ORIGINAL ARTICLE

INFLUENCE OF THE DURABLE APPLICATION OF PROTONIC PUMP INHIBITORS ON CYTOKINE CONCENTRATION IN RAT BLOOD SERUM

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ABSTRACT

The aim: Of the work was to determine the content of pro- and anti-inflammatory cytokines in the blood serum of the control group rats and after 28 days of inhibiting HCl secretion in the stomach by proton pump blockers "Omeprazole" and "Pantoprazole".

Materials and methods: The studies were performed on 30 white non-linear male rats weighing 160-180 g, divided into three groups with 10 animals in each. The control (group 1) were injected intraperitoneally with water for injections within 28 days once a day. Group 2 was administered omeprazole. Group 3 was administered pantoprazole. The concentration of cytokines in the blood serum of rats was determined by the enzyme immunoassay method. For statistic data processing, Student's t-criterion for independent samples was applied.

Results: After prolonged administration of omeprazole and pantoprazole, the blood serum concentrations of IFNγ, TNFα, IL-1 in rats increased by 58.5% and 3.41%, 73.3% and 48.4%, 80.2% and 40.8%, respectively, and IL-12B 40p decreased by 36.6% when using omeprazole and was almost indistinguishable from the control values when pantoprazole was administered. With administration of omeprazole, IL-4 concentration decreased by 39.8% and that of pantoprazole increased by 3.86% compared to the control. Administration of omeprazole and pantoprazole did not affect IL-6 concentration.

Conclusion: Inhibition of hydrochloric acid secretion in the stomach of rats for 28 days using omeprazole and pantoprazole led to an imbalance between pro- and anti-inflammatory cytokines. The adverse effect of pantoprazole was less pronounced than that of omeprazole.

KEY WORDS: hypochlorhydria, pantoprazole, omeprazole

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INTRODUCTION

An important function of gastric juice is inactivation of ingested microorganisms [1], reducing the secretion of hydrochloric acid (HCl) in the stomach contributes to infectious diseases caused by various infectious agents [2].

An increase in intragastric pH over 4 for any reason leads to excessive bacterial growth in all parts of the digestive tract [3, 4, 5], and hypergastrinemia. Prolonged hypergastrinemia and dysbiosis interfere with the motor-evacuation function of the digestive tract [6, 7, 8], which enhances the development of chronic inflammatory process and plays a decisive role in the development of tumors [9]. Both hypergastrinemia and dysbiosis can develop due to long-term administration of antisecretory drugs (proton pump inhibitors) [10, 11]. Acid-dependent diseases often require prolonged administration of proton pump inhibitors, which inhibit the protective action of hydrochloric acid. [6, 7, 8].

Chronic inflammation is at the heart of gastric and neoplastic diseases of the stomach as well as inflammatory bowel diseases. The correlation between *H. pylori* infection and cytokines has been well studied [12, 13]. Levels of IL-1β, IL-6, IL-8, TNF-α and IFN-γ grow in the gastric mucosa with *H. pylori* infection [14]. Against this background, the negative feedback between gastrin secretion and the pH of gastric contents is broken, and acid secretion begins to retard due to the action of the pro-inflammatory cytokines IL-1β and TNFα [15, 16].

IL-1β is by 100 times more potent than proton pump inhibitors, and by 6,000 times more potent than H2-histamine receptor blockers, it inhibits the hydrochloric acid secretion in the stomach [17]. TNFα and IL-1β are necessary to initiate chronic inflammation. This indicates that human gastric epithelial cells that promote the induction of proinflammatory cytokines after the action of *H. pylori* urease are involved in the mucosal inflammatory process which is accompanied by *H. pylori* infection [18]. Concerning the role of cytokines in the inflammatory process in the stomach and intestine, which develops against the background of prolonged gastric juice hypoacidity in the absence of *H. pylori* infection, the literature sources are limited and apply only to individual cytokines.

THE AIM

The aim of the work was to determine the content of pro- and anti-inflammatory cytokines in the blood serum of

the control group rats and after 28 days of inhibiting HCl secretion in the stomach by proton pump blockers "Omeprazole" and "Pantoprazole".

