Structural changes of gastric mucosa in patients with intestinal metaplasia and nitrogen oxide hyperproduction

Abstract. Background. The purpose was to analyze structural changes in gastric mucosa and to assess the role of nitric oxide system in these processes in patients with chronic gastritis and intestinal metaplasia. Materials and methods. Morphological study was conducted in 38 patients with chronic gastritis with atrophy, intestinal metaplasia located in the antrum and diffuse intestinal metaplasia. Concentration of nitric oxide was measured in gastric contents and in the blood of all patients. Results. Morphometric study showed a marked decrease (to $(0.12 \pm 0.04)\%$) in the nuclear-cytoplasmic ratio in patients with intestinal metaplasia compared to patients with atrophy, while the ellipticity of the native cell nuclei also decreased with the development of intestinal metaplasia, and in patients without metaplastic changes was equal to $(0.76 \pm 0.04)\%$, for cases of antrum-localized intestinal metaplasia $(0.65 \pm 0.11)\%$, and for the diffuse metaplasia — $(0.41 \pm 0.12)\%$. It was found that in patients with antrum-localized intestinal metaplasia, the level of nitric oxide in gastric juice increased and was 24–65 % higher than the control values. A possible mechanism of gastric mucosa cell death with apoptosis and necrosis under hyperproduction of nitric oxide is considered. Conclusions. The inhibitory effect of nitric oxide on acidic gastric secretion is realized at the expense of its stimulating effect on the activity of cyclooxygenase and the synthesis of prostaglandins.

Keywords: gastric intestinal metaplasia; nitrogen oxide; chronic gastritis; dysplasia

Introduction
Chronic gastritis — the presence of aggressive inflammation in the gastric mucosa (GM), which often results in its destruction, is one of the most common pathologies affecting the upper digestive system. Progressive specific changes in the GM, both functional (acid-forming disorder) and structural (atrophy), are the highest independent risks for gastric cancer known so far [1, 3].

According to the modern international scientific position, atrophy is defined as “loss of the corresponding gland structure”. This definition covers both the “loss” of the natural glands (fibrosis) and the metaplastic changes of the corresponding glands, as a result of which they acquire a characteristic structure and lose the ability to perform their function [1].

All the changes that occur in chronic gastritis include cell death (from the structural breakdown point of view) and the development of oxidative stress [2, 3]. Nitric oxide (NO) is a membrane-permeable gaseous secondary messenger that participates in signal transduction of the central and autonomic nervous systems and regulates many respiratory, genitourinary and gastrointestinal functions [4–7].

In the human stomach, NO and a number of other bioactive nitrogen oxides are formed from precursors that come with food and saliva [8–10, 16]. Hydrochloric acid secreted by the parietal cells of the gastric mucosa is a key catalyst for many reactions involving nitric oxide and its derivatives. The key link here is the protonation of nitrites in the lumen of the stomach and the formation of nitric acid. This acid itself is essentially a nitrosating agent, but can also spontaneously disintegrate with the formation of NO and other nitrogen oxides [11, 12]. In the stomach, due to the transformation of nitrates and nitrites, nitrosation products are formed, which can be transported to the blood and act as a powerful vasodilator, capable of lowering arterial pressure [13, 18].
The biological significance of NO formation and its effect on preserving the integrity of GM remains not yet fully understood, but its role in the regulation of the periodic motility of the stomach is realized through diffusion signal transmission [20]: NO, as a secondary mediator, provides vasodilator effects of the vagus nerve, acts as a neurotransmitter in the peripheral nerves innervating the gastrointestinal tract [20, 21]. However, despite the predominantly positive role of NO, the amount of nitric oxide released in the stomach is critically important. The negative effect of NO begins to occur when its concentration drops sharply or increases, which leads to functional and structural damage to the organ [20].

It should be noted that the literature data on the study of NO functional activity are based mostly on the results of studying the final products of metabolism (nitrites and nitrates) in the blood serum. Meanwhile, these data may depend on a number of factors, in particular, the presence of concomitant pathology of other organs and body systems. The study of the NO levels in gastric juice in diseases of the upper gastrointestinal tract, as a marker for assessing the activity of destructive processes in the GM, is of considerable interest.

The purpose of this study was to analyze structural changes in gastric mucosa and to evaluate the role of nitric oxide system in these processes in patients with chronic gastritis and intestinal metaplasia.

Materials and methods

The present study was undertaken in order to analyze the morphological and biochemical changes in 38 patients with chronic gastritis divided into three groups: group I — atrophy of the GM (n = 6); group II — intestinal metaplasia located in the antrum (n = 12); group III — diffuse intestinal metaplasia (n = 20). The control group consisted of apparently healthy persons.

