ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Changes in Sensitivity of Parietal cell to Secretagogues after Correction of Gastric Hypoacidity with Probiotic.

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ABSTRACT

The aim of this work was to investigate the sensitivity of stomach secretory cells to gastric secretion stimulants after withdrawal of omeprazole (H+-K+ -ATPase blocker) and after simultaneous prolonged administration of omeprazole and multiprobiotic. The study was carried out on white nonlinear male rats. In 24 hours period after last administration of omeprazole gastric basal and stimulated (by carbachol, pentagastrin, histamine) acid output were measured in acute experiments by method of perfusion of isolated stomach by Ghosh and Shild. It was shown that 28 days injection of omeprazole caused hypergastrinemia, due to which sensitivity of parietal cells to pentagastrin and histamine had decreased, but secretory response induced by carbachol didn't changed. In rats, which got omeprazole and multiprobiotic "Symbiter acidophilic" concentrated simultaneously, basal and stimulated acid output did not significantly differ from control. It was concluded that multiprobiotic prevented structural and functional changes in parietal cells that was caused by hiperhastrinemia.

Keywords: gastric acid secretion, omeprazole, multiprobiotic, hypergastrinemia.

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ISSN: 0975-8585

INTRODUCTION

Over the last few years due to the lack of effective means more and more attention has been paid to treatment of gastrointestinal diseases, that are associated with decreased gastric secretion.

Except atrophic gastritis, hypoacidity of gastric juice may develop during treatment with drugs that reduce the secretion of hydrochloric acid (HCl), especially proton pump inhibitors [1,2]. Indications for their long-term use is Zollinger-Ellison syndrome, gastroesophageal reflux disease, pancreatitis and others [2–5]. As is known, continued reduction of acid gastric secretion initiates increase of gastrin concentration in blood. Gastrin causes trophic effect on the mucous membrane of the digestive tract and is mitogenic factor for epithelial cells growth [6,7]. Therefore, long-term hypergastrinemia may became risk factor for cancer development [8–10]. However, such point of view exists among the authors, that development of gastric carcinogenesis in patients with hypoacidity is connected not only with hypergastrinemia, but with excessive bacterial growth [11]. It was shown that excessive growth of pathogenic flora leads to the formation of nitrite from food and saliva nitrates with further production of mutagenic and carcinogenic N-nitroso compounds [12] and other well known carcinogen – acetaldehyde [13]. Also the relationship between bacterial infection and stomach secretion of gastrin is proved [14]. *Helicobacter pylori* colonization of the stomach initiates the immune response of T-helper cells of the first order (Th-1) [Ernst P.B., Gold B.D.2000] with release of interferon-gamma (IFNγ), which stimulates gastrin secretion [15].

In our previous work it was shown that 28 day administration of omeprazole had leaded to hypergastrinemia, due to which morphological changes in the gastric mucosa appeared and basal acid output (BAO) also changed [16,17].

Prolonged decline of hydrochloric acid secretion in stomach except hypergastrinemia also resulted in microbiocoenosis disturbance and excessive growth of pathogenic flora [11,18]. Taking into account these facts, we conducted several series of studies where the effects of hypochlorhydria such as dysbiosis and hypergastrinemia were corrected by multiprobiotic. As the result, we observed positive changes: the normalization of microflora quantitative and qualitative composition and moderate gastrin reduction in the blood [19,20].

However, stimulated secretory activity of the stomach after long-term stomach hypoacidity is poorly studied, because each of the standard stimulants (carbachol, gastrin, histamine) acts specifically to each phase of gastric secretion [21,22].

That is why the aim of this work was to investigate the sensitivity of stomach secretory cells to gastric secretion stimulants after withdrawal of omeprazole and after simultaneous prolonged administration of omeprazole and multiprobiotic.

MATERIALS AND METHODS

The study was carried out on white nonlinear male rats. The average weight of animals was 150-230 g. The investigations were done in accordance with the recommendations of European convention and national laws about biomedical research [23].

