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EFFECT OF LONG-TERM USE OF PROTON PUMP INHIBITORS ON THE CONTENT OF NITRITE IONS IN THE BLOOD SERUM AND MUCOUS COATING OF THE GASTROINTESTINAL TRACT OF RATS

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The purpose of the study was to determine the content of nitrite ions in blood serum and mucous membranes of the stomach and colon in rats under the conditions of long-term use of proton pump inhibitors Omeprazole and Pantoprazole. The study was performed on 30 white non-linear male rats, divided into three groups. The control (first group) received water for injections intraperitoneally once a day for 28 days. The second – Omeprazole. The third – Pantoprazole. After long-term administration of omeprazole and pantoprazole, the concentration of NO^{2-} in blood serum increased by 24 % ($p<0.05$) and 13 % compared to the control group. The increase in the concentration of NO^{2-} in the mucous membranes of the stomach and colon after 28-day suppression of HCl secretion in the stomach by Omeprazole and Pantoprazole was much more pronounced and amounted in the stomach to 144 % ($p<0.05$) and 85 % ($p<0.05$) more compared to the control group. In the mucous membrane of the large intestine, it was 159 % ($p<0.05$) and 119 % more than in the control group. Long-term inhibition of hydrochloric acid secretion in the stomach of rats by proton pump blockers Omeprazole and Pantoprazole caused excessive generation of nitric oxide in blood serum and mucous membranes of the digestive tract of rats. The negative effect of pantoprazole was less pronounced than that of omeprazole.

Key words: hypochlorhydria, pantoprazole, omeprazole, inflammatory process.

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ВПЛИВ ТРИВАЛОГО ЗАСТОСУВАННЯ ІНГІБІТОРІВ ПРОТОННОЇ ПОМПИ НА ВМІСТ НІТРИТ-ІОНІВ У СИРОВАТЦІ КРОВІ ТА СЛИЗОВІЙ ОБОЛОНЦІ ШЛУНКОВО-КИШКОВОГО ТРАКТУ ЩУРІВ

Метою дослідження було визначити вміст нітрит-іонів у сироватці крові та слизових оболонках шлунку та товстої кишки щурів за умов тривалого застосування інгібіторів протонної помпи омепразолу та пантопразолу. Дослідження проводили на 30 білих нелінійних щурах-самцях, розділених на три групи. Контроль (перша група) отримував воду для ін'єкцій внутрішньочеревно 1 раз на добу протягом 28 днів. Другий – Омепразол. Третій – Пантопразол. Після тривалого застосування омепразолу та пантопразолу концентрація NO2- у сироватці крові зросла на 24 % ($p<0,05$) та на 13 % порівняно з контрольною групою. Підвищення концентрації NO2- у слизових оболонках шлунка та товстої кишки після 28-денного пригнічення секреції HCl у шлунку омепразолом та пантопразолом було значно більш вираженим і становило у шлунку 144 % ($p<0,05$) та на 85 % ($p<0,05$) більше порівняно з контрольною групою. У слизовій оболонці товстого кишечника він становив на 159 % ($p<0,05$) і на 119 % більше, ніж у контрольній групі. Тривале пригнічення секреції соляної кислоти в шлунку щурів блокаторами протонної помпи омепразолом і пантопразолом викликало надмірне утворення оксиду азоту в сироватці крові та слизових оболонках травного тракту щурів. Негативний ефект пантопразолу був менш вираженим, ніж омепразолу.

Ключові слова: гіпохлоргідрія, пантопразол, омепразол, запальний процес.

The work is a fragment of the research project "The role of TRPV-4 receptors in the regulation of the gastro-intestinal tract", state registration No. 0118U004306.

Proton pump blockers contribute to bacterial overgrowth in every part of the digestive tract [3], as well as hypergastrinemia [9].

Prolonged hypergastrinemia and bacterial imbalance disrupt digestive motility, adversely affecting the ability to evacuate through the intestines [4, 10]. This leads to the development of a chronic inflammatory process in the intestines due to conflicting motor functions and bacterial imbalance [2].