Structural changes were analyzed in GM biopsies obtained during gastroscopy (2 biopsies from the fundus and antrum of the stomach on lesser and greater curvatures, and 1 biopsy from the incisura angularis). Imprints for bacteriological examination for the presence of helicobacter infection were made in all patients, and in the presence of helicobacter in the biopsy samples of patients from group III. In this group, according to the histological study, hyperplasia of the GM glands was detected in 42.9 % of cases. A morphometric study showed a significant reduction in NCR to (0.12 ± 0.04) % (Fig. 1), with a slight decrease in NCR even in cases where no marked hyperplastic changes were noted in the histological and endoscopic examination.

Another sign of the initial changes in the native structure of the GM is NEC. The accuracy of NEC is slightly higher than that of the NCR, but does not depend on the overall size of the cells, which reduces the margin of error from the orientation of the sections. In our study, the NEC of native cells decreased with the development of IM. Therefore, for group I, it was equal to (0.76 ± 0.04) %, for group II — (0.65 ± 0.11) %, and for group III — (0.41 ± 0.12) % (Fig. 2). Statistical analysis revealed a strong correlation between this parameter and NCR (r = 0.55, p ≤ 0.05). The NEC of metaphasic cells ranged from (0.66 ± 0.01) % to (0.89 ± 0.09) %, regardless of the study group.

Pleomorphism of the nuclei, which is the hallmark of more severe structural changes in the GM, is characterized by significant fluctuations in the mean diameter of the epithelial cell nucleus and the increase in NCR.

The formation of NO in the stomach on lesser and greater curvatures, and 1 biopsy during gastroscopy (2 biopsies from the fundus and antrum of the stomach). Imprints for bacteriological examination for the presence of helicobacter infection were conducted separately for metaphasic cells and for native cells in the zones bordering those affected by intestinal metaplasia.

Changess in the NCR are among the first and most precise signs of intestinal metaplasia and dysplasia in GM. Thus, if NCR of unchanged gastric glands was equal to (0.18 ± 0.02) %, in cells affected by intestinal metaplasia it was significantly increased — to (0.33 ± 0.03) % (p < 0.05). It is important to note that the increase of NCR in metaphasic cells was most often accompanied by its decrease in adjacent areas. This difference was particularly noticeable in the biopsy samples of patients from group III. In this group, according to the histological study, hyperplasia of the GM glands was detected in 42.9 % of cases. A morphometric study showed a significant reduction in NCR to (0.12 ± 0.04) % (Fig. 1), with a slight decrease in NCR even in cases where no marked hyperplastic changes were noted in the histological and endoscopic examination.

The comparative analysis was conducted with the indicators in the control group consisted of apparently healthy persons. The evaluation of the studied indicators was given in accordance with their changes: NO in gastric juice — (14.32 ± 19.48) µmol/l; NO in the blood — (25.0 ± 58.3) µmol/l; cholic acid in gastric juice — (0.000 ± 0.006) mmol/l.

Results and discussion

In order to improve the accuracy of the histological study, the following morphometric indices were calculated on the microphotographs obtained with the help of 1000× imaging lens: nuclear-cytoplasmic ratio (NCR), nuclear ellipticity coefficient (NEC) and the nuclear homogeneity coefficient. Given the structural difference between the native cells of the stomach and intestinal glands, these indices were calculated separately for metaphasic cells and for native cells in the zones bordering those affected by intestinal metaplasia.

The purpose of this study was to analyze structural changes in gastric mucosa and to evaluate the role of nitric oxide system in these processes in patients with chronic gastritis and intestinal metaplasia.

Factors that affect the production of NO and other reactive nitrogen oxides in the stomach include pH, the amount of nitrates, the presence of vitamin C, thioanate, polyphenols, the availability of proteins, thiols, and oxygen pressure. The formation of NO in the stomach is also an acid-dependent process, which can be suppressed by proton pump inhibitors that increase the pH of the stomach [19].

The concentration of NO in the gastric contents was determined by the method of Reinhold and Wilson. The analysis of the results was performed using the methods of descriptive statistics: the calculation of the mean, 95% interval and the standard error in the sample, the result was recorded as M ± m. Comparison of the mean values was performed using parametric methods. To determine the relationship between the two indicators, a correlation analysis was conducted to determine the reliable Pearson correlation coefficients and Spearman rank coefficients.