Three series of experiment were performed. Three groups of animals were used in each series. All drugs were administered for 28 days long. In 1st group (control) rats were injected with 0.2 ml of saline intraperitoneally (IP) and 0.5 ml of saline was administrated orally, 2nd – omeprazole (Sigma Chemichal Co, St. Louis, USA) IP at the dose 14 mg/kg diluted in 0.2 ml of saline, 3rd - simultaneous administration of omeprazole IP and multiprobiotic "Symbiter® acidophilic" concentrated orally (“O.D. Prolisok” Kyiv, Ukraine) at dose 0.14 ml/kg diluted in 0.5 ml of saline.

In 24 hours period after last injection gastric basal (BAO) and stimulated acid output (SAO) were measured in acute experiments by method of perfusion of isolated stomach by Ghosh and Shild. Carbachol (10 μg/kg), pentagastrine (26 μg/kg) and histamine (3 mg/kg) were used to stimulate gastric secretion [24]. Rats were anaesthetized by urethane (Sigma Chemichal Co, St. Louis, USA) at the dose 1.15 g/kg IP. The stimulators were injected IP after 120 min of BAO measurement and stimulated acid output was measured for 120 min

ISSN: 0975-8585

more. Acidity of 10 minutes samples was determined by ionomer ЭВ-74 with use of 0.01 N Sodium hydroxide (NaOH) solution. Quantity of NaOH, that was used to titrate 10 min samples, showed BAO in this sample. To calculate total BAO and SAO all values of separate samples were sumed and transferred in µmole. The rats were sacrificed with urethane (3 g/kg) IP.

For the investigation of the data distribution type Shapiro-Wilk's W criterion was used. Mean of value (M) and standard error of the mean (m) were calculated as all datas were parametric. *Post-hoc* analysis included Student's t-test for parametric data. Probability of a type I error was $\alpha > 0.05$ [25].

RESULTS AND DISCUSSION

It was shown that after 28 days administration of saline in the control group BAO was 30.4±10.2 µmole/120 min. In our next investigations BAO of both control and experimental groups we consider as 100%. In control group carbachol stimulated acid output (CAO) was 74.9±18.1 µmole/120 min, that was by 162±79.7% ($p<0.001$) higher compare to BAO.

BAO in rats, which got omeprazole for 28 days, had wide range of data that is why animals were divided into two groups with low and high levels of secretory activity: LSA-group and HSA-group correspondently. The distribution was made similar to our previous study where the results were analyzed by morphological and electron microscopic techniques and were divided into groups. Thus, in gastric mucous hyperplasia of ECL cells was observed in the group of rats with high secretory activity and in group with low secretory activity the epithelial cells with signs of keratinization and nuclear-cytoplasmic ratio disturbance were found. That might indicate signs of metaplasia [16,17,26,27].

BAO decreased by 52±3% ($p<0.01$) up to 14.5±6.8 µmole/120 min in LSA group of rats, which were injected with omeprazole for 28 days. At the same time, in HSA group secretion increased to 61.2±9.6 µmole/120 min or by 102±7.4% ($p<0.01$) compare to control rats. CAO increased by 101.6±75.2% (27.9±7.1 µmole/120 min) in LSA-group, on the contrary in rats of HSA-group CAO increased by 199.4±55.9% (180.6±26.4 µmole/120 min) (fig.1).

