# PROBIOTIC CONCEPT FOR TREATMENT OF GENERALIZED CHRONIC PERIODONTITIS IN WOMEN

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**Summary**
On the grounds of the experimental and clinical studies parodontoprotective effect of multiprobiotic has been substantiated, indicated by normalization of proteinase-inhibitory balance and NO-system, inhibition of oxidative stress and endotoxicosis, prevention of depolymerization of periodontal connective tissue biopolymers.

**Keywords**
Periodontitis, biofilm, multiprobiotic.

The normal oral microbiocenosis is crucial in maintaining the integrity of the periepithelial biofilm of periodontal tissues and plays an important role in protecting against the aggression of transient pathogenic microflora [1, 2]. Admittedly, breaking of epithelial covering by microbiota representatives and spread of inflammatory infiltrate in the periodontal connective tissues beyond the sulkus gingival is the main pathogenetic element, and the brink of protective biofilm transformation, formed by endogenous oral microbiota. Therefore, we hypothesize that current comprehensive therapy of generalized chronic periodontitis does not lead to sustained remission. We believe that the basis for pathogenetic treatment of periodontal tissues diseases is the multistrain live probiotics who are able to normalize the oral microbiocenosis, contributing to the succession of periodontal pathogens due to high antagonistic action.

Current pharmaceutical market has a wide range of probiotic agents, but most of them are not multistrain ones, and microorganisms are in dried condition that is contraindicated for use in dental practice. Consequently, for correction and treatment of periodontal tissues diseases we have chosen a current domestic multistrain «Simbiter-omega» probiotic [3]. Its main advantages are the following: 1. The living culture. 2. Multistrain diversity due to mutual symbiosis. 3. The highest concentration of $2x10^{10}$ CFU live cells. 4. 200 mg of Montmorillonite clay-based high-purity bentonite gel. 5. 250 ml of flax and wheat sprouts oils, which are the source of ω-3 and ω-6 essential polyene fatty acids.

We believe that the probiotic concept of correction of colonization resistance of the oral cavity is aimed at sustainable recovery of the physiological imbalance of microbiological and immunological links of colonization resistance, considering the specific features of the microbiota.

**The purpose of the research** was aimed at the experimental study and clinical observations to substantiate the application of the multiprobiotic in treatment of periodontal tissue diseases.

## Materials and Methods

Periodontal tissues of 46 Wistar rats, weighted 180-220 g, were studied during the experiment. Animal housing and experiments on them have been carried out in compliance with requirements of international principles of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986). The rodents were kept on a regular ration and in standard condition of vivarium. The animals were killed under urethane anesthesia (50 mg/kg body weight intraabdominally) by bloodletting.

All animals were assigned into groups. Group I was a control group. They were intraabdominally administered with 0.2 ml water for injection once daily during 28 days. Animals from Group II were intraabdominally administered with 14 mg/kg body weight omeprazole, dissolved in 0.2 ml water for injection, daily during 28 days. Animals from Group II were perorally administered with 14 mg/kg body weight omeprazole and «Simbiter-omega» daily during 28 days. Rats with spontaneous periodontitis were not involved into the experiment.

The total activity of NO-synthase [KF 1.14.13.19] has been measured in periodontal tissues by Hevel's method (Hevel J.M. et al., 1991); the content of nitrite-anions has been measured by the Griss method [10].

Proteinase-inhibitory balance has been studied according to indices of total proteolytic activity [5] and total antitryptic activity [6].

The content of intermediate mass molecules has been measured according to Gabrielyan N.I. [7]; content of oxygen modified proteins has been measured according to Dubinina E.E. and Burmistrov S.O. technique [8]; content of TBA-reactants has been measured according to Stalnaya I. method [9]; catalase activity [KF 1.11.1.6] has been determined according to Korolyuk M.A., et al. [10]; superoxide dismutase activity [KF 1.15.1.1] — according to Brusov O.S. [10]; ornithinedecarboxylase activity has been determined by Chinard method, modified by Khramov V.A. [11].

Content of free oxyproline in rat periodontal tissues has been measured by Tetyanets S.S. method [12]; content of glycosaminoglycans in rat periodontal tissues has been measured by Sharayev P.N. method (1987) [13]; content of free fucose in rat periodontal tissues has been measured by Sharayev P.N. et al., method (1997) [14].

51 patients with generalized chronic periodontitis of I-II degree and 20 patients of reference group have been involved into the experiment. Patients have been assigned into following clinical groups according to the therapy of generalized chronic periodontitis of I-II degree: 1) group of apparently healthy patients (n=20); 2) patients with generalized chronic periodontitis of I-II degree (conventional treatment), who were prescribed with «Metrogil-denta» gel use locally and «Fitodent» mouthwash during 5 days (n=15); 3) patients with generalized chronic periodontitis of I-II degree, who were prescribed with «Simbiter-omega» multiprobiotic locally in dentoalveolar trays overnight during 20 (n=36).