There is ample evidence that nitric oxide plays a key role in the digestive system [1]. This includes data collected in our current study, in which we found that NO levels are increased in the gastric and colonic mucosa of rats. This change in NO levels can lead to relaxation of the muscles of the digestive tract wall [7]. When NOS-null mice were fed a high-fat diet, their digestive tracts developed gastromegaly, suggesting the importance of NOS in the regulation of digestion. Morphological studies of the stomachs of various animals showed several observations. First of all, contractile changes in smooth muscle tissue and hypertrophy of the pyloric section were observed in the stomach of these animals. It has been suggested that NOS deficiency leads to pyloric stenosis in humans. This was later proven when researchers observed people with pyloric stenosis and found that they lacked intramuscular NOS.

So, after 28 days of taking omeprazole and pantoprazole [7, 10], the colon and stomach no longer functioned normally. This may be due to an increase in the level of nitric oxide in the digestive tract. Therefore, it was necessary to study this phenomenon on rats.

The purpose of the study was to determine the content of nitrite ions in blood serum and mucous membranes of the stomach and colon in rats under the conditions of long-term use of proton pump inhibitors Omeprazole and Pantoprazole.

Materials and methods. The study was carried out on 30 white non-linear male rats weighing 160 to 180 grams. Each rat was placed in one of three groups of 10 individuals. The research was conducted in accordance with international and national recommendations for conducting medical and biological researches.

For the first group of experiments, control rats received intraperitoneally 0.2 ml of water for injections. Rats of the second group were administered omeprazole at a dose of 14 mg/kg (manufactured by Sigma-Aldrich, USA), which was mixed with 0.2 ml of injection solution. The third group received 0.57 mg/kg pantoprazole ("Ulsepan" manufactured by World Medicine, Great Britain) once a day for 28 days. It was mixed with 0.2 ml of water for injections and administered intraperitoneally.

Determination of the content of nitrite ions in blood serum

Using the Griess method with modifications for the analysis of nitrite ions in blood serum, 200 µl of 4 % NaOH solution was added to 200 µl of serum. Then, 400 µl of distilled water and 1.2 ml of 4 % ZnSO4 were added to the mixture. The mixture was cooled for 10 minutes before centrifugation for 20 minutes at 0°C to +4°C at 15,000 rpm. 1.4 ml of the supernatant was taken, 1.4 ml of Gries's reagent was added, consisting of a 1:1 mixture of 0.1 % solution of α-naphthylethylenediamine in 5 % orthophosphoric acid and 1 % solution of sulfanilic acid in 5 % orthophosphoric acid. The sample was incubated for 10-15 minutes in a dark place. Extinction was measured at 550 nm. The $NO2^-$ content was determined according to the calibration graph, which was constructed using different concentrations of $NaNO_2$.

Determination of the content of nitrite ions in the mucous membranes of the stomach and colon.

The content of nitrite ions was determined according to the Griess method [9] with modifications.

1.8 ml of distilled water, 0.2 ml of a 1 % solution of sulfanilic acid in a 5 % solution of phosphoric acid was added to 0.2 ml of homogenate of cells and mucous membranes. It was left for 7 minutes in a dark place at room temperature. 0.2 ml of a 1 % aqueous solution of alpha-naphthylethylenediamine was added, mixed, left in a dark place at room temperature for 10 minutes. After that, the extinction was measured on a spectrophotometer at a wavelength of 539 nm. The $NO2^-$ content was determined according to the calibration graph, which was constructed using different concentrations of $NaNO_2$.

For each of the samples, it was checked whether the distribution of the studied indicator was normal, using the Shapiro-Wilk W test. According to this criterion, it was determined that if the distribution of sample data did not correspond to the Gauss distribution, then the Mann-Whitney U-test was used to compare samples when comparing two independent samples, and the Wilcoxon test was used to compare dependent samples. The obtained data are presented as a median with a range (from 25 % to 75 %) [2].

Given the normal distribution of the studied indicator, the significance of the difference in indicators was assessed using the Student's t-distribution. The mean (M) and standard error of the mean (m) were calculated. The difference was considered statistically significant at $p<0.05$.

The conclusion on the compliance of experimental studies with generally accepted bioethical norms in compliance with relevant international provisions was approved at the meeting of the bioethical commission of the Poltava V. G. Korolenko National Pedagogical University.

Results of the study and their discussion. The results of the study showed that the concentration of nitrite ions (NO2-) in the blood serum of control rats was 4.64±0.42 nmol/mg of protein (Fig. 1).