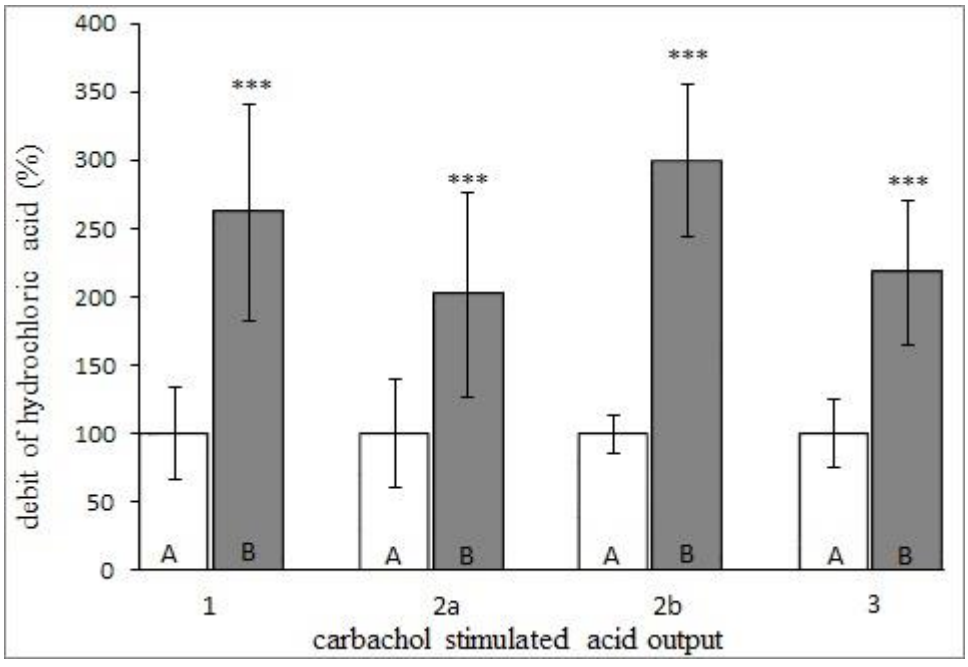

Figure 1. Basal (A) and Carbachol (B) stimulated gastric acid output (M±SD):

1 – control (n=10), 2a – rats with low level of gastric secretory activity (n=10), 2b - rats with high level of gastric secretory activity (n=10), 3 – simultaneous administration of omeprazole and multiprobiotic "Symbiter";
*** - $p \leq 0.001$ compare to control.

ISSN: 0975-8585

However, after CAO comparison in LSA- and HSA-group with control it was concluded that in percentage ratio response to carbachol action between the groups had not statistically significant difference. Thus, omeprazole administration for 28 days did not alter the sensitivity of the secretory cells to the stimulator. Although it should be noted that the level of gastrin in blood serum of both LSA- and HSA-groups increased by 2.9 times compared to the control (fig.2), in other word hypergastrinemia was observed after long-term injection of H^+-K^+-ATPase blocker.

