

Effect of multiprobiotic “Symbiter® acidophilic” concentrated on morphofunctional changes in stomach evoked by 28-days introduction of omeprazole

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Abstract: Experiments on rats showed that 28-day injection of the antisecretory preparation omeprazole (14 mg/kg) resulted in a statistically significant increase in the gastrin level in blood plasma, which was accompanied by a significant imbalance between indices of stomach contamination by obligate and facultative microflora, by imbalanced stomach secretory function as well as by essential morphological changes in stomach. Multiprobiotic “Symbiter® acidophilic concentrated” introduced simultaneously with omeprazole during 28 days prevented the formation of disbiotic and partially morphological and functional changes in stomach. It was concluded that multiprobiotic “Symbiter® acidophilic concentrated” use was advisable in patients with hypergastrinemia of different genesis (hypoacidity/anacidity, atrophic gastritis, long-term use of antisecretory drugs) and it can also be included in complex therapy of acid dependent diseases of the gastrointestinal tract to prevent development of disbacteriosis in the stomach, and as a result, to prevent morphological and functional changes in the stomach.

Key words: stomach, gastric acid, hypoacidity, omeprazole, gastrin, probiotics

INTRODUCTION

Preparations of the class of proton pump inhibitors are widely used in clinical practice as basic remedies for the treatment of acid dependent diseases (peptic ulcers of stomach and duodenum, symptomatic erosions and ulcers, pancreatitis, gastroesophageal reflux disease, etc.) [6]. However, due to feedback, hypoacidity results in hypergastrinemia. According to contemporary data, long-term use of omeprazole may lead to negative morphological changes of the stomach mucous membrane and even to the development of polyps, gastrinoma [8, 9, 10, 11] and carcinoids localized on acid producing mucous membrane of the human stomach which are hormonally induced (by gastrin) [2]. ECL carcinoid tumours often develop in patients with hypergastrinemia, such as autoimmune gastritis and Zollinger-Ellison syndrome (gastrinoma) [21]. Hydrochloric acid plays also important role in the prevention of stomach bacterial colonization. It protects the organism from microorganisms that enter into stomach with food [17]. Lowered acidity favours infectious diseases [13, 22]. Bacterial overgrowth in the proximal part of the digestive tract can also induce an additional risk of malignization in stomach [16] because the reduction of nitrates to nitrites and the subsequent production of N-nitroso compounds has been the principal focus of the effect of bacterial overgrowth in the stomach. N-nitroso compounds are recognized carcinogens

in animals and can induce gastric cancer in rodents. This is why it is important to investigate the influence of probiotic morphological and functional changes in stomach.

In this connection, our aim was to study the effect of multiprobiotic “Symbiter® acidophilic” concentrated on microbiocenose, structural-morphological changes in the stomach, and basal gastric acid secretion against a background of 28 days inhibition of gastric secretion by omeprazole.

MATERIALS AND METHODS

The study was carried out on 30 white rats maintained in accordance with the guidelines of the Animal Ethical Research Committee of Taras Shevchenko National University in Kiev. The rats were divided into 3 groups. The animals of the first (control) group during 28 days were injected with 0.2 ml water (intraperitoneally (i.p.)) and 0.3 ml water per os once a day. The rats of the next two groups were given omeprazole («Sigma», USA) (14 mg/kg, i.p., once a day) during 28 days. Omeprazole was solved in 0.2 ml water for injections. In addition, the animals of the third group, simultaneously with omeprazole, received multiprobiotic “Symbiter® acidophilic concentrated” (Firm OD Prolosok Ltd) at a dose of 0.14 ml/kg which was solved in 0.3 ml water for injections.

Symbiter is a concentrated fluid biomass of bioplasts of symbiosis of 14 microorganisms strains. The composition of one dose (10 ml) of Symbiter is a concentrated biomass of bioplasts of bacteria symbiosis CFU/cm³, no less: Lactobacillus and Lactococcus – 6.0×10¹⁰, Propionic bacterium – 3.0×10¹⁰, Bifidobacterium – 1.0×10¹⁰, Acetic bacterium – 1.0×10⁶.