The comparative analysis was conducted with the indicators in the control group consisted of apparently healthy persons. The evaluation of the studied indicators was given in accordance with their changes: NO in gastric juice — (14.32 ± 19.48) µmol/l; NO in the blood — (25.0 ± 58.3) µmol/l; cholic acid in gastric juice — (0.000 ± 0.006) mmol/l.
In our study, there were no significant differences between the values of NCR in the analyzed groups (Fig. 3a). However, in one case, against the background of intestinal metaplasia, which was characterized by a large area of diffusion (> 75 % of the total biopsy area), but a reduced number of goblet cells, a significant increase in NCR was noted (Fig. 3b) that allowed considering this case as dysplasia of the GM.

NO, the production of which may increase at the expense of Helicobacter life-sustaining activity or decrease due to the development of oxidative stress accompanying IM [3],...
activity of cyclooxygenase and the synthesis of prostaglandins. A possible mechanism of gastric mucosa cell death due to apoptosis and necrosis under conditions of hyperproduction of nitric oxide is considered.

**Conclusions**

Morphometric study showed a significant decrease (to (0.12 ± 0.04) %) in the nuclear-cytoplasmic ratio of patients with intestinal metaplasia compared to patients with atrophy, while the ellipticity of the nuclei of the native cells also decreased with the development of intestinal metaplasia, and in patients without metaplastic changes was equal to (0.76 ± 0.04) %, for cases of antrum-localized intestinal metaplasia — (0.65 ± 0.11) %, and for the diffuse metaplasia — (0.41 ± 0.12) %. It was found that in patients with antrum-localized intestinal metaplasia, the level of nitric oxide in gastric juice increased and was 24–65 % higher than the control values. It is assumed that the inhibitory effect of nitric oxide on acidic gastric secretion is realized at the expense of the stimulating effect of nitric oxide on the activity of cycooxygenase and the synthesis of prostaglandins.

**References**


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**Table 1 — Mean value of nitric oxide in the blood and gastric juice for study groups**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.75 ± 0.25</td>
<td>4.95 ± 1.36*</td>
<td>2.94 ± 0.51</td>
<td>4.66 ± 0.63*</td>
</tr>
<tr>
<td>Cholic acid, mmol/l</td>
<td>0.41 ± 0.15</td>
<td>0.50 ± 0.40</td>
<td>0.30 ± 0.09</td>
<td>0.52 ± 0.27</td>
</tr>
<tr>
<td>NO in gastric juice, µmol/l</td>
<td>74.76 ± 0.76</td>
<td>73.20 ± 0.07</td>
<td>86.84 ± 9.00</td>
<td>73.03 ± 10.62</td>
</tr>
<tr>
<td>NO in the blood, µmol/l</td>
<td>20.82 ± 0.72</td>
<td>26.20 ± 1.27</td>
<td>23.80 ± 1.00</td>
<td>22.16 ± 1.48</td>
</tr>
</tbody>
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Note. * — р < 0.05 — likelihood of changes between parameters in patients compared to the control group.
Структурні зміни слизової оболонки шлунка в пацієнтів із кишковою метаплазією та гіперпродукцією оксиду азоту

Резюме. Мета: проаналізувати структурні зміни слизової оболонки шлунка та оцінити роль системи оксиду азоту в цих процесах у пацієнтів із хронічним гастритом та кишковою метаплазією. Матеріали та методи. Морфологічне дослідження проводилося в 38 пацієнтів із хронічним гастритом та атрофією, морфометричне дослідження — в 100 пацієнтів.

Результати. Концентрація оксиду азоту була вищою в умовах гіперпродукції оксиду азоту в шлунковому соку збільшується і на 24–65 % перевищує контрольну величину. Розглянуто можливий механізм захисту клітин слизової оболонки шлунка від некрозу за рахунок його стимулюючого впливу на активність циклооксигенази та синтез простагландинів.

Ключові слова: кишкова метаплазія шлунка; оксид азоту; хронічний гастрит; дисплазія

Структурні зміни слизової оболонки шлунка у пацієнтів із кишковою метаплазією та гіперпродукцією оксиду азоту

Резюме. Целью являлося проанализировать структурные изменения слизистой оболочки желудка и оценить роль системы оксида азота в этих процессах у пациентов с хроническим гастритом и кишковой метаплазией. Материалы и методы. Морфологическое исследование проводилось у 38 пациентов с хроническим гастритом и кишевебам метаплазией. Морфометрическое исследование проводилось у 100 пациентов.

Результаты. Концентрация оксида азота была высока в условиях гиперпродукции оксида азота в желудочном соке и на 24–65 % превышала контрольную величину. Рассмотрены возможные механизмы защиты клеток слизистой оболочки желудка за счет его стимулирующего влияния на активность циклооксигеназы и синтез простагландинов.

Ключевые слова: кишевебанная метаплазия; оксид азота; хронический гастрит; дисплазия