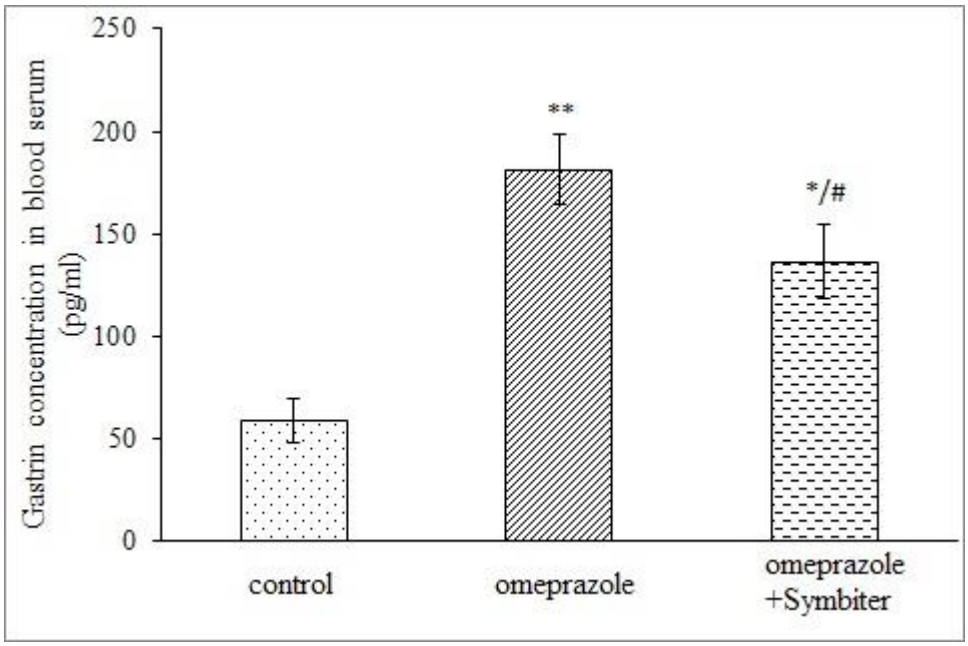

Figure 2. Gastrin concentration in blood serum (M±SD)

* - p ≤0.05, ** - p ≤0.01 compare to control, # - p ≤0.05 compare to isolated omeprazole administration.

As mentioned above, colonization of stomach with pathogenic flora was promoted by gastrin increase in the blood of rats. In our experiment to prevent changes in gastric microbiota omeprazole and multiprobiotic "Symbiter® acidophilic" concentrated were administered simultaneously for 28 days. Thus, the results demonstrated that BAO (49±12.1 µmole/120 min) and CAO (108.3±36.6 µmole/120 min) in this group didn't significant differ from control group. It should be noted, that multiprobiotic reduced concentration of gastrin in blood serum, but didn't return it to normal (fig. 2).

Investigation of McColl K El-Omar E. showed that the most sensitive to hypoacidity was pentagastrin stimulated gastric secretion [28]. Therefore, we decided to research pentagastrin stimulated acid output (PAO) after long-term omeprazole administration.

Similar to the previous series, after 28 days administration of omeprazole we received two groups of rats with low (LSA) and high (HSA) levels of stomach secretory activity (34.8±10.3 µmole/120 min and 135.6±43 µmole/120 min, respectively). PAO in LSA group increased by 59.4±24.4% ($p<0.01$) and in HSA rats was higher by 75.8±48.4% ($p<0.01$). Pentagastrin made similar effect, without statistically significant difference, in both groups. So we combined obtained data and the average PAO increased by 67.7±38.2% ($p<0.01$) in comparison with control animals.

Pentagastrin increased secretion by 246.4±110% ($p<0.001$) in animals of control group, in researched group this data decreased by 178.7± 25.4% ($p<0.01$) in comparison with control. Similar response to pentagastrin was observed by other scientists after long term ranitidine administration [29]. Pentagastrin dose remained unchanged in all series of experiment. Considering this fact such effect could be explained by lost of sensitivity to gastrin by parietal cell in rats after prolonged hypochlorhydria (fig. 3). Ramus and Williamson in their work observed similar situation. Response on pentagastrin decreased when concentration of gastrin was high in blood after selective vagotomy [30].

