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Experimental correction of metabolic changes in mouth at long-term hypoacidity by multiprobiotic «symbiter acidophilic»

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It is known, that long decrease in gastric secretion leads to the development of hypergastrinemia, dysbiosis and to pathological changes in digestive organs. Very important there is a search of ways to correction of these undesirable consequences. Long-term usage of omeprazole leads to metabolic disorders in periodontium tissues and salivary glands, such as development of NO-ergic system disbalance and activation of free-radical oxidation, that are positively corrected by multiprobiotic of new generation "Symbiter acidophilic".
Key words: periodontium, salivary glands, omeprazole, hypoacidity, hypergastrinemia, dysbiosis, "Symbiter acidophilic ".

The leading role in development of pathological changes in the oral cavity organs at long-term administration of proton pump inhibitor (PPI), probably, belongs to hypoacidity of gastric juice which assists to the development of dysbiosis of digestive tract different parts. As all of parts of digestive organs are connected by the general plan of histological structure, ontogenetic development, and difficult neuro-humoral mechanisms of intercommunication, violation of microecology in one part certainly influences on biotops of others in particular on oral cavity [1].

With the purpose of correction of mouth dysbiosis at the long application of PPI omeprazole in complex treatment of acid-dependent diseases of digestive system organs we applied probiotic, which not only corrects violation of biocenosis of digestive organs, but also has positive influence on immune and endocrine systems of organism [2, 3].

Multiprobiotic of the last generation «Symbiter Acidophilic» shows by itself the mutualistic symbiosis of 14 cultures of probiotic bacteria (bifidobacterium, lactobacillus, lactococcus and propionibacteria) with the high concentration of viable cells ($10^{11\text{-}12}$ CFU/dos.), has a wide spectrum of physiological valuable properties with synergism of the most essential probiotics properties [1]. Concentrated biomass of living cells of microorganisms symbiosis, CFU/sm^3, not less than: lactobacillus and lactococcus – $6{,}0\times10^{10}$, propioniacidic bacteria – $3{,}0\times10^{10}$, bifidobacteria – $1{,}0\times10^{10}$, acetoacidic bacteria – $1{,}0\times10^{6}$ is a content of 1 dose of «Symbiter Acidophilic» (10 ml). It does not need additional activating, but begins to show the action from the oral cavity, because it is a living biomass of cells, but not liofilisate, in which microorganisms are in anabiosis. By modern presentations, the mechanism of positive action of probiotic is based on variability properties of indigenic microflora [4, 5, 6].

The purpose of our research was to study the influence of multiprobiotic «Symbiter Acidophilic» on the oral cavity organs at long-term hypoacidity of gastric juice.

Materials and methods. Experiments were carried out with the observing of recommendations about carrying out of medical-biological researches according to European convention on 42 rats-males of the "Wistar" line, weight of 180-250 g. Euthanasia of animal was carried out under urethane anaesthesia by bloodletting. Omeprazole ("Sigma", USA) was injected intraperitoneally to experimental rats in a dose of 14 mg/kg daily for 28 days separately or together with multiprobiotic «Symbiter Acidophilic» (0,14 ml/kg per os once a day, "O.D. Prolisok", Ukraine), to control rats during this time 0,2 ml of water was injected. Objects of research were soft, bone periodontium tissues and salivary glands of animals in homogenates of which were determined the activity of NO-synthase (NOS) (EC: 1.14.13.39) [8], maintenance of nitrite-anions [7], oxidative-modified proteins (OMP) [8] and middle mass molecules (MMM) [9]. In blood plasma gastrin concentration was determined by a radioimmunoassay technique, using an analytical kit of "MP Biomedicals, LLC", USA. We determined, that gastrin concentration in a blood plasma of rats of the control group was 59,0±35,05 pg/ml, in the experimental animals – 170,7±90,7 pg/ml. Thus, hypergastrinemia is observed as a result of long-term hypoacidity of gastric juice [10].

During the bacterial researches were studied the features of contamination of intestine mucous shell by conditionally pathogenic and normal microflora after 28 days from the beginning of experiment. The study of intestine microbiocenosis included the analysis of specific and quantitative composition of microflora. The quantitative indexes of intestine microflora were studied by sowing of 1 ml from every breeding (1:10) on differential diagnostic environments (varieties of agar etc.). Got results were analyzed with the use of variation statistics methods.