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One day after the last introduction of omeprazole, omeprazole + Symbiter or water, an experiment was carried out to determine the level of basal acid secretion. Each experiment started in the morning, on an empty stomach, after a day of starvation but free access to water. Animals were narcotized with urethane (Sigma, USA) at a dose of 1.1 g/kg (i.p.). Stomach secretion was studied using the method of isolated stomach perfusion [4].

Rat euthanasia was carried out through the introduction of a lethal dose of narcotics. Blood plasma gastrin concentration was measured by the radioimmunoassay method with use of analytical kits from MP Biomedicals, LLC (USA).

The characteristics of contamination of stomach mucosa with opportunistic and normal microflora were evaluated after 28 days from the beginning of the experiment. Study of stomach microbiocenosis included analysis of specific and quantitative content of microflora. Analysis and evaluation of results of the study of stomach biocenosis in rats were evaluated according methodical recommendations "Diagnostics and treatment of intestine dysbacterioses", (Moscow, 1991) and Order No. 535 of the Ministry of Health of the USSR from 1985, and Order No 59 of the Ministry of Health of the Ukraine from 2003. Quantitative indices of stomach microflora were studied by means of inoculating 1 ml of each dilution (1:10) on differential-diagnostic medium: Endo's, Ploskiriev's and BCA to identify pathogenic enterobacteria; bile-saline agar and Saburo's medium to determine staphylococci and fungi; Endo's and Simonce's citrate to discover colibacillus and opportunistic enterobacteria; 5% blood agar and EDDS medium to identify enterococci; Blaurok's medium for bifidobacteria and MKS for lactobacilli.

Research of morphological changes of stomach mucosa was carried out on 10 stomach fragments of rats, which were introduced placebo during 28 days, on 10 stomach fragments of each rat from all groups. Stomach fragments were fixed in 10% neutral formalin solution and the material was embedded in paraffin. Serial section of 5-7 μ m thickness were made on rotor microtome and then stained with hematoxylin and eosin [12]. Histological preparations were analyzed at microscope magnification $\times 120$, $\times 160$, $\times 180$, $\times 200$ and $\times 400$. Calculations were carried out on microphotographs using the software UTHSCSA ImageTool.

Statistical analysis. Normal distribution of studied parameter for each sampling was checked using Shapiro-Wilka's criteria. Average value (M) error and standard deviation (SD) were calculated to discover significant changes of indices of blood plasma gastrin concentration and basal gastric acid secretion, as well as morphological and bacteriological indices. Sampling comparison was performed using the unpaired Student's t-test. Differences among values were considered statistically significant if $p < 0.05$.

RESULTS

The day after the last introduction of omeprazole to the rats there was observed an increase of gastrin basal concentration in blood serum from 59.0 ± 35.05 to 170.7 ± 90.7 pg/ml ($p < 0.05$).

Analysis of stomach microflora in control animals evidenced a scanty spectrum of bacteria in the content of this organ biocenosis (Table 1). Most frequently, the stomachs of control

animals were contaminated with fungi of the genus *Candida*, enterococcus, colibacillus and lactobacteria. Representatives of opportunistic microflora were not practically seeded from stomach. *Citrobacter* was revealed only in 10 % of animals. Quantitative indices of seeding enterococcus, escherichia and fungi of the genus *Candida* from the stomachs were not of a high level and were within the limits 10^2 - 10^3 cfu/g. Concentration of lactobacteria isolated from the stomachs was also negligible (10^2 cfu/g).

Table 1 Quantitative indices of rat stomach biocenosis after 28-day introduction of omeprazole (14 mg/kg) and joint introduction of Symbiter and omeprazole (lg cfu/g, M \pm SD).

