mg)	23.9 ± 1.0	27.8 ± 1.3	24.9 ± 0.9#	27.9 ± 1.5
CAT, (mmol H_2O_2/min×mg)	16.9 ± 1.6	22.4 ± 2.6**	20.9 ± 1.2	20.0 ± 2.4
MPO, (U/mg)	1.2 ± 0.3	5.0 ± 0.5*	1.6 ± 0.5#	4.3 ± 1.2

was reduced for 20% ($P < 0.05$), compared to their activity in indomethacin induced damages. Contents of NO and MDA also showed a tendency to decrease ($P < 0.05$).

Many reports showed that several H_2S-based therapeutics corresponded to target disorders and were characterized by oxidative stress and associated tissue injury. It was shown that their cytoprotection was accompanied by the decrease of mRNA expression for pro inflammatory cytokines, such as IL-10 or TGF-b. They also prevent inflammation by decrease of MDA content, increase GSH level and decrease of NO, IL-6 and TNF-a secretion in intestine mucosa [1].

In experimental model of indomethacin-induced injury characteristic damages in distal part of small intestine was observed. In our investigation we demonstrated that novel 4-thiazolidinones displayed significant cytoprotective effect, manifested by the decreased area of the MMSI lesions. Normalization of NO-synthases activities and the intensity of lipoperoxidation processes were found. Also administration of investigated compounds was associated with increase of H_2S level in serum blood. Thus,

novel 4-thiazolidinones showed cytoprotective, anti-inflammatory effects and antioxidant properties, and may be promising substances for new pharmacological preparations.

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