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ACTION OF HYDROGEN SULFIDE DONORS ON NITROSO-OXIDATIVE PROCESSES IN SMALL INTESTINE OF RATS WITH METHOTREXATE-INDUCED ENTEROPATHY

Introduction. *Medication-induced enteropathy plays an important part among factors leading to the development of small intestinal injury. There are some evidences indicating a potential preventive action of hydrogen sulfide (H_2S) donors against drug-induced enteropathies based on that fact that the use of the most of enterotoxic medications including anti-tumor drugs leads to the suppression of this gaseous mediator production.*

The aim of the study *– to compare the action of H_2S donors in small intestine of rats on parameters of NO-synthase system and oxidative stress under condition of methotrexate-induced enteropathy.*

Research Methods. *The experimental procedures were carried out on rats which on the background of methotrexate-induced enteropathy received H_2S donors NaHS (1 and 10 mg/kg) and L-cysteine. Following biochemical parameters were measured in small intestinal mucosa: activity of NO-synthases, myeloperoxidase, superoxide dismutase and catalase; concentrations of NO_x (nitrite/nitrate) and malonic dialdehyde. H_2S concentration was determined in blood serum.*

Results and Discussion. *Administration of methotrexate didn't cause any visible changes of small intestine surface, however led to serious biochemical changes. NO concentration increased as a result of iNOS activation (more than fivefold ($p \leq 0.01$). Simultaneously concentration of H_2S decreased in blood serum. Administration of H_2S donors practically returned these parameters to their normal value. Methotrexate-induced enteropathy caused the increase of myeloperoxidase activity by 66 %, $p \leq 0.01$, indicating of inflammatory process formation and activation of lipid peroxidation. Administration of NaHS didn't cause any serious changes in myeloperoxidase activity, however increased SOD activity and practically retuned it to its norm.*

Conclusions. *Nirtoso-oxidative stress plays the key role in enteropathy formation resulted in methotrexate administration. H_2S donors modulate parameters of NO-synthase system and activity of SOD.*

KEY WORDS: **enteropathy; methotrexate; hydrogen sulfide; NO-synthases oxidative stress.**

INTRODUCTION. Increased toxicity of different medications in the gastrointestinal tract is a common and serious medical problem; the number of drugs that can harm the gastrointestinal tract is impressive [1]. Enteropathy is known to be one of the most commonly appeared pathologies resulting in the use of medications such as: nonsteroidal anti-inflammatory drugs (NSAIDs), anti-tumor drugs and hypotensive medications [2]. Among anti-tumor drugs methotrexate and 5-fluorouracil is known by its ability to damage the small intestinal mucosa by preventing crypt mitotic activity, inhibiting dihydrofolate reductase and leading to the development of malabsorption syndrome and diarrhoea [3]. There are some evidences indicating a potential preventive action of hydrogen sulfide (H2S) donors against drug-induced enteropathies [2, 4] based on that fact that the use of the most of enterotoxic medications including anti-tumor drugs leads to the suppression of this gaseous mediator production. The most common way to generate H_2S for experiments is to use common salts such as NaHS and its precursors such as L-cysteine [5]. Cytoprotective effects of donors of H_2S were previously demonstrated on the model of indomethacin-induced small intestinal injury in rats [2, 4]. However, their effects in methotrexate-induced enteropathy are still poorly studied.

The aim of the study – to evaluate the action of H_2S donors in small intestine of rats on parameters of NO-synthase system and oxidative stress under condition of methotrexate-induced enteropathy.

RESEARCH METHODS. The structure of this study and the experimental procedures performed on the animals were approved by the Ethical Committee of Lviv National Medical University. The experimental procedures were carried out in accordance with the international guidelines for the use and care of laboratory animals. Male, outbred

albino rats weighing 200–220 g were used. Animals were randomly divided into 5 groups: group 1 – intact animals were used as controls; 2 – methotrexate, an anti-tumour drug was introduced in a single dose of 10 mg/kg, intraperitoneally [6] to induce enteropathy; animals in the groups 3, 4 and 5 groups received NaSH (1 mg/kg), NaSH (10 mg/kg) and L-cysteine were introduced intraperitoneally twice after methotrexate. 3 days after injection of methotrexate enteropathy had developed.