MATERIALS AND METHODS

The studies were performed on 30 white non-linear male rats weighing 160-180 g, which were randomly divided into three groups of 10 animals in each. Animals were handled and kept in a vivarium in compliance with international guidelines and national legislation on biomedical research.

Controls (group 1) were rats that were injected intraperitoneally intravenously (iv) with 0.2 ml of water for injection over 28 days. Group 2 rats were administered once a day for 28 days omeprazole 14 mg / kg (manufactured by "Sigma-Aldrich", USA), which was dissolved in 0.2 ml of water for injection. Rats of group 3 were administered pantoprazole at the dose of 0.57 mg / kg once a day for 28 days ("Ulsepan" produced by "World Medicine", UK) dissolved in 0.2 ml of water for injection.

The concentration of IFN-γ, TNF-α, IL in the blood serum of rats was determined by enzyme-linked immunosorbent assay using commercial kits ("GE Healthcare: Amersham", UK) [19]. The immunological plates (96 wells), which were pre-immobilized with antibodies to these cytokines, were filled with 50 μl of the blood serum sample and, in parallel, 50 μl of the respective cytokine recombinant standard was added to individual wells to construct a calibration graph. A standard solvent containing 0.1% sodium azide was then added to all wells and incubated at 25 ° C for 1 h.

After incubation, the wells were washed 3 times with washing buffer, antibodies labeled with biotin (100 μl in each well) were added to each well and incubated at 25 ° C for 30 min. Next, the wells were washed 3 times with washing buffer, with 100 μl of horseradish streptavidin peroxidase solution and incubated at 25 ° C for 30 min. Then the wells were washed 3 times with the buffer, the amount of 100 μl of tetramethylbenzidine-substrate was added to each well and incubated in the dark at 25 ° C for 30 min. After incubation, the reaction was stopped by introducing 100 μl of stop reagent containing 0.18 M sulfuric acid into each well and the absorption was measured with the reader (ELx800, Bio-Tek Instruments, USA) at 450 and 550 nm.

The calculation took into account the correction for the optical error of the plate by subtracting the values at 550 nm from the value at 450 nm. The cytokine concentration was calculated according to a calibration graph constructed according to the recombinant standard. All samples were analyzed in triplicate. The concentration of cytokines was expressed in pg / ml of the blood serum.

For each sample, the distribution of the studied index was checked using the Shapiro-Wilk W test. According to this criterion, it was determined that if the distribution of the sample data did not correspond to the Gaussian distribution, then a nonparametric method was used to compare the samples – the Mann-Whitney rank groups U-test for comparison of two independent samples and the Wilcoxon test for the dependent ones. In this case, the received data is presented as Median; (25% ... 75%) [20].

In the normal distribution of the studied index, the significance of the indices difference was assessed using the Student's t-test. The mean (M) and the standard error of the mean (m) were calculated. The difference at $p < 0.05$ was considered statistically significant [21].

The conclusion about the conformity of the experimental studies with the generally accepted bioethical standards with the observance of the relevant international regulations was approved at the meeting of the Bioethical Commission at the Poltava V.G. Korolenko National Pedagogical University.

RESULTS

Studies have shown that, after 28 days of omeprazole administration, the concentration of proinflammatory cytokine IFN-γ in the rat blood serum increased by 58.5% ($p < 0.05$). With the continuous administration of pantoprazole, the concentration of IFN-γ in the blood serum of rats was by 55.1% ($p < 0.05$) lower than that in rats administered omeprazole and it did not statistically significantly different from that in the control group rats (fig. 1).

IFN-γ is not only a mediator of macrophage activation. It enhances phagocytosis and production of other proinflammatory cytokines, influences neuropeptide secretion [22]. In this regard, we also determined the concentration of other cytokines in the blood serum.

Concentration of other proinflammatory cytokines also increased – TNF-α with the introduction of omeprazole. This increase made 73.3% ($p < 0.05$).