ISSN: 0975-8585

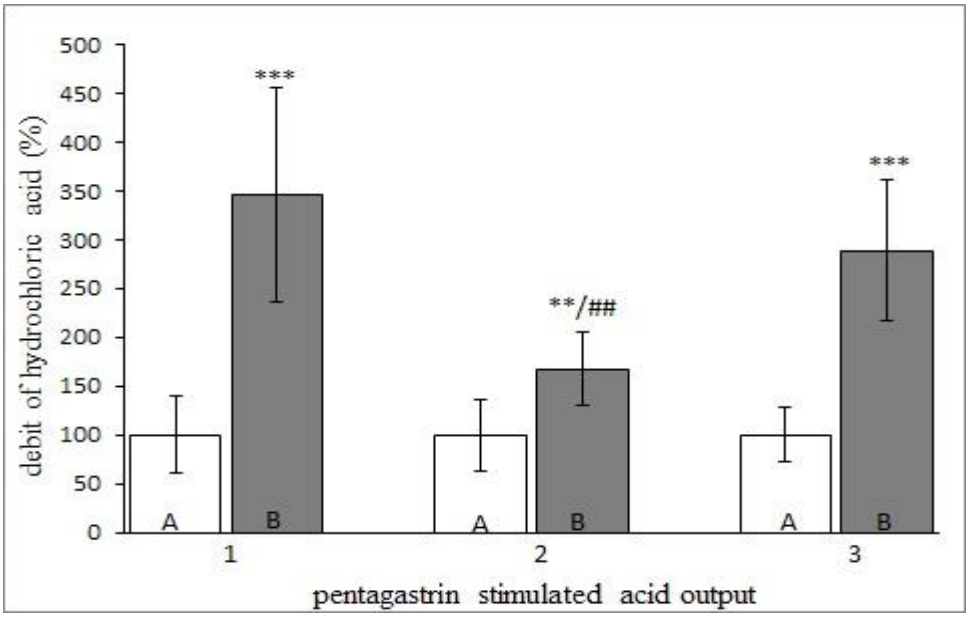

Figure 3. Basal (A) and Pentagastrin (B) stimulated gastric acid output (M±SD):

1 – control (n=10), 2 - omeprazole (n=10), 3 – simultaneous administration of omeprazole and multiprobiotic "Symbiter"; ** - p ≤0.01, *** - p ≤0.001 compare to control, # - p ≤0.05 compare to stimulator action in control group.

Independently of its nature (H2 blockers, blockers of H^+-K^+-ATPase, vagotomy) hypochlorhydria reduces PAO.

It was noted that in rats that had got omeprazole and multiprobiotic "Symbiter acidophilic" simultaneously, multiprobiotic didn't completely eliminated the hypergastrinemia (fig. 2), although BAO and PAO did not significantly differ from control (fig. 3). So, it was concluded that multiprobiotic prevented structural and functional changes that was caused by hiperhastrinemia.

In next series of experiments, we used histamine as stimulant. It is known that gastrin stimulates HCl secretion both acting directly on the G-receptors of parietal cells and with help of histamine. The release of gastrin in the blood had stimulated ECL cells, as the result histamine secreted. Histamine interacted with H_2 receptors of parietal cells, that resulted in HCl secretion. Thus, ECL-cells played the role of intermediaries between the G and parietal cells [31].

Hypergastrinemia, that was the result of prolonged decrease of gastric acid secretion caused by omeprazole, leaded to increase of histamine synthesis by ECL cells. Intensification of histamine secretion might be the reason of pathological changes in ECL and parietal cells. So, the aim of further experiments was to determine how gastric secretion had changed after exogenous histamine administration.

Thus, in control group, BAO was 25.4±11.1 µmole/120 min. Histamine stimulated acid output (HAO) was 69.4±15.6 µmole/120 min (or increased by 203±101% ($p<0.001$) compare to BAO). As result of 28 days omeprazole administration, we got two groups of animals with low (18.2±7.4 µmole/120 min) and high (75.1±12.9 µmole/120 min) basal acid output. Histamine injection to rats with low BAO increased gastric secretion by 137±105% and by 92±31.2% in rats with high BAO. We combined groups with low and high BAO in one as there were no statistically significant difference between the them in response to histamine action. Histamine increased acid secretion by 110.7±63.4% ($p<0.001$) in rats of both groups. In the same time, HAO in experimental group decreased by 92.3±17% ($p<0.001$) in comparison with control group (fig. 4).