Microorganisms	Control n=10	Omeprazole n=10	Omeprazole+ Symbiter; n=10
<i>Escherichia coli</i>	3.1 \pm 0.02	5.0 \pm 0.04 *	3.0 \pm 0.03
<i>Escherichia coli</i> with modified enzyme properties	2.1 \pm 0.04	—	—
<i>Escherichia coli</i> lactose (-)	—	4.4 \pm 0.02	—
<i>Klebsiella</i>	—	4.0 \pm 0.03	—
<i>Citrobacter</i>	4.0 \pm 0.06	4.3 \pm 0.05 *	2.0 \pm 0.04#
<i>Proteus</i>	—	5.1 \pm 0.03	—
<i>Enterobacter</i>	3.2 \pm 0.02	4.0 \pm 0.04	2.4 \pm 0.03#
<i>Staphylococcus aureus</i>	—	4.2 \pm 0.02	—
<i>Staphylococcus epidermidis</i> hemolytic	—	2.8 \pm 0.03	—
<i>Staphylococcus epidermidis</i>	2.8 \pm 0.03	4.0 \pm 0.03 *	2.1 \pm 0.01#
Enterococcus	2.2 \pm 0.02	3.4 \pm 0.02 *	2.6 \pm 0.04#
<i>Candida</i>	2.0 \pm 0.04	4.1 \pm 0.02 *	1.6 \pm 0.05#
<i>Lactobacillus</i>	2.1 \pm 0.03	1.6 \pm 0.02 *	3.8 \pm 0.01#

Note: * – $p < 0.05$, with respect to control; # – $p < 0.05$ with respect to omeprazole

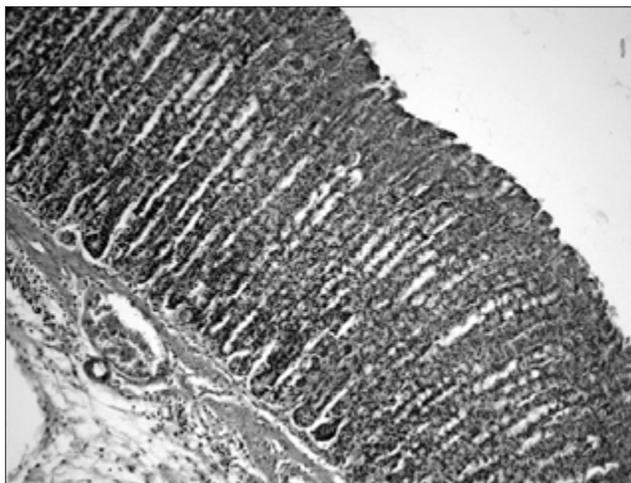
Comparison of the state of stomach microflora in the rats of the control group with the group to which was introduced omeprazole during 28 days, showed that after 28 days of omeprazole introduction the rats were characterized by a significant decrease of lactobacteria seeding, and a tendency to increased colonization by opportunistic microflora and fungi from the genus *Candida*. There were shown statistically significant lowered quantitative indices of normal microflora seeding from stomach. In 40% of animals, normal microflora was not seeded at all. Stomach bacterial spectrum was enlarged due to seeding clebsiella, enterobacter and proteus. There was also registered an increased contamination of the stomachs by *Staphylococcus aureus*. Concentration of these opportunistic bacteria reached a high level (10^4 - 10^5 cfu/g). Our findings indicate a lowering of colonizational resistance of stomach mucous membrane in animals with hypergastrinemia, which may present the possibility for the transit of microflora from intestine to stomach through an upward current.

Analysis of stomach morphological state of control group rats which were given the placebo showed normal morphology of gastric mucosa (Fig. 1). However, the increase of depth of epithelium by 54% ($p < 0.001$), area of nucleus section of parietal cells by 84.2% ($p < 0.001$) and area of nucleus section of endocrine cells in fundus by 59.2% ($p < 0.001$) were registered in the 2nd group of animals which were given omeprazole during 28 days (Table 2). The obtained data

Table 2 Influence of multiprobiotic "Symbiter® acidophilic" on basal gastric secretion and structural state of gastric mucosa in omeprazole-treated rats.

	Control Mean + SD	Omeprazole (14 mg/kg, once a day during 28 days) Mean + SD	Omeprazole (14 mg/kg, once a day, during 28 days) + Symbiter (during 28 days) Mean + SD
Basal gastric acid output (mM/120 min)	40.3+21.0 n=26	172.5+ 91.6*** n=8	90.3+ 43.1***/# n=10
Depth of epithelium (µm)	200.0+11.66 n=10	308.0+9.97*** n=10	192.9+9.38 ### n=10
Area of nucleus section of parietal cells (µm ²)	2.66+0.071 n=10	4.9+0.24*** n=10	3.3+0.22***/### n=10
Area of nucleus section of endocrine cells in fundus (µm ²)	2.5+0.08 n=10	3.98+0.2*** n=10	2.3+0.06***/### n=10

SD – standard deviation;
*** statistically significant difference (p<0.001) vs. control group;
statistically significant difference (p<0.05) and ### significant difference (p<0.001) compared with group of omeprazole-treated rats; n – number of animals.