With prolonged administration of pantoprazole, the concentration of TNF-α in the rats' blood serum was lower by 24.9% ($p < 0.05$) compared to the group of rats receiving omeprazole for the same time. Compared to the control group, rats treated with pantoprazole for 28 days, they had a higher by 48.4% ($p < 0.05$) TNF-α concentration in the blood serum (fig. 2).

It was found that after 28 days of administering omeprazole to rats, the concentration of the proinflammatory cytokine IL-1β increased by 80.2% ($p < 0.05$) compared to the control.

When pantoprazole administered to rats, the blood serum IL-1β concentration was lower by 39.4% ($p < 0.05$) compared to the rats administered omeprazole. However, it remained by 40.8% ($p < 0.05$) higher in comparison with the concentration of IL-1β in the blood serum of the control group rats (fig. 3).

Since the proinflammatory cytokines IFN-г, TNF-б, IL-1в play a key role in protecting the body against pathogens [23], we can argue that the increase in the concentration of these cytokines in the blood serum with administration of omeprazole testifies to a rapid development of inflammation in the intestinal tract mucosa as a response to mucosal contamination with pathogenic and opportunistic microflora. Pantoprazole, due to its slow activation, contributes less to dysbiosis and inflammation.

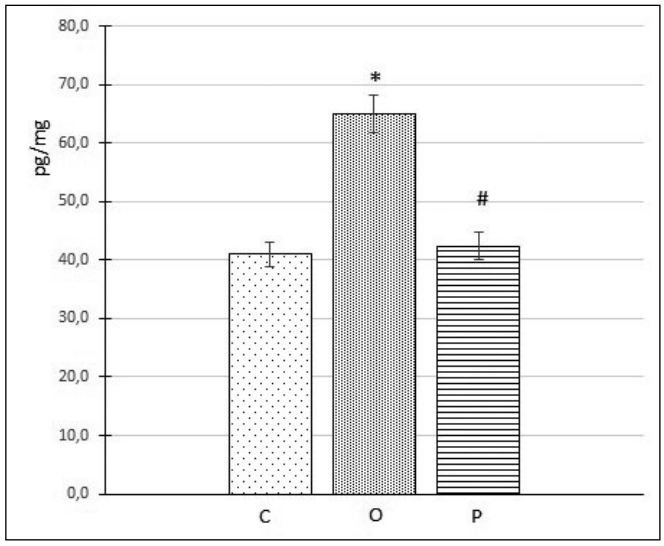

Fig. 1. Concentration of IFN-γ in the blood serum after 28 days of omeprazole and pantoprazole administration, M±m (in each group n = 10)
Notes: C – control group; O – group of rats administered Omeprazole for 28 days; P – group of rats administered Pantoprazole for 28 days.
* – p <0.05, ** – p <0.01, *** – p <0.001 compared to the control; # – p <0.05, ## – p <0.01 compared to the group of animals administered omeprazole.

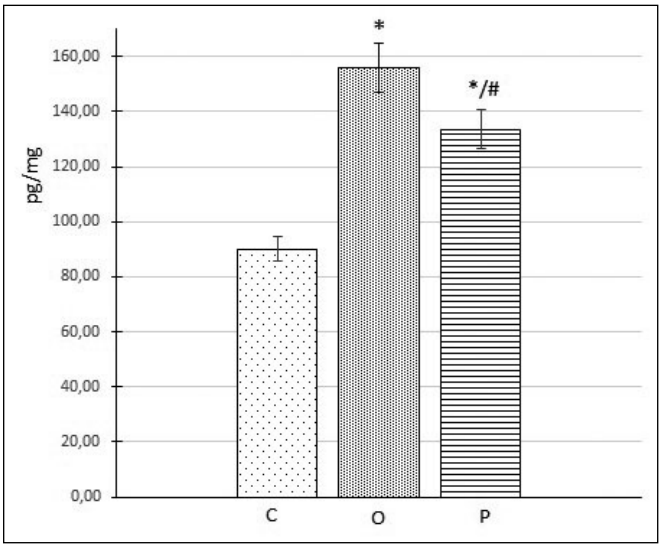

Fig. 2. TNF-α concentration in the blood serum after 28 days of omeprazole and pantoprazole administration, M±m (in each group n = 10)
Notes: C – control group; O – group of rats administered Omeprazole for 28 days; P – group of rats administered Pantoprazole for 28 days.
* – p <0.05, ** – p <0.01, *** – p <0.001 compared to the control; # – p <0.05, ## – p <0.01 compared to the group of animals administered omeprazole.