Rats were anesthetized with 1 ml of urethane at a dose of 1.1 mg/kg injected intraperitoneally and sacrificed by cervical dislocation. A blood sample from the cervical vessel was immediately collected into vials containing 0.1 ml of heparin. The samples of small intestinal mucosa were homogenised in phosphate buffer pH 6.0 1:4 and centrifuged at 3000 rpm, supernatant was used to determine values of biochemical parameters. Activity of NO-synthase isoenzymes (inducible iNOS and constitutive cNOS) was measured by the method described in detail [7]. NOS activity was expressed in nmol L-citrylline/min×mg of protein. NO*x* (nitrite/nitrate) concentration in homogenates of small intestine was assayed by the Griess reaction-dependent method of [8]. In order to determine total (NO_2/NO_3) concentration to deproteinised homogenates (1:100) of zinc for reduction of nitrate to nitrite or manganese sulphate for measurement of nitrate-anion were added. Naphthyl-ethylenediamine was used to perform Griess reaction. The absorbance was read in a Statfax at 520-560 (550) nm. Concentration of stable products of NO was expressed as nitrite+nitrate (mmol/g). Myeloperoxidase (MPO) activity in small intestinal homogenates was assayed spectrophotometrically by the method [9] with some modifications. The MPO activity was analysed spectrophotometrically as follows: 1 ml of homogenate was added to 2.9 ml of 0.1 M K_3PO_4 buffer (pH 6.0) involving O-dianisidine dihydrochloride (0.167 mg/ml) and 0.005 % hydrogen peroxide of the resection mixture was recorded at a wave length of 450 nm. One unit (U) of activity was defined as that degrading 1 μmole of peroxide/mg of protein. Lipid peroxidation levels were determined as malonic dialdehyde (MDA) concentration in homogenates of gastric mucosa, according to the procedure [10]. MDA levels were expressed as mmol/l. Activity of superoxide dismutase (SOD) was determined by the reaction of reduction of nitrotetrazolium blue to nitroformazan [11]. 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 [12]. Catalase activity was expressed in mmol H_2O_2/min×mg of protein. H_2S concentration was determined by reaction with para-phenylenediamine [13].

The statistical processing of the data was done by conventional methods for analysis of variance using MS Excel software for Student's test. The difference was considered to be significant at $p \le 0.05$.

RESULTS AND DISCUSSION. Administration of methotrexate didn't cause any visible changes of small intestine surface. It should be pointed out that methotrexate-treated animals were suffering from severe enterotoxicosis manifested by diarrhoea and vomiting.

Methotrexate-induced enteropathy was accompanied by significant changes of gaseous mediators production manifested by the decrease of H_2S concentration in blood serum by 20 % ($p \le 0.01$) and the increase of NO_x concentration in small intestinal mucosa by 40 % ($p \le 0.01$) (Fig. 1). In recent years it was shown, that hydrogen sulfide plays an impor-