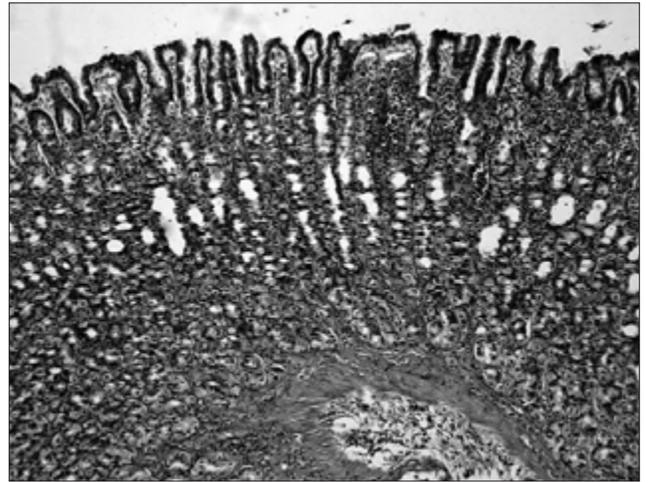
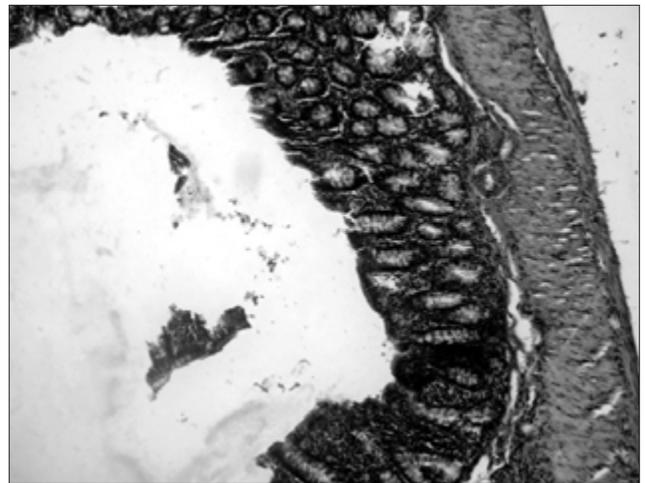
**Figure 1** Gastric mucosa (fundus) of control group of rats. Magnification × 120.

argues for the development of hyperplasia of gastric mucosa (Fig. 2). One case in the 2nd group was characterized by marked lymphoid-cell infiltration with gland replacement. Events of intestine metaplasia of glands also occurred in the 2nd group of animals, when goblet cells covered gastric pits in gastric mucosa (Fig. 3).

Our study on effect of 28-days hypergasrinemia on the secretory function of the stomach showed that a 28-day introduction of omeprazole led to an increase of basal gastric acid secretion by 328.0% (p<0.001) one day after the last introduction of the blocker (Table 2).

In the next series of investigations, there was analyzed the effect of 28 days joint introduction of omeprazole and Symbiter on parameters of stomach microecology, as well as on basal stomach secretion and the morphological state of stomach mucous membrane.

Our findings showed that the indices of stomach seeding with opportunistic flora in rats that were administered with omeprazole simultaneously with Symbiter did not differ

**Figure 2** Gastric mucosa (fundus) of rats administered omeprazole during 28 days. Magnification × 120.**Figure 3** Intestine metaplasia of glands in fundus of rats administered omeprazole during 28 days. Magnification × 120.

practically from control (Table 1). The specific spectrum of enterobacteria isolated from stomach narrowed due to the absence of clebsiella and proteus. All examined animals had no stomach seeding with *Staphylococcus aureus*. As in control animals, stomach microbiocenosis in rats of the 3rd group included mainly enterococcus, colibacillus, and fungi of the genus *Candida* in insignificant concentrations (10¹–10² cfu/g). Seeding of lactobacteria from stomach in this group of animals increased to 10⁴ cfu/g. Thus, investigations aimed at analyzing the effect of multiprobiotic Symbiter on the state of digestive tract microbiocenosis in animals against the background of 28 days injection of omeprazole show that Symbiter is able to stabilize effectively stomach colonization resistance, and normalize the balance between main species of obligatory and opportunistic microflora.