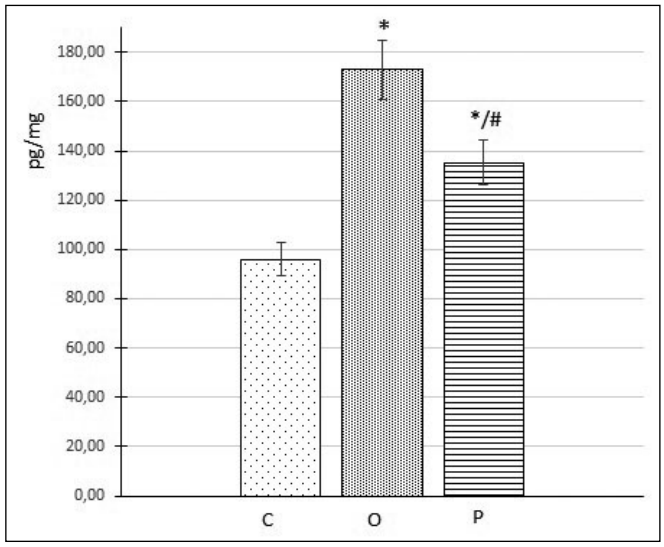

Fig. 3. Concentration of IL-1β in the blood serum after 28 days of administering omeprazole and pantoprazole, M±m (in each group n = 10).
Notes: C – control group; O – group of rats administered Omeprazole for 28 days; P – group of rats administered Pantoprazole for 28 days.
* – p <0.05, ** – p <0.01, *** – p <0.001 compared to the control; # – p <0.05, ## – p <0.01 compared to the group of animals administered omeprazole.

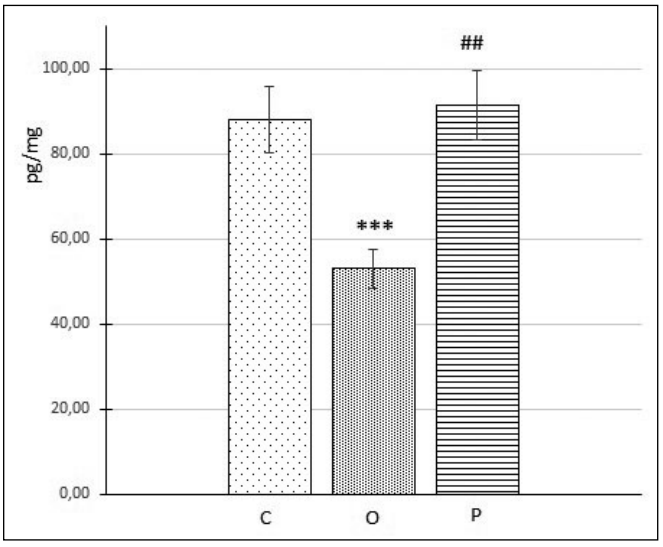

Fig. 4. Concentration of IL-4 in the blood serum after 28 days of administering omeprazole and pantoprazole, M±m (in each group n = 10).
Notes: C – control group; O – group of rats administered Omeprazole for 28 days; P – group of rats administered Pantoprazole for 28 days.
* – p <0.05, ** – p <0.01, *** – p <0.001 compared to the control; # – p <0.05, ## – p <0.01 compared to the group of animals administered omeprazole.

Long-term administration of omeprazole reduced the rats' blood serum anti-inflammatory cytokine IL-4 concentration by 39.8% (p <0.001) compared to the controls. Pantoprazole had almost no effect on the concentration of IL-4 in the blood serum of rats, which was the same as that in the control group (fig. 4).