One day after the last dose of omeprazole and pantoprazole, serum NO2- concentrations increased by 24 % ($p<0.05$) and by 13 % compared to control groups. After omeprazole and pantoprazole inhibited

gastric HCl secretion for 28 days, the increase in NO2- concentration in gastric and colon mucosa was more pronounced, reaching 144 % ($p<0.05$) and 85 % ($p<0.05$) in the stomach) more than the control group. (Fig. 2).

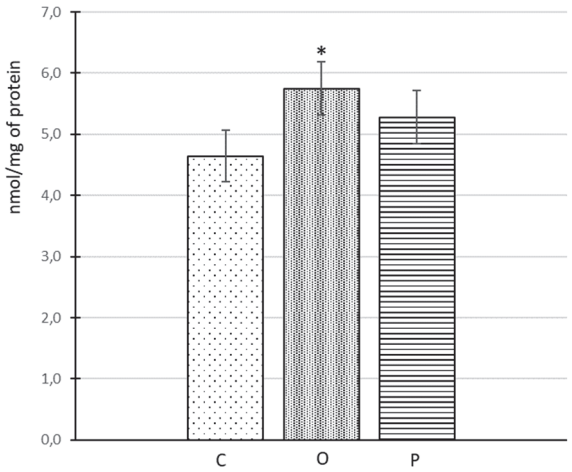

Fig. 1. Nitrite-ions content in blood serum of rats, (M+m)

Notes: K – control group; O – a group of rats that were injected Omeprazole for 28 days; P – a group of rats that were injected with Pantoprazole for 28 days. * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ compared to the control; # – $p<0.05$, ## – $p<0.01$ compared to the group of animals that were injected with omeprazole.

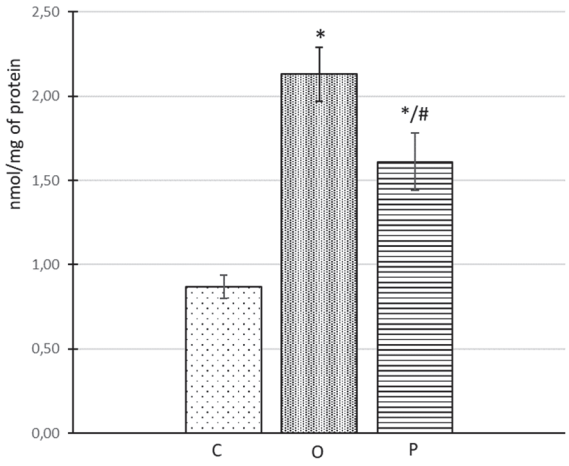

Fig. 2. Nitrite-ions content in the gastric mucosa of rats, (M+m)

Notes: K – control group; O – a group of rats that were administered Omeprazole for 28 days; P – a group of rats that were injected with Pantoprazole for 28 days. * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ compared to the control; # – $p<0.05$, ## – $p<0.01$ compared to the group of animals that were injected with omeprazole.

In the mucous membrane of the large intestine, they were 159 % ($p<0.05$) and 119 % higher than the control group (Fig. 3), respectively.

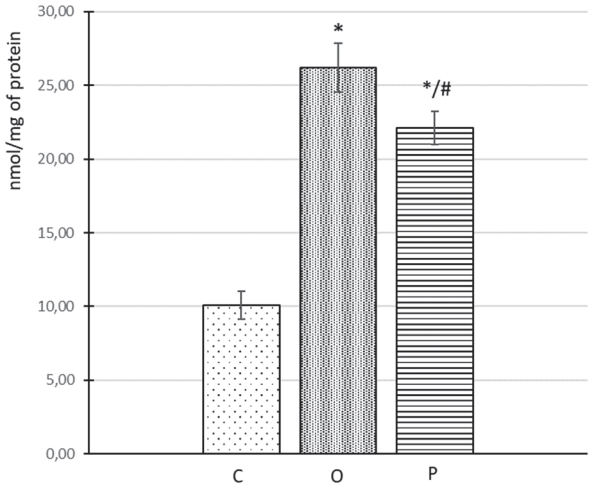

Fig. 3. Nitrite-ions content in the mucous membrane of the colon of rats, (M+m)

Notes: K – control group; O – a group of rats that were administered Omeprazole for 28 days; P – a group of rats that were injected with Pantoprazole for 28 days. * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ compared to the control; # – $p<0.05$, ## – $p<0.01$ compared to the group of animals that were injected with omeprazole.