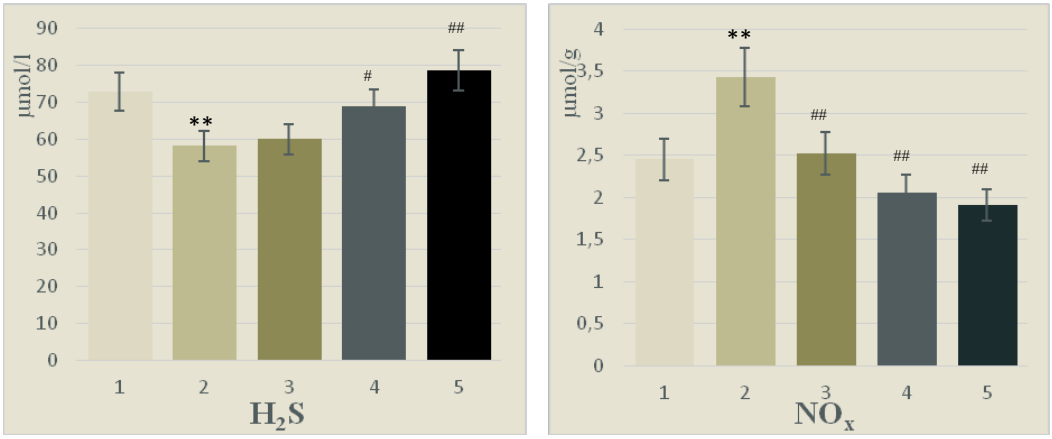

Fig. 1. Concentrations of H_2S in blood serum and stable products of NO in homogenates of small intestinal mucosa at the background of methotrexate-induced enteropathy of rats of the following groups: group 1 – control group, group 2 – methotrexate-induced enteropathy, group 3 – methotrexate +1 mg/kg of NaSH , group 4 – methotrexate+ 10 mg/kg NaSH, group 5 – methotrexate+L-Cys. Mean±SD, n=8 in each group of animals. * – $p \le 0.05$, ** – $p \le 0.01$, in relation to control animals; # – $p \le 0.05$, ## – $p \le 0.01$ as compared to the methotrexate action.

tant role in promoting resolution of inflammation, and restoration of normal tissue function [14], thus the suppression of its synthesis in medication-induced small intestinal injury may play a crucial role for development of enteropathy.

H_2S donor NaHS displayed the tendency to increase of H_2S concentration whereas L-cysteine administration returned it to its normal level. H_2S donors dose-dependently decreased NO_x concentration in correspondence to the existence of metabolic relationship between both gaseous mediators.

Changes of NO_x concentration in group of methotrexate-treated animals were resulted by the increase of iNOS activity (more than fivefold ($p \leq 0.01$) as compared with induced of control group) (Fig. 2). Simultaneously cNOS activity decease by 15 % ($p \leq 0.05$). All studied inhibitors decreased iNOS activity and practically returned it to its normal levels.

Enterotoxic action of methotrexate was accompanied by the development of oxidative stress in small intestine manifested by the rise of MDA concentration by 61 % ($p \leq 0.01$) (Fig. 3) and the significant decrease of antioxidant enzyme SOD (Fig. 4). Simultaneously the activity of MPO was increased by 66 % ($p \leq 0.01$) as compared to the control group which suggests neutrophil infiltration resulting in the development of inflammatory process in small intestine.

None of studied H_2S donors decreased MPO activity as compared to methotrexate action. It should be pointed out that ability of hydrogen sulfide to modulate MPO activity was previously shown [15]. However in our study only H_2S precursor L-cysteine has demonstrated the tendency to de-

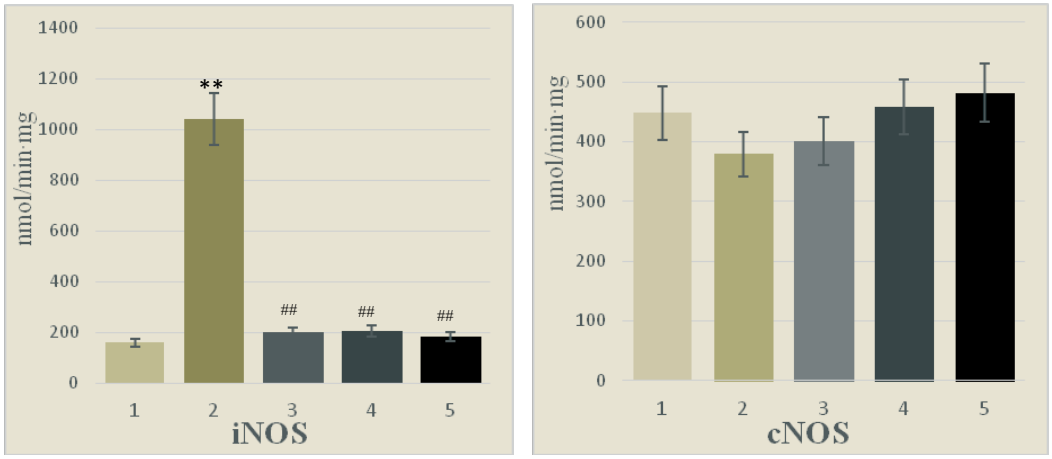

Fig. 2. Activity of nitric oxide synthases (iNOS and cNOS) in homogenates small intestinal mucosa at the background of methotrexate-induced enteropathy of rats of the following groups: group 1 – control group, group 2 – methotrexate-induced enteropathy, group 3 – methotrexate+1 mg/kg of NaSH, group 4 – methotrexate+10 mg/kg NaSH, group 5 – methotrexate+L-Cys. Mean±SD, n=8 in each group of animals. * – $p \leq 0.05$, ** – $p \leq 0.01$, in relation to control animals; # – $p \leq 0.05$, ## – $p \leq 0.01$ as compared to the methotrexate action.