Prolonged gastric juice hypoacidity induced by omeprazole and pantoprazole did not affect the concentration of proinflammatory cytokine IL-6 in the blood serum of rats (fig. 5).

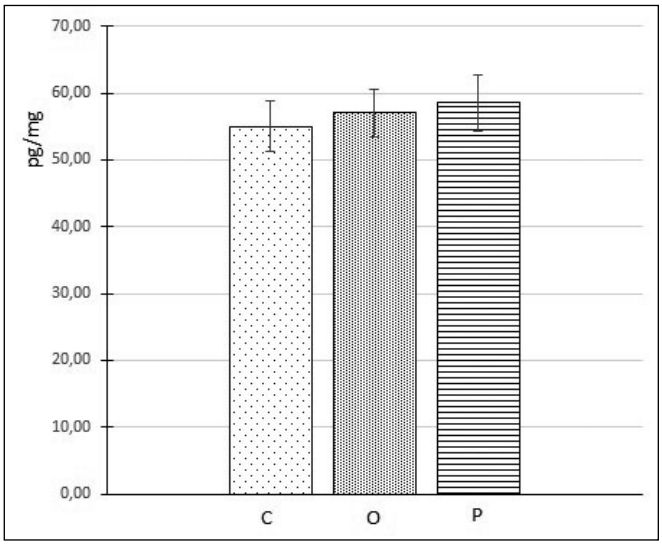

Fig. 5. Concentration of IL-6 in the blood serum after 28 days of administering omeprazole and pantoprazole, M±m (in each group n = 10).
Notes: C – control group; O – group of rats administered Omeprazole for 28 days; P – group of rats administered Pantoprazole for 28 days.
* – p <0.05, ** – p <0.01, *** – p <0.001 compared to the control; # – p <0.05, ## – p <0.01 compared to the group of animals administered omeprazole.

DISCUSSION

A characteristic feature of the inflammatory process in the intestine is the increased expression of IFN-γ, IL-1β and TNF-α genes [24]. These data are consistent with our results regarding the increase in the concentration of IFN-γ, IL-1β and TNF-α in the blood serum of rats after prolonged administering omeprazole and pantoprazole, since we state that dysbacteriosis caused by prolonged suppression of HCl secretions by proton pump blockers in the stomach is the cause of the inflammatory process in all the mucous membranes of the digestive tract, including and intestine [6]. Dysbacteriosis and, as a consequence, the inflammatory process was more pronounced under the conditions of administering omeprazole than pantoprazole.

We have suggested that dysbiotic changes in the stomach may be the cause of the immune response and the intense production of proinflammatory cytokines. This hypothesis is favored by the work of Brzozowski et al. [25], which showed that excessive colonization of the stomach by the *Candida* genus fungi in rats can be achieved by administering the ranitidine antisecretory drug to them. In this case, candidiasis was accompanied by increased expression and release of IL-1β and TNFα, which was indicated by their content increase in the blood plasma.

We observed a significant increase in gastric colonization by *Candida* fungi after 28 days of administering another antisecretory drug, Omeprazole, to rats, whereas when Pantoprazole was administered, the inoculation rates of *Candida* fungi from both the stomach and colon were at the control group level [6]. Lipopolysaccharides, which are the major components of the outer membrane of aerobic gram-negative bacteria *E. coli* (endotoxins), have a 1000-fold greater effect on the secretion of proinflammatory cytokines than lipopolysaccharides of *H. pylori* [26]. Bacterial lipopolysaccharides disrupt the healing processes in the gastric mucosa by reducing blood flow in the gastric mucosa, increasing the expression and release of the pro-inflammatory cytokines IL-1β and TNF-α [27].