Such an increase in the concentration of NO2- in the mucous membrane of the gastrointestinal tract indicates the development of an inflammatory process in the mucous membrane of the gastrointestinal tract, which is primarily the result of dysbacteriosis, which occurs against the background of the effect of prolonged inhibition of HCl. allocation in the stomach [7].

The lowest was the increase in the concentration of NO2- in blood serum. This can be explained by the fact that NO, which is synthesized during inflammatory processes, performs its biological function in tissues, precisely in those tissues where the inflammatory process develops. In our case, it is the mucous membrane of the digestive tract, where NO affects cellular structures [6].

The increase in NO2 synthesis is a consequence of increased NOS activity. It is known that bacterial products in the gastrointestinal tract stimulate iNOS activity in immunocompetent, epithelial, and other cells [6].

Unlike constitutive NOS isoforms, iNOS does not require elevated Ca2+ concentrations to initiate its activity [3]. Induction of iNOS in immune cells, endothelial cells, smooth muscle cells, epithelial cells and other cells can be initiated by inflammatory cytokines – IFNγ, TNFα or IL-1. It was shown that IFNγ leads to the expression of the iNos gene in intestinal epithelial cells. The enzyme encoded by iNOS catalyzes the formation of NO from L-arginine. In addition, some bacteria are able to form NO from nitrogenous products.

In fact, we observed something similar in previous experiments: in the context of dysbacteriosis and hypergastrinemia caused by 28 days of omeprazole and pantoprazole, we found that pro-inflammatory cells Factors IFNγ, TNFα, IL-1 in the serum. This leads to an increase in iNOS activity in

the gastric and colonic mucosa, with a subsequent increase in serum and nitrite ion levels in the gastric and colonic mucosa.

Considering that NO can inhibit the secretion of HCl acid in the stomach and stimulate the synthesis of gastrin, we hypothesized that the extremely pronounced hypergastrinemia after 28 days of omeprazole taking was the result of two factors: the first is the direct effect of omeprazole and pantoprazole (blocking of H +-K+-ATPases, high pH in the stomach, stimulation of pH-sensitive receptors on gastrin cells, gastrin secretion), with further mediation of gastrin secretion by omeprazole and pantoprazole by increasing NO production due to dysbacteriosis at high pH in the stomach.

NO is involved not only in the regulation of immune reactions and the development of inflammation, it also takes part in the regulation of many physiological functions and the pathogenesis of many diseases, including the digestive tract. It has been shown that nitric oxide inhibits the contraction of the smooth muscles of the stomach. Suppression of gastric motility by nitric oxide is accompanied by relaxation of the lower esophageal sphincter, which, in our opinion, will contribute to the colonization of the stomach by bacteria of oropharyngeal origin.

In conclusion, nitric oxide (NO) plays a multifaceted role in the body, extending beyond its involvement in immune regulation and inflammation. It has been demonstrated to influence various physiological functions and contribute to the pathogenesis of numerous diseases, including those affecting the digestive tract. Specifically, NO has been found to exert inhibitory effects on the contraction of smooth muscles in the stomach, a phenomenon supported by existing research.

One notable consequence of this inhibitory effect is the relaxation of the lower esophageal sphincter (LES), a critical muscular structure that separates the esophagus from the stomach. The relaxation of the LES due to nitric oxide may have significant implications. It is hypothesized that this relaxation could facilitate the colonization of the stomach by bacteria originating from the oropharynx. This observation underscores the intricate interplay between NO and gastrointestinal physiology, highlighting its potential role in influencing microbial colonization within the digestive tract.

The broader understanding of nitric oxide's involvement in digestive processes and its influence on the lower esophageal sphincter may have implications for our understanding of conditions related to gastric motility and microbial populations in the stomach. Further research is warranted to elucidate the precise mechanisms and clinical significance of these interactions in the context of digestive health and disease.

Conclusions

1. The proton pump blocker omeprazole inhibits the secretion of gastric hydrochloric acid in rats for a long time, which leads to an increase in the production of nitric oxide in the blood serum and mucous membrane of the digestive tract of rats compared to the control group.

2. Proton pump blocker pantoprazole inhibits the secretion of gastric hydrochloric acid in rats for a long time, and also increases the production of nitric oxide in the blood serum and mucous membrane of the digestive tract of rats, but the statistical significance is less than when using omeprazole.

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