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DYNAMICS OF CHANGES IN NITROGEN OXIDE DERIVATIVES IN YOUNG PATIENTS WITH GASTROESOPHAGEAL REFLUX DISEASE

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Current research is devoted to studying the role of nitric oxide and NO synthase in ensuring the functioning of the gastrointestinal tract. The work aims to study the importance of the role of nitric oxide in the pathogenesis of gastroesophageal reflux disease to analyze the clinical symptoms and state of endothelial dysfunction of the esophageal mucosa depending on the variant of the disease course by determining the concentration of nitric oxide derivatives and levels of NO-synthase activity. The results obtained by us indicate an increase in the level of inflammatory markers in young patients regardless of the presence of erosive lesions of the esophageal mucosa. Further studies are needed to assess the selective effect of NO-synthase isoforms to determine their role in the management and treatment of patients with gastroesophageal reflux disease at a young age.

Key words: gastroesophageal reflux disease, nitric oxide, NO-synthase, nitrites.

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ДИНАМІКА ЗМІН ПОХІДНИХ ОКСИДУ АЗОТУ У ХВОРИХ НА ГАСТРОЕЗОФАГЕАЛЬНУ РЕФЛЮКСНУ ХВОРОБУ МОЛОДОГО ВІКУ

Сучасні дослідження присвячені вивченню ролі оксиду азоту та NO-синтази у забезпеченні функціонування шлунково-кишкового тракту. Метою роботи було вивчити важливість ролі оксиду азоту в патогенезі гастроезофагеальної рефлюксної хвороби, проаналізувати клінічні симптоми та стан ендотеліальної дисфункції слизової оболонки стравоходу в залежності від варіанту перебігу захворювання шляхом визначення концентрації похідних оксиду азоту та рівнів активності NO-синтаз. Отримані нами результати свідчать про підвищення рівня маркерів запалення у пацієнтів молодого віку незалежно від наявності ерозивного ураження слизової оболонки стравоходу. Необхідні подальші дослідження для оцінки селективного впливу на ізоформи NO-синтаз для визначення їх ролі у веденні та лікуванні хворих на гастроезофагеальну рефлюксну хворобу молодого віку.

Ключові слова: гастроезофагеальна рефлюксна хвороба, оксид азоту, NO-синтаза, нітрити.

The work is a fragment of the research project "Optimization of diagnostics, treatment, and rehabilitation of patients with diseases of internal organs", state registration No. 0124U000096.

Gastroesophageal reflux disease (GERD) is a common chronic disease of the gastrointestinal tract (GI) that affects 10 to 30 % of the world's population and is increasingly diagnosed in young people [11]. In contrast to the past, when researchers believed that the consequences of gastric reflux were caused by the direct effect of acid, today there are assumptions that the complications of reflux are associated with impaired functioning of the immune system and inflammatory processes. An important pathogenetic mechanism of impaired motility and tone of the smooth muscles of the GI tract is endothelial dysfunction, an element of which is NO – an inhibitory non-adrenergic, non-cholinergic neurotransmitter that, in addition to regulating secretion, significantly affects the relaxation and motility of the smooth muscles of the gastrointestinal tract [5, 6].

One of the substances that is involved in this is nitric oxide synthase (NOS). This enzyme produces nitric oxide during the conversion of L-arginine to L-citrulline [3, 11]. Currently, methods for treating many pathological processes of the digestive system are being developed using both stimulants and inhibitors of nitric oxide (NO) synthesis, but their role and effects have not been studied sufficiently. This is primarily due to the rather complex organization of nitric oxide metabolism and the diversity of roles of this molecule in the gastrointestinal tract. According to modern views, pro-inflammatory cytokines induce the expression of iNOS, which leads to hyperproduction of NO, which is subject to oxidation with the formation of aggressive free radicals. The latter have a direct cytotoxic effect on the cells of the esophageal mucosa with the development of its erosive lesions. This mechanism is of great importance in the pathogenesis of erosive forms of GERD, eosinophilic esophagitis, Barrett's esophagus, and erosive-ulcerative lesions of the stomach [2, 10]. At the same time, there are studies that confirm the gastroprotective effect of NO synthesized by constitutive NOS on the damaged mucosa. In the stomach, constitutive isoforms of NOS are localized: neuronal – on the surface of the cells of the gastric mucosa, and endothelial – in the capillary cells in the lower layer of the gastric glands and in the submucosa.