ISSN: 0975-8585

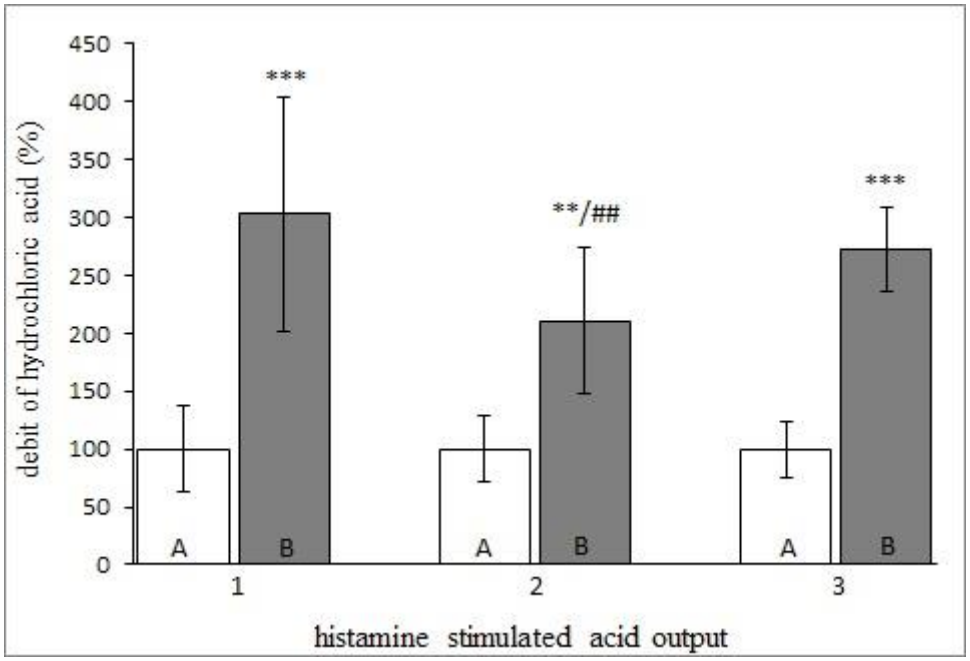

Figure 4. Basal (A) and Histamine (B) stimulated gastric acid output (M±SD):

1 – control (n=10), 2 - omeprazole (n=10), 3 – simultaneous administration of omeprazole and multiprobiotic "Symbiter"; ** - p ≤0.01, *** - p ≤0.001 compare to control, # - p ≤0.05 compare to stimulator action in control group.

It was noticed that our conclusion had analogs in works of other scientists [30,32]. James W. Freston and authors demonstrated that after treatment with omeprazole levels of gastrin in blood were similar to those that occur after gastric vagotomy. It was showed that in case of hypergastrinemia histamine injection in maximal dose caused no significant increase in acid secretion. Thus, to overcome the effects of long term hypoacidity, the increase of gastrin level had to be prevented. As gastrin increases in case of dysbiosis, we used "Symbiter® acidophilic" concentrated to prevent this increase.

In the group of rats, which simultaneously with omeprazole received multiprobiotic for 28 days long, BAO and HAO did not significantly different from control (fig. 4), although the concentration of gastrin did not completely returned to normal (fig. 2). Normalization of gastric secretion in rats that were simultaneously treated with omeprazole and multiprobiotic could be explained by action of short-chain fatty acids (SCFAs). SCFAs are the main end products of fermentation of not fissionable polysaccharides and dietary fiber by Lactobacillus and Bifidobacterium in the colon. Acetic and propionic acid causes inhibition of gastric secretion by inhibiting tonic activity of the nervus vagus.

The positive effect of multiprobiotic on the secretory function of the stomach was the result of the normalization of qualitative and quantitative composition of the stomach microflora, decreased IFN-γ level [33], which makes certain contribution to the development hypergastrinemia, and through activation of PPARγ caused by certain strains of microorganisms [34] that are part of the "Symbiter® acidophilic" concentrated.

CONCLUSION

It was shown that 28 days injection of omeprazole caused hypergastrinemia, due to which sensitivity of parietal cells to pentagastrin and histamine had decreased, but secretory response induced by carbachol didn't chang. In rats, which got omeprazole and multiprobiotic "Symbiter acidophilic" concentrated simultaneously, basal and stimulated acid output did not significantly differ from control. It was concluded that multiprobiotic prevented structural and functional changes in parietal cells that was caused by hiperhastrinemia.

ISSN: 0975-8585

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