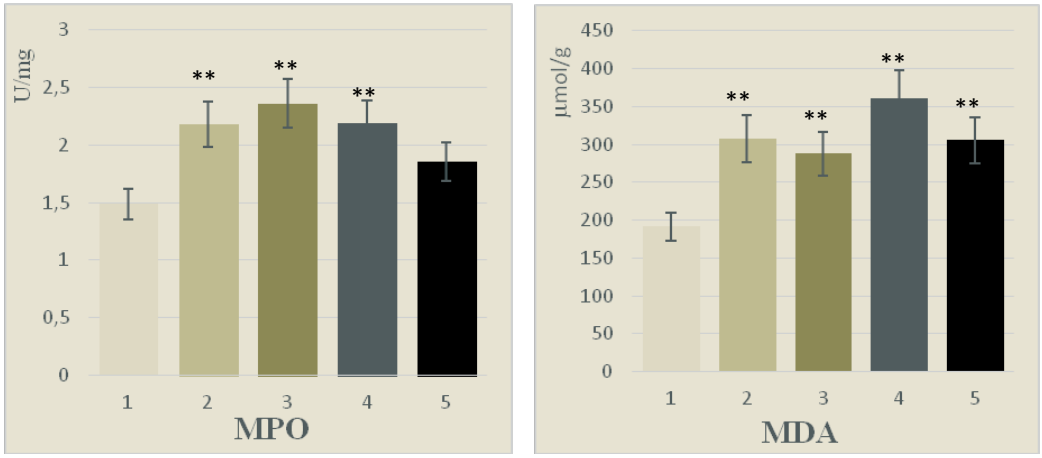

Fig. 3. Activity of MPO and concentration of MDA in homogenates small intestinal mucosa at the background of methotrexate-induced enteropathy of rats of the following groups: group 1 – control group, group 2 – methotrexate-induced enteropathy, group 3 – methotrexate+1 mg/kg of NaSH, group 4 – methotrexate+10 mg/kg NaSH, group 5 – methotrexate + L-Cys. Mean±SD, n=8 in each group of animals. * – $p \leq 0.05$, ** – $p \leq 0.01$, in relation to control animals.

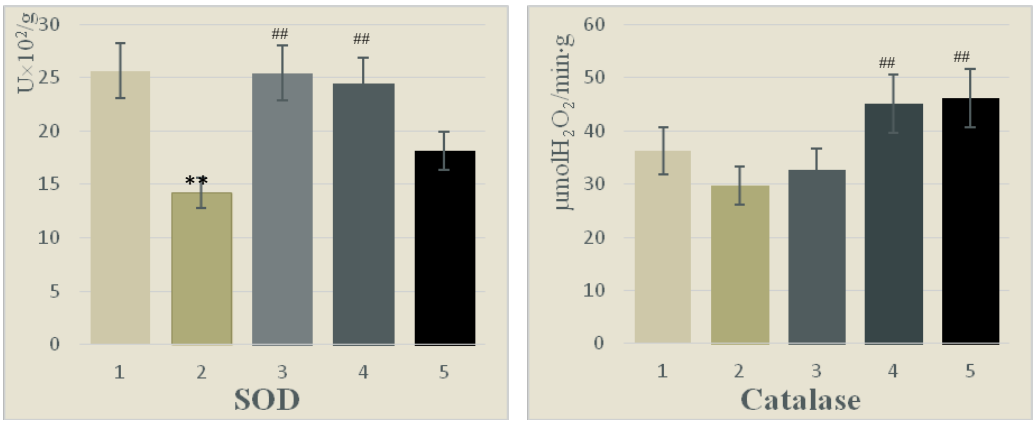

Fig. 4. Activity of SOD and catalase in homogenates small intestinal mucosa at the background of methotrexate-induced enteropathy of rats of the following groups: group 1 – control group, group 2 – methotrexate-induced enteropathy, group 3 – methotrexate+1 mg/kg of NaSH, group 4 – methotrexate+10 mg/kg NaSH, group 5 – methotrexate+L-Cys. Mean±SD, n=8 in each group of animals. * – p≤0.05, ** – p≤0.01, in relation to control animals; # – p≤0.05, ## – p≤0.01 as compared to the methotrexate action.

crease of MPO activity in small intestine of methotrexate-treated rats (Fig. 3).