In analyzing the morphological picture of mucous membrane in the 3rd group of rats that were given simultaneously omeprazole and Symbiter, we should note that the depth of epithelium did not change from the control group (Fig. 4). The area of the nucleus section of parietal cells was decreased in comparison with omeprazole-treated animals, also it remained higher by 24.1% (p<0.001) of the area of nucleus section of parietal cells in the control group. As for the area of nucleus section of endocrine cells in the fundus, this was decreased



Figure 4 Gastric mucosa (fundus) of rats simultaneously administered omeprazole and Symbiter during 28 days. Magnification $\times 120$.

by 8% ($p < 0.001$). Thus, Symbiter prevented the development of hyperplasia in gastric mucosa; however, the prevention was incomplete.

Study of basal gastric secretion in rats with simultaneous introduction of omeprazole and Symbiter showed a decrease of basal gastric secretion by 47.6%, compared with the group which was given only omeprazole, but it was higher by 124.1% compared with the control group.

DISCUSSION

Our experiments have shown that 28 days introduction of the antisecretory preparation omeprazole resulted in a significant increase in gastrin blood level, the trophic action of which led to essential morphological changes in the stomach. Hyperplasia evoked by trophic influence of gastrin on gastric mucosa resulted in an increase of basal gastric acid secretion. These results are in accordance with studies by other investigators [3, 23].

Hydrochloric acid also plays an important role in the prevention of stomach bacterial colonization [17, 22, 24]. There are three possible sources of bacteria for the gastric lumen: 1) they can be injected with food, 2) originate from the oropharyngeal cavity, or 3) migrate from the small or large bowel and enter the stomach via enterogastric reflux. Bacterial overgrowth is associated with an increased risk of non-cardia gastric cancer, as seen in patients with hypochlorhydria due to pernicious anaemia, atrophic gastritis, or partial gastrectomy. The principal hypothesis of carcinogenesis is based on evidence that bacterial overgrowth results in the production of nitrite from nitrate, with subsequent production of carcinogenic N-nitroso compounds [16].

Multiprobiotic "Symbiter® acidophilic concentrated" introduced simultaneously with omeprazole during 28 days prevented the formation of disbiotic and partially morphological and functional changes in stomach. We suggested that one of the mechanisms of the protective action of Symbiter is normalization of stomach microbiocenosis and, as a result, prevention of the formation of carcinogenic N-nitroso compounds.

Additionally, experiments on culture of intestine mucous membrane from patients with neoplasia showed that

lactobacilli can decrease synthesis of the tumour necrosis factor [2, 15, 19]. Some strains of *L. casei* and *L. bulgaricus*, as well as nonpathogenic colibacillus, can influence the synthesis of cytokines through explant of stomach mucous membrane [18]. Signals formed due to the interaction of *L. casei* with epithelium may inhibit inflammatory processes in the mucous membrane [20]. It has also been shown that acid-milk bacteria that interact with the normal mucous membrane of the large intestine can generate strain specific changes in genetic mechanisms of local immunity [1, 5, 7].

The protective functions of normoflora are not limited only by antagonism to pathogenic agents. Lactobacteria are able to stimulate differentiation of interepithelial intestine lymphocytes closer to suppressor and cytotoxic phenotypes [5, 14].

CONCLUSION

Our findings have shown that long-term inhibition of hydrochloric acid secretion in stomach due to 28 day introduction of omeprazole, blocker of proton pump, results in an increase of blood gastrin level. The trophic action of gastrin causes the development of hyperplasia in stomach and as a result the basal gastric acid secretion is increased. Simultaneously, an excessive growth of opportunistic microflora is registered in the stomach. Multiprobiotic concentrated "Symbiter® acidophilic" introduced partially prevented development of morphological and functional changes in the stomach. Our findings allow the recommendation that multiprobiotic concentrated "Symbiter® acidophilic" should be administered to people with hypergastrinemia and disbacteriosis of different genesis.

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