Microecology of control rats intestine was characterized by a wide spectrum of transit microflora. The different representatives of enterobactery (esherihia, citrobacter, klebsiella, proteus, enterobacter et all.) entered in the composition of conditionally-pathogenic bacteria. S*taphilococcus*, enterococcus and Candida fungi were also selected. In 90% of intact animals pathogenic microflora from an intestine practically was not shown. The concentration of lactobacillus and bifidobacterium arrived at a high level – 10^7-10^9 CFU/g. Bacteriological researches of rats intestine maintenance with hypoacidity allowed to find out the negative changes of microecology, which consisted in a disbalance between the indexes of conditionally-pathogenic and normal microflora. The increase of frequency and general amount of staphilococcus and enterobacters (Proteus, Klebsiella, Citrobacter, Enterobacter) is registered, and also Kandida fungi. Found out a tendency to the decrease of quantitative indexes of inoculation of normal intestinal E. coli and enterococcus. The deficiency of indigenic microflora is registered in 70% of rats, its concentration made only 10^4-10^5 CFU/g. Application of «Symbiter Acidophilic» was accompanied by normalization of intestine contamination indexes by the representatives of normal and conditionally-pathogenic microflora: the quantitative indexes of staphilococcus goldish, enrerobacteries and Candida fungi sowing were decreased, population of lacto- and bifidobacteries was increased.

Consequently, multiprobiotic «Symbiter Acidophilic» is able effectively stabilize the colonization resistance and normalize the balance between basic types of obligative and conditionally-pathogenic microflora at long-term hypoacidity in the intestine and in the oral cavity.

Results of research and their discussion. Nitrogen oxide is the important regulator of extra- and intercellular processes in living organisms [11, 12]. One of the mechanisms of metabolic violations development in periodontium tissues , which result to periodontitis, is disorder of local blood flow, one of which reasons is vasoconstriction. Generally known, that endotelial dysfunction plays the important role in the origin of many pathological processes, in the development of which the leading role

has a disbalance of NO-system. Metabolism of bone periodontium tissues is substantially differs from the metabolism of soft periodontium tissues, because of vascularisation features, intensivity of basic metabolism, processes of remodulation, functional loading, existence of intercommunication between bone periodontium tissues and cement of teeth roots, redistribution of the dental loading on bone tissues [13].

For the research of NO-ergic system of periodontium tissues and salivary glands of rats at long-term omeprazole-induced hypergastrinemia we determined the activity of NO-synthase and maintenance of NO_2^-, which is the final product of NO metabolism.

From the table 1 is evidently, that the long introduction of PPI to animals for 28 leads to decrease 1,2 times the activity of NO-synthase in soft periodontium tissues compared to the control. The highest activity of NO-synthase is marked for animals which obtained for 28 days «Symbiter Acidophilic» – in 5,26 times higher, than for control animals (table 1). At 28-daily introduction of «Symbiter Acidophilic» the activity of NO-synthase increases 1,23 times in bone periodontium tissues in comparing with animals to which during this time only PPI was entered (tab. 1). In tissues of salivary glands the activity of NO-synthase at 28-daily omeprazole introduction increased 1,45 times, and at correction with the application of multiprobiotic «Symbiter Acidophilic» the activity of NO-synthase on 28th day of experiment increased 1,18 times ($p<0.05$) in comparing to rats without correction (tabl. 1).

NO_2^- is the final product of NO metabolism in organism. In the free state the period of its life is limited by 6-30 seconds, after that this gas form is oxidated to NO_2^- and other higher oxides. The increase of the NO-production systems power helps in adaptation to the factors of environment. At inflammatory processes, activation of macrophages and neutrophils, and also at development of pathogenic microflora of digestive system organs, maintenance of NO_2^- and NO_3^- ions considerably increase [12].

On 28th day of experiment the content of nitrite anion in soft periodontium tissues of rats with a correction grew 3 times accordingly in comparing with rats which at this time obtained PPI that is explained by the high activity of NOS-enzyme (tabl. 2). At 28-daily introduction of «Symbiter Acidophilic» the maintenance of

Table 1. The activity of NO-synthase in peridontium tissues and salivary glands of rats at long-term usage of PPI and correction by «Symbiter Acidophilic» (M±m)