It is well known that H_2S exhibits strong antioxidant properties. In our study an antioxidant action of H_2S inhibitor NaHS was manifested by the increase of SOD activity as compared to methotrexate group (Fig. 4). Surprisingly, L-Cys didn't demonstrate such action. However all studied donors didn't decrease MDA concentration. Catalase activity significantly increased as a result of 10 mg/kg NaSH and L-Cys action. (Fig.4)

Thus, although methotrexate is widely used in clinics as an anticancer agent, it's utility is limited by its gastrointestinal toxicity which is one of the most serious side effects in its treatment. However, the mechanism of the toxicity has not been completely clarified [16]. On the other hand, the oxidative stress is known to play an important role in various diseases and drug-induced side effects. In our study the administration of methotrexate induced the development of oxidative stress. On the other hand many studies have suggested an important role of nitric oxide in methotrexate-induced injury [17]. Our results allow us to suggest the potential role of the decrease of endogenous H_2S concentration in the mechanisms of methotrexate induced enterotoxicity. Thus, donors of H_2S may significantly regulate both oxidative stress and iNOS activation in methotrexate models. NaHS is commonly used in in vivo and in vitro experiments as a source of H_2S to study the possible physiologic functions of endogenous H_2S. NaHS immediately dissociates and forms the hydrosulfide anion HS^-, which then reacts with H^+ to form H_2S [18]. In our study NaHS displayed the regulatory effect upon iNOS and SOD activity without any significant effect of MDA concentration and MPO activity.

CONCLUSIONS. Nirtoso-oxidative stress plays the key role in small intestine of rats in mechanisms of enteropathy development resulted in methotrexate administration. H_2S donors modulate parameters of NO-synthase system and activity of SOD of methotrexate-treated rats.

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ЛЬВІВСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ ДАНИЛА ГАЛИЦЬКОГО

ВПЛИВ ДОНОРІВ ГІДРОГЕНУ СУЛЬФІДУ НА НІТРОЗО-ОКСИДАТИВНІ ПРОЦЕСИ В ТОНКІЙ КИШЦІ ЩУРІВ ПРИ МЕТОТРЕКСАТІНДУКОВАНІЙ ЕНТЕРОПАТІЇ

Резюме

Вступ. *Серед чинників, що призводять до розвитку ентеропатій, важливе місце посідають медикаментозні, зумовлені прийманням низки фармацевтичних препаратів. Зокрема, виражені ентеротоксичні властивості має протипухлинний препарат "Метотрексат". Потенційними чинниками корекції його токсичного впливу могли б бути донори гідрогену сульфіду (H_2S), оскільки біохімічні зміни в тонкій кишці при різних медикаментозних ентеропатіях проявляються зниженням продукування ендогенного H_2S, що призводить до втрати його цитопротективних властивостей.*

Мета дослідження *– порівнять вплив донорів H_2S на показники NO-синтазної системи та ступінь оксидативного стресу в тонкій кишці щурів на тлі метотрексатіндукованої ентеропатії.*

Методи дослідження. *Досліди виконували на щурах, яким на тлі ентеротоксичної дії метотрексату вводили донори H_2S: NaHS у дозах 1 та 10 мг/кг, L-цистеїн у дозі 30 мг/кг. У слизовій оболонці тонкої кишки визначали активність синтаз оксиду азоту, мієлопероксидази, супероксиддисмутази і каталази; концентрацію стабільних метаболітів оксиду азоту та ТБК-активних продуктів; у сироватці крові – концентрацію H_2S.*