However, there is not enough convincing data that could explain the ambiguous role of NO and iNOS in the inflammatory process of mucous shells in the esophagus in GERD [1, 9]. Since NO has both

cytoprotective and cytotoxic effects on tissues, determination of NO levels is necessary to assess its function and effects on tissues in various diseases [2, 11].

The purpose of the study was to establish the role of nitric oxide system disorders in the formation of non-erosive and erosive forms of gastroesophageal reflux disease in young people.

Materials and methods. 60 young patients with GERD were examined, including 37 (61.7 % men) and 23 (38.3 % women). Patients were treated in the conditions of the Municipal Enterprise "Poltava Regional Clinical Hospital named after M.V. Sklifosovsky of Poltava regional Council". The diagnosis of GERD in patients was established in accordance with the requirements of the unified clinical protocol, approved by the order of the Ministry of Health of Ukraine dated 31.10. 2013 No. 943 [4]. Clinical and endoscopic manifestations of GERD were assessed. The presence and severity of GERD symptoms were analyzed using the GERD-Q scale: 0-2 points correspond to 0 % probability of GERD; 3-7 points – 50 % probability; 8-10 points – 79 % probability; and 11-18 points – 89 % probability of GERD. All patients filled out the GERD-Q questionnaire, which included questions about their well-being over the past 7 days. When processing the results of the questionnaire, the total score of the questionnaire and a separate score for each of the six questions were noted: the presence and frequency of heartburn, regurgitation, epigastric pain, nausea, heartburn at night, and the frequency of taking medications.

All patients underwent upper endoscopy, the results of which were formed into two groups:

Group I (n = 30) – patients with erosive GERD;

Group II (n = 30) – patients with the non-erosive form of GERD.

The average age of patients with erosive GERD in group I was 30.8±7.2 years; the ratio of men to women was 23 (76.7 %) / 7 (23.3 %). The average age of patients in group II with non-erosive GERD was 31.7±7.6 years; the ratio of men to women was 14 (46.7 %) / 16 (23.3 %).

The control group consisted of 15 practically healthy individuals, including 7 (46.7 %) men and 8 (53.3 %) women, aged 24.3±5.25 years. H. pylori status was assessed based on the results of H. pylori antigen detection in stool. The study included patients with H. pylori-negative status.

All patients had NO system parameters determined in blood plasma: activity of total nitric oxide synthase (gNOS), inducible form of NOS (iNOS), neuronal (nNOS), and endothelial (eNOS) forms of NOS.

The obtained data were entered and subjected to statistical processing using Microsoft programs. Excel and GraphPad Prism 10 (GraphPad Software, Inc., San Diego, CA, USA), assuming normal data distribution, the results were presented as arithmetic means (M) and their standard deviation (SD). The significance of differences was calculated using Student's t-test and Mann–Whitney U-test. Differences were considered statistically significant at $p < 0.05$.

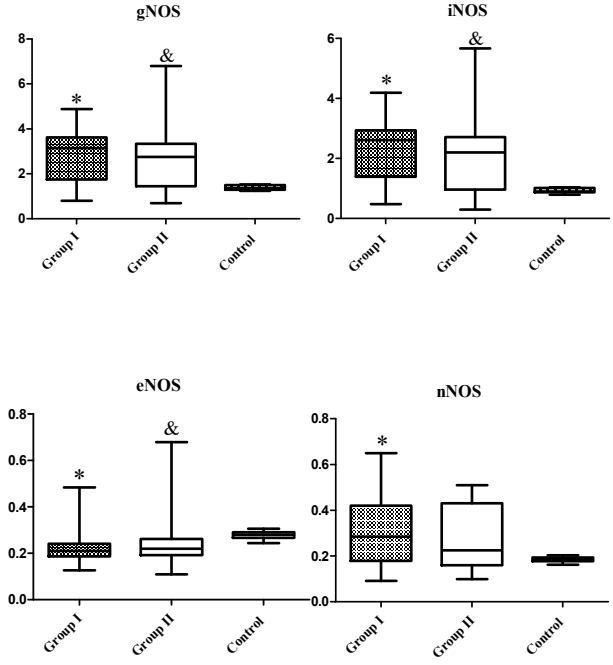

Fig. 1 Activity of NOS isoforms in patients with erosive and non-erosive gastroesophageal reflux disease (nmol/min per g protein). Note: significant differences between indicators: * $p<0.05$ – patients in groups and practically healthy individuals; & $p<0.05$ – patients of group I and practically healthy individuals.