Groups of animals	The activity of NO-synthase in soft periodontium|mild| tissues, nmol $[NO_2]$/g*min	The activity of NO- synthase | in bone periodontium tissues, | nmol $[NO_2]$/g*min	The activity of NO- synthase | in salivary glands, nmol $[NO_2]$/g*min
1. Control (n=12)	0,123 ± 0,020	0,154 ± 0,012	3,97±0,11
2. Omeprazol 28 days (n=17)	0,103 ± 0,031	0,174 ± 0,018	5,76±0,25
3. Omeprazol + Symbiter 28 days (n=8)	0,338 ± 0,079	0,181 ± 0,024	6,77±0,15
4. Symbiter 28 days (n=5)	0,647 ± 0,379	0,217 ± 0,033	3,90±0,08
Statistical index	$P_{1-2}>0,05$ $P_{1-4}>0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}>0,05$	$P_{1-2}>0,05$ $P_{1-4}>0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$	$P_{1-2}<0,05$ $P_{1-4}>0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$

Note: n – is amount of animals.

Table 2. The maintenance of NO_2^- in periodontium tissues and salivary glands of rats at long-term usage of PPI and correction by «Symbiter Acidophilic» (M±m)

Groups of animals	Contents of NO_2^- in soft periodontium tissues, mmol/g	Contents of NO_2^- in bone periodontium tissues, mmol/g	Contents of NO_2^- in salivary glands, mmol/g
1. Control (n=12)	0,062 ± 0,012	0,058 ± 0,006	0.164 ± 0.007
2. Omeprazol 28 days (n=17)	0,066 ± 0,010	0,069 ± 0,005	0.194 ± 0.006
3. Omeprazol + Symbiter 28 days (n=8)	0,198 ± 0,023	0,144 ± 0,015	0,198 ± 0,003
4. Symbiter 28 days (n=5)	0,113 ± 0,020	0,109 ± 0,020	0,164 ± 0,004
Statistical index	$P_{1-2}>0,05$ $P_{1-4}<0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$	$P_{1-2}>0,05$ $P_{1-4}<0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$	$P_{1-2}<0,05$ $P_{1-4}>0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$

Note: n – is amount of animals.

nitrites in bone periodontium tissues reliable grows 1,58 times compared with animals, which during this time obtained only PPI (tabl. 2). In salivary glands at omeprazole-induced hypergastrinemia the maintenance of NO_2^- increased 1,18 times ($p<0.05$), and at conditions of correction by multiprobiotic "Symbiter Acidophilic" the maintenance of nitrites had not significant changes.

Consequently, the administration to the rats multiprobiotic "Symbiter Acidophilic" at long-term omeprazole-induced hypoacidity promotes the normalization of bloodstream and local regulatory processes in periodontium tissues and salivary glands.

The universal mechanism of tissues damage under the action of different factors is activating of free-radical oxidation (FRO) [14, 15], the indicator of which isdetermination of OMP maintenance[16]. Activation of FRO processes results in the increase of MMM maintenanceand causes endogenous intoxication. Endotoxemia of different genesis causes the increase of MMM concentration and the level of MMM correlates with a severeness of disease [17]. Among the sources of intoxication the basic attention is related to the hearths of inflammatory destruction, ischemised tissues, areas of pathogenic microflora vegetation in organism [18, 19].

Analysing the maintenance of OMP in soft periodontium tissues is marked there significant decrease 2,9 times at the omeprazole administration at the same time with «Symbiter Acidophilic» on the 28th day of experiment compared with animals without correction (tabl. 3). The reliable decrease of OMP maintenance 4 times is marked in periodontium tissues of rats which obtained «Symbiter Acidophilic» for 28 days in comparing to those which obtained omeprazole for 28 days ($P<0,05$) (tabl. 3). From the table 3 is evidently, that maintenance of the oxidative-modified proteins in the salivary glands of rats [20] at conditions of omeprazole-induced hypergastrinemia on the 28th day of omeprazole introduction was increased 1,33 times ($P<0.05$) compared with control, and the usage of multiprobiotic «Symbiter Acidophilic» caused the reliable decrease of OMP maintenance of OMP in salivary glands tissues compared to rats without correction.