After 28 days of gastric secretion inhibition by omeprazole, the concentration of *E. coli* (with altered enzymatic properties) in the intestine increased more than twice, the concentration of *E. coli* (lactose-negative) increased by almost 4 times. In the stomach, the concentration of *E. coli* almost doubled, and *Escherichia coli* (lactose-negative) occurred, with the concentration of 10^4 CFU/g. Meanwhile, Pantoprazole administration did not alter *Escherichia coli* inoculation rates, which were the same as in the control [6]. Therefore, we have every reason to believe that the development of dysbacteriosis in different biotopes of the digestive tract, caused by inhibition of HCl secretion in the stomach, is one of the main reasons for the increase of proinflammatory cytokines concentration in the blood serum of rats.

Proinflammatory cytokines directly affect parietal cells and inhibit HCl secretion, stimulate gastrin synthesis and secretion [13], suppress digestive tract motility [31]. With regard to gastrin secretion, there is an important work showing that IL-1β and TNF-α can directly regulate the expression of gastrin gene through mitogen-activated protein kinase and protein kinase C-dependent mechanism [13]. The authors of this work suggested that IL-1β and TNF-α may directly influence the development of hypergastrinemia caused by *Helicobacter pylori*. Our findings regarding the development of hypergastrinemia and dysbacteriosis in the stomach and intestine induced by prolonged gastric hypoacidity [6, 7], and the detected increase in IL-1β and TNF-α levels in the blood serum of rats, permit to make a more generalizing conclusion: IL-1β and TNF-α may play a role in hypergastrinemia induced by gastric hypoacidity of any genesis.

Growth in the mucous membrane of the gastrointestinal tract and, as a consequence, in the blood serum, of proinflammatory cytokines IL-1β and IFN-γ may be one of the mechanisms for suppression of the digestive tract motility in the conditions of long-term hypoacidity of gastric juice. And lower concentrations of proinflammatory cytokines IFN-γ, IL-1β and TNF-α when pantoprazole is administered, explain the more pronounced motility of the stomach and colon [7, 8].

The proinflammatory cytokines IFN-γ, TNF-α, IL-1β and IL-6 play a key role in protecting the body from pathogenic microorganisms [23]. Under the conditions of inflammatory process development cytokines are synthesized in the following sequence: TNF-α, IL-1β, IFN-γ and IL-6. Further, IL-6 inhibits the secretion of TNF-α and IL-1β, thus activating the humoral level of immunity [28]. In this regard, IL-6 is considered both as a pro-inflammatory and anti-inflammatory cytokine. In contrast to IL-6, IFN-γ is considered to be a major stimulator of the cell-mediated

immune response [29]. In our studies, the concentration of IL-6 in the blood serum of rats after 28 days of administering omeprazole and pantoprazole did not change, indicating a possible absence of a significant inhibitory effect of IL-6 on TNF-α and IL-1β secretion. Therefore, under these conditions, the cell-mediated link of immunity is activated first of all.

The results obtained for changes in the concentration of cytokines in the blood serum of rats after prolonged administration of omeprazole and pantoprazole are consistent with the literature data, according to which one of the inflammatory process mechanisms in the intestine is to disrupt the balance between pro- and anti-inflammatory cytokines [30].

Our further studies will focus on determining nitrite ions in the mucous membranes of the stomach, colon, and the blood serum of rats after 28 days of administering omeprazole and pantoprazole.

CONCLUSIONS

1. Suppression of hydrochloric acid secretion in the stomach of rats for 28 days with omeprazole resulted in an imbalance between pro- and anti-inflammatory cytokines: the concentrations of proinflammatory cytokines IFN-γ, TNF-α, IL-1β significantly increased, and IL-6 concentration did not change. The concentration of the anti-inflammatory cytokine IL-4 decreased.
2. Suppression of hydrochloric acid secretion in the stomach of rats for 28 days with pantoprazole to a lesser extent than omeprazole administration disrupted the balance between pro- and anti-inflammatory cytokines. IFN-γ concentration was at the level of the control, that of proinflammatory cytokines TNF-α, IL-1β increased, but significantly lower than in the group receiving Omeprazole. Pro-inflammatory cytokine concentrations – IL-6 and anti-inflammatory – IL-4 were at the control levels.

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The Author declare no conflict of interest.

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