Results of the study and their discussion. According to the results of our study, patients with both erosive and non-erosive GERD had an increase in gNOS activity. Thus, in patients of groups I and II, gNOS activity in blood plasma increased 2 times (2.83±1.08) nmol/min per g protein ($p = 0.0005$) and 1.9 times (2.63±1.35) nmol/min per g protein ($p = 0.003$) compared with practically healthy individuals (1.39±0.1) nmol/min per g protein without significant changes in the comparison groups (Fig. 1).

It is important that the increase in iNOS activity in patients with exacerbation of erosive and non-erosive forms of GERD occurred due to iNOS. Thus, in patients of group I, we observed a 2.5-fold increase in iNOS activity (2.29±0.97 versus 0.92±0.09 nmol/min per g of protein; $p = 0.0002$) in blood plasma compared to practically healthy subjects.

However, according to the results of our study, an increase in iNOS activity in the blood plasma of patients of group II with non-

erosive GERD was recorded by 2.3 times (2.12±1.24 versus 0.92±0.09 nmol/min per g of protein; p = 0.003) compared to the norm. We did not detect significant changes in iNOS activity in the comparison groups depending on the presence or absence of erosive changes in the esophagus. This fact may be of particular importance in understanding the pathogenesis of the non-erosive form of GERD. It can be assumed that the non-erosive form of GERD, under the conditions of its long-term course and the continuation of the action of risk factors, can transform into the erosive form. It should be noted that the study included young patients who did not have severe concomitant pathology that could potentiate the occurrence of erosions in the esophageal mucosa. Therefore, non-erosive and erosive forms of GERD share a common pathogenetic mechanism induced by pro-inflammatory cytokines, namely increased iNOS activity.

NOS was assessed in patients with GERD depending on the presence of erosive changes in the esophagus. It is important that in patients with the erosive form of GERD of group I, the activity of nNOS in blood plasma increased by 2 times ((0.3±0.14) vs. (0.19±0.01) nmol/min per g of protein; p = 0.01) compared to a group of practically healthy individuals. At the same time, in patients with the non-erosive form of GERD in group II, the activity of nNOS in blood plasma did not significantly differ from the norm (0.29±0.14 vs. 0.19±0.01 nmol/min per g of protein; p = 0.06). It should be noted that nitric oxide, which is formed under the influence of nNOS, is an inhibitory neurotransmitter responsible for vasodilation due to nerve stimulation of smooth muscles. The NO released as a result of this process regulates peristalsis and sphincter tone. Thus, according to the results of our study, it is the erosive form of GERD that is accompanied by increased activity of nNOS, which can lead to prolonged relaxation of the lower esophageal sphincter and impaired esophageal motility with deterioration of its clearance. This pathogenetic mechanism causes prolonged acidification of the esophagus with an increased risk of erosive lesions.

According to the results of our study, the activity of eNOS in blood plasma decreased by 1.2 times (0.24±0.09 nmol/min per g protein) in patients of group I with the erosive form of GERD (p = 0.0005) and (0.24±0.1 nmol/min per g protein) in patients of group II with the non-erosive form of GERD (p = 0.0007) versus (0.28±0.02 nmol/min per g protein) in practically healthy individuals. The results of our study indicate a significant role of eNOS in the processes of vasodilation.

Considering that we found a decrease in eNOS activity in patients with both erosive and non-erosive forms of GERD, we can confirm common pathogenetic mechanisms of their development. The presence of the non-erosive form is also accompanied by endothelial dysfunction, which leads to a violation of protective and regenerative mechanisms in the mucosa.

In turn, NO, which is endogenously synthesized by constitutive NOS, relaxes smooth muscle cells, including the lower esophageal sphincter, which leads to a decrease in muscle tone and reflux of acidic gastric contents into the esophagus. Also, NO, produced by constitutive isoforms of NOS, in the presence of an ulcer, exerts such protective effects as maintaining an appropriate level of blood flow (reducing vasoconstriction caused by catecholamines), regulating motility, and activating the synthesis of protective proteins HSP 70.