On 28th day of experiment the maintenance of MMM is increased 1,06 times ($P<0,05$) in periodontium tissues of rats which obtained omeprazole for 28 days comparing to the control group, while for animals, who obtained only «Symbiter Acidophilic» during this time

Table 3. Maintenance of OMP in periodontium tissues and salivary glands of rats at long-term usage of PPI and correction by «Symbiter Acidophilic» (M±m)

Groups of animals	Contents of OMP in soft periodontium tissues, c.u.	Contents of OMP in salivary glands, c.u.
1. Control (n=12)	0,059 ± 0,008	0,363 ± 0,026
2. Omeprazol 28 days (n=17)	0,211 ± 0,007	0,484 ± 0,023
3. Omeprazol + Symbiter 28 days (n=8)	0,072 ± 0,006	0,449 ± 0,012
4. Symbiter 28 days (n=5)	0,053 ± 0,005	0,338 ± 0,017
Statistical index	$P_{1-2}<0,05$ $P_{1-4}>0,05$ $P_{1-3}>0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$	$P_{1-2}<0,05$ $P_{1-4}>0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$

Note: n – is amount of animals.

its reliable decrease 1,14 times compared to rats without correction (tabl. 4). Contents of MMM in the salivary glands of rats at 28-daily omeprazole application increased 1,32 times ($P<0,05$) compared with control. Analyzing the maintenance of MMM in tissues of rats salivary glands [21] at the usage of multiprobiotic «Symbiter Acidophilic» is reliable decrease of its content compared to animals without correction (tabl. 4).

Thus, application of multiprobiotic «Symbiter Acidophilic» inhibits the processes of FRO and reduces the endotoxemic effect of long omeprazole administration in periodontium tissues and salivary glands.

Thus, the experimental efficiency of probiotics therapy at long-term hypoacidity is proved on the basis of endotelial dysfunction normalization, inhibition of free-radical oxidation processes and warning of endotoxemia development, due to decrease of MMM maintenance in periodontium tissues and salivary glands.

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ЕКСПЕРИМЕНТАЛЬНА КОРЕКЦІЯ МЕТАБОЛІЧНИХ ЗМІН В РОТОВІЙ ПОРОЖНИНІ В УМОВАХ ТРИВАЛОГО ГІПОАЦИДИТЕТУ МУЛЬТИПРОБІОТИКОМ «СИМБІТЕР АЦИДОФІЛЬНИЙ»

З часом зниження шлункової секреції призводить до розвитку гіпергастринемії, дисбіозу та патологічних змін в

Table 4. The maintenance of MMM in periodontium tissues and salivary glands of rats at long-term usage of PPI and correction by «Symbiter Acidophilic» (M±m)

Groups of animals	Contents of MMM in periodontium tissues, c.u.	Contents of MMM in salivary glands, c.u.
1. Control (n=12)	0,174 ± 0,002	0,243 ± 0,016
2. Omeprazol 28 days (n=17)	0,185 ± 0,004	0,321 ± 0,024
3. Omeprazol + Symbiter 28 days (n=8)	0,175 ± 0,001	0,290 ± 0,012
4. Symbiter 28 days (n=5)	0,163 ± 0,002	0,228 ± 0,009
Statistical index	$P_{1-2}<0,05$ $P_{1-4}<0,05$ $P_{1-3}>0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$	$P_{1-2}<0,05$ $P_{1-4}>0,05$ $P_{1-3}<0,05$ $P_{2-3}<0,05$ $P_{2-4}<0,05$

Note: n – is amount of animals.

органах травної системи. Важливим є пошук корекції цих змін. Довготривале застосування омепразолу спричинює метаболічні порушення в тканинах пародонта та слинних залоз, а саме: розвиток дисбалансу NO-ергічної системи та активація вільно-радикальних процесів, які позитивно корегуються мультипробіотиком нового покоління «Симбітер ацидофільний».
Ключові слова: періодонт, слинні залози, омепразол, гіпоацидитет, гіпергастринемія, дисбіоз, «симбітер ацидофільний».

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ЭКСПЕРИМЕНТАЛЬНАЯ КОРРЕКЦИЯ МЕТАБОЛИЧЕСКИХ ИЗМЕНЕНИЙ В РОТОВОЙ ПОЛОСТИ ПРИ ДЛИТЕЛЬНОМ ГИПОАЦИДИТЕТЕ МУЛЬТИПРОБИОТИКОМ «СИМБИТЕР АЦИДОФИЛЬНЫЙ»

Со временем снижение желудочной секреции приводит к развитию гипергастринемии, дисбиозу и патологическим изменениям в органах пищеварительной системы. Важным есть поиск коррекции этих изменений. Длительное применение омепразола приводит к таким метаболическим нарушениям в тканях пародонта и слюнных железах, как развитие дисбаланса NO-эргической системы и активация свободно-радикальных процессов, которые позитивно коррегируются мультипробиотиком нового поколения «Симбитер ацидофильный».
Ключевые слова: пародонт, слюнные железы, омепразол, гипоацидитет, гипергастринемия, дисбиоз, «Симбитер ацидофильный».

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