Результати й обговорення. *Введення протипухлинного препарату "Метотрексат", хоч і не спричинювало змін поверхні тонкої кишки, проте призводило до суттєвих біохімічних змін. Зокрема, концентрація оксиду азоту зростала внаслідок активації індуцибельної синтази оксиду азоту (більше ніж у 5 разів, $p \geq 0{,}01$), при цьому спостерігали зниження концентрації H_2S у сироватці крові. Введення донорів H_2S практично повертало ці показники до норми. Метотрексатіндукована ентеропатія зумовлювала підвищення активності мієлопероксидази на 66 % ($p \leq 0{,}01$), що свідчило про формування запального процесу та активацію процесів ліпопероксидації. Введення NaHS не чинило суттєвого впливу на активність мієлопероксидази, проте підвищувало активність супероксиддисмутази, практично повертаючи її до показників норми.*

Висновки. *Ключовою біохімічною зміною, що призводить до формування ентеропатії при введенні протиракового препарату "Метотрексат", слугує нітрозо-оксидативний стрес. Донори H_2S чинять модулюючий вплив на показники NO-синтазної системи та активність супероксиддисмутази.*

КЛЮЧОВІ СЛОВА: **ентеропатія; метотрексат; гідрогену сульфід; синтази оксиду азоту; оксидативний стрес.**

Ю. А. Склярова, И. С. Фоменко
ЛЬВОВСКИЙ НАЦИОНАЛЬНЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ ИМЕНИ ДАНИЛА ГАЛИЦКОГО

ВЛИЯНИЕ ДОНОРОВ СЕРОВОДОРОДА НА НИТРОЗО-ОКСИДАТИВНЫЕ ПРОЦЕССЫ В ТОНКОЙ КИШКЕ КРЫС ПРИ МЕТОТРЕКСАТИНДУЦИРОВАННОЙ ЭНТЕРОПАТИИ

Резюме

Вступление. *Среди факторов, приводящих к развитию энтеропатий, важное место занимают медикаментозные, обусловленные употреблением ряда фармацевтических препаратов. В частности, выраженными энтеротоксическими свойствами владеет противоопухолевый препарат "Метотрексат". Потенциальными факторами коррекции его токсического воздействия могли бы быть доноры сероводорода (H_2S), поскольку биохимические изменения в тонкой кишке при различных медикаментозных энтеропатиях проявляются снижением продуцирования эндогенного H_2S, что приводит к потере его цитопротективных свойств.*

Цель исследования *– сравнить влияние доноров H_2S на показатели NO-синтазной системы и степень оксидативного стресса в тонкой кишке крыс на фоне метотрексатиндуцированной энтеропатии.*

Методы исследования. *Опыты выполняли на крысах, которым на фоне энтеротоксического действия метотрексата вводили доноры H_2S: NaHS в дозах 1 и 10 мг/кг, L-цистеин в дозе 30 мг/кг. В слизистой оболочке тонкой кишки определяли активность синтаз оксида азота, миелопероксидазы, супероксиддисмутазы и каталазы; концентрацию стабильных метаболитов оксида азота и ТБК-активных продуктов; в сыворотке крови – концентрацию H_2S.*

Результаты и обсуждение. *Введение противоопухолевого препарата "Метотрексат", хотя и не вызывало изменений поверхности тонкой кишки, однако приводило к существенным биохимическим изменениям. В частности, концентрация оксида азота возрастала вследствие активации индуцибельной синтазы оксида азота (более чем в 5 раз, $p \geq 0,01$), при этом наблюдали снижение концентрации H_2S в сыворотке крови. Введение доноров H_2S практически возвращало эти показатели к норме. Метотрексатиндуцированная энтеропатия обусловливала повышение активности миелопероксидазы на 66 % ($p \geq 0,01$), что свидетельствовало о формировании воспалительного процесса и активации процессов липопероксидации. Введение NaHS не оказывало существенного влияния на активность миелопероксидазы, однако повышало активность супероксиддисмутазы, практически возвращая ее к показателям нормы.*

Выводы. *Ключевым биохимическим изменением, приводящим к формированию энтеропатии при введении противоопухолевого препарата "Метотрексат", служит нитрозо-оксидативный стресс. Доноры H_2S оказывают модулирующее влияние на показатели NO-синтазной системы и активность супероксиддисмутазы.*

КЛЮЧЕВЫЕ СЛОВА: **энтеропатия; метотрексат; сероводород; синтазы оксида азота; оксидативный стресс.**

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