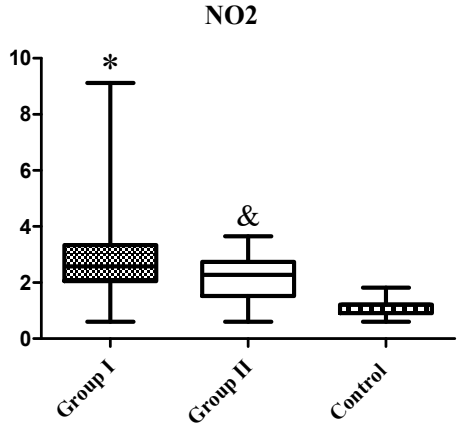

Fig. 2. Nitrite concentration in patients with erosive and non-erosive GERD compared to practically healthy individuals. Note: significant differences between indicators: * p<0.05 – patients in groups and practically healthy individuals; & p<0.05 – patients of group I and practically healthy individuals.

According to the results of our study, the concentration of nitrites ($_{NO_2}$) in the blood plasma of patients in group I increased by 2.5 times (2.86±1.5 nmol/l vs. 1.14±0.3 nmol/l; p < 0.0001) compared to practically healthy individuals. In the non-erosive form of GERD in patients of group II, the content of nitrites ($_{NO_2}$) in the blood plasma increased by 1.9 times (2.16±0.74 nmol/l vs. 1.14±0.3 nmol/l; p<0.0001) compared to the control group. It is important that the indicator of nitrites ($_{NO_2}$) under the conditions of development of erosive changes of the esophagus in patients of group I had a tendency to increase by 1.3 times compared to patients of group II (p>0.05) (Fig. 2).

Thus, the development of GERD is accompanied by disorders of the NO system, which are characterized by increased activity of gNOS mainly due to the isoform iNOS, which can be explained by the activation of inflammatory processes and the production of pro-inflammatory cytokines. The latter activates macrophages

with increased activity of iNOS and, accordingly, the synthesis of NO from L-arginine. Excessive production of NO is accompanied by an increase in the concentration of nitrites ($_{NO_2}$), which was recorded in patients of both study groups. These results are consistent with previous studies [7, 12].

Disturbances in the nitric oxide system play a significant role in the pathogenesis of erosive and non-erosive forms of GERD. Under normal conditions, NO participates in maintaining the protective and reparative properties of the gastroduodenal mucosa. However, excessive production of NO, which occurs under the action of NOS, leads to its oxidation products. NO reacts with superoxide (O_2–), which leads to the formation of peroxynitrite (ONOO–), which belongs to aggressive free radicals. Our results coincide with other studies that demonstrated that the activity of iNOS in the serum of patients with GERD increases in direct proportion to the duration of the disease and the severity of erosive lesions of the esophageal mucosa [1, 2, 10, 11]. However, according to some scientists, it is non-erosive reflux that is accompanied by the maximum increase in iNOS activity in esophageal tissues compared to normal and erosive forms of GERD [9]. This can probably be explained by the fact that in patients with non-erosive GERD, this pathogenetic mechanism plays a significant role in the relaxation of the lower esophageal sphincter.

An important result of our study was the detection of increased nNOS activity in patients with erosive GERD. This fact requires further study to confirm the role of this pathogenetic factor in the formation of erosive lesions of the esophageal mucosa. [5]. Common pathogenetic links in the development of erosive and non-erosive forms of GERD are confirmed by the decrease in eNOS activity in both comparison groups. Our findings indicate a significant role of eNOS in the processes of vasodilation, which is consistent with the data of previous studies [6, 13]. The obtained data also confirm the results of the study by T. Pochinok [7], which established a significant increase in NO_2 in blood plasma and esophageal mucosa in the presence of GERD.

Conclusions

1. In patients with erosive GERD, total NOS activity (gNOS) was almost 2 times higher ($p = 0.0005$) compared to a group of practically healthy individuals, and in patients with non-erosive GERD, it was 1.9 times higher ($p = 0.003$), respectively.

2. In patients with erosive GERD, iNOS activity was 2.5 times higher ($p = 0.0002$), and in patients with non-erosive GERD – 2.3 times higher ($p = 0.003$) compared to practically healthy subjects. At the same time, in patients with erosive GERD, nNOS activity was almost 2 times higher ($p = 0.01$) compared to the group of practically healthy subjects.

3. In both endoscopic variants of GERD, the eNOS activity index was 1.2 times lower than in the control group ($p=0.0005$ in the group with erosive form, $p=0.0007$ in the group with NERD).

4. The concentration of NO_2 in the erosive form of GERD was 2.5 times higher ($p < 0.0001$), and in the non-erosive form, 1.9 times higher ($p < 0.0001$) compared to the control group.

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STUDY OF THE POSSIBILITY OF USING CATGUT AS A CARRIER SUBSTANCE FOR ANTISEPTICS TO PREVENT RECOLONIZATION OF PATHOGENIC BACTERIA

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To achieve high efficiency in the treatment of patients with chronic generalized periodontitis, in recent years, therapeutic agents immobilized on natural or synthetic polymers, which are placed directly in the periodontal pocket, have been used. In this regard, the possibility of using catgut as a carrier substance for antiseptics to prevent recolonization of pathogenic bacteria was assessed. The studies were conducted on standard cultures of Staphylococus aureus, Streptococcus gallolyticus, Streptococcus agalactiae and Porphyromonas gingivalis. It was found that catgut has the ability to be saturated with antiseptic solutions, as evidenced by the long-term antibacterial effect of chlorhexidine and decasan on periodontal microorganisms. This allows us to significantly expand the scope of application of catgut as a stable complex for the transport of antiseptic substances.

Key words: generalized periodontitis, periodontal tabs, catgut, chlorhexidine, decasan.

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ВИВЧЕННЯ МОЖЛИВОСТІ ВИКОРИСТАННЯ КЕТГУТУ ЯК СУБСТАНЦІЇ-НОСІЯ ДЛЯ АНТИСЕПТИКІВ З МЕТОЮ ПОПЕРЕДЖЕННЯ РЕКОЛОНІЗАЦІЇ ПАТОГЕННИХ БАКТЕРІЙ

Для досягнення високої ефективності лікування пацієнтів із хронічним генералізованим пародонтитом в останні роки застосовуються лікувальні засоби, іммобілізовані на природних чи синтетичних полімерах, що розміщуються безпосередньо в пародонтальній кишені. У зв'язку з цим була здійснена оцінка можливості використання кетгуту як субстанції-носія для антисептиків із метою попередження реколонізації патогенних бактерій. Дослідження проводили на стандартних культурах Staphylococus aureus, Streptococcus gallolyticus, Streptococcus agalactiae та Porphyromonas gingivalis. Було встановлено, що кетгут має здатність насичуватись розчинами антисептиків, про що свідчить тривала антибактеріальна дія хлоргексидину та декасану щодо пародонтогенних мікроорганізмів. Саме це дозволяє значно розширити сферу застосування кетгуту як стабільного комплексу для переносу антисептичних речовин.

Ключові слова: генералізований пародонтит, пародонтальні вкладки, кетгут, хлоргексидин, декасан.

The study is a fragment of the research project "Development of pathogenetic prevention of pathological changes in the oral cavity of people with internal diseases", state registration No. 0121U108263.

Despite the significant successes of modern methods of treating periodontitis, and today the high frequency of progression of this inflammatory process and its complications remains an unresolved problem in dentistry [11]. The most important stage of treatment of patients with generalized periodontitis is professional oral hygiene. However, hardware and instrumental treatment of the pocket and root is not able to completely eliminate periodontal pathogens. To achieve high efficiency of scaling, general and local use of agents of different pharmacological groups is necessary, which would have a prolonged antimicrobial and fungicidal effect, not injure the surrounding tissues and promote their regeneration [2, 3].

To prevent re-infection of the pocket for many years, periodontologists have used the systemic administration of high doses of antibiotics to achieve the desired concentration of the drug in the gingival fluid. However, this tactic not only prevented the recolonization of pathogenic bacteria in the pocket, but also led to pathological changes in the gastrointestinal tract, allergic reactions and the emergence of resistant strains of bacteria.