*«Simbiter® omega»* multiprobiotic is produced by the «O.D. Prolisok» scientific production company. It comprises of 14 strains of probiotic bacteria that belong to *Bifidobacterium, Lactobacillus,* and *Lactococcus*, *Propionibacterium*, and *Acetobacter*, and are in the form of steady mutual symbiosis. Additionally, the agent consists of 5% solution of flax and wheat sprouts oils, which are the main source of omega-3 and omega-6 essential polyene fatty acids. Probiotic activity of the agent is determined by the high antagonistic activity relative to wide range of pathogenic and opportunistic microorganisms, synthesis of vitamins, short-chain fatty acids, exopolysaccharides, glycopeptides, etc. The *«Simbiter® omega»* multiprobiotic contains not less than $10^{12}$ live cells of probiotic bacteria in one doze (10 $cm^3$) and is indicated to children above 3 years old and adults. One doze (10 $cm^3$) of *«Simbiter® omega»* contains concentrated biomass of microorganisms' symbiosis live cells, CFU/$cm^3$: lactobacilli and lactococci not less $6{,}0x10^{10}$, propionic-acid bacteria not less $3{,}0x10^{10}$, bifidus bacteria not less $1{,}0x10^{10}$, vinegar bacteria not less $1{,}0x10^{6}$.

For effective application of multiprobiotic with its antagonistic action on the majority of opportunistic pathogenic and pathogenic microorganisms, the custom-made dentoalveolar trays were used for direct contact of agent's symbiotic microflora with periodontal pathogens.

The dentoalveolar trays, filled with *«Simbiter® omega»* multiprobiotic overnight during 20 days, were made of polyethylene on the *«Ultraform»* dental vacuum composer.

Unstimulated oral fluid, collected from the patients on an empty stomach has been biochemically examined to measure the content of oxygen modified proteins [8] and catalase activity [10].

The degree of endogenous intoxication has been determined according to the content of intermediate mass molecules [7]; proteinase-inhibitory potential has been measured through proteinase activity [5]

and total antitryptic activity [6], ornithine decarboxylase activity [11].

Statistical processing of the results has been made on the «Intel Pentium 4» PC, using the «Microsoft Exel» software for «Windows Professional» with estimation of the average values of the (M) parameters and (±m) mean error.

## Results

The model of omeprazole-induced hypoacidity has been used for simulation of rat oral dysbiosis. Notwithstanding some specificity of microbiocenoses in different biotopes, one should take into account a close correlation between the local microbial systems. In this way each system, being a part of the whole microecological system, is integrated into it by the compound, extensively branched links of interaction between the local biocenoses and each microbial ecosystem with the whole macroorganism. Therefore, violations of microbial ecology in any biotope will inevitably involve the other ones into pathological process, as well as organs and systems that are mutually related and dependent on the microbial ecology.

**Table 1**

Biochemical indices in rat periodontal soft tissues in conditions of prolonged administration of omeprazole and correction with «Simbiter-omega» multiprobiotic (M±m)

| No | Biochemical indices | 1. Control (n=12) | 2. Omeprazole 28 days (n=17) | 3. Omeprazole + «Simbiter-omega» 28 days (n=17) | Statistical indicator |
|---|---|---|---|---|---|
| 1. | GAG content, мсmol/g | 1.117±0.067 | 1.526±0.106 | 0.809±0.016 | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 2. | Fucose content, мсmol/g | 1.757±0.259 | 1.219±0.333 | 1.091±0.843 | $p_{1-2}>0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 3. | Oxyproline content, мсmol/g | 3.292±0.142 | 6.151±0.205 | 4.140±0.150 | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 4. | Antitryptic activity, g/kg | 39.798±0.542 | 32.503±0.961 | 3.010±0.050 | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 5. | Activity of NO-synthase, nmol ($NO_2$)/g*min | 0.123±0.020 | 0.103±0.031 | 0.89±0.047 | $p_{1-2}>0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 6. | $NO_2^-$ content, мmol/g | 0.062±0.012 | 0.066±0.010 | 0.208±0.006 | $p_{1-2}>0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 7. | Content of oxygen modified proteins, a.u. | 0.059±0.008 | 0.211±0.007 | 0.056±0.003 | $p_{1-2}<0.05$<br>$p_{1-3}>0.05$<br>$p_{2-3}<0.05$ |
| 8. | Content of intermediate mass molecules, a.u. | 0.174±0.002 | 0.185±0.004 | 0.071±0.005 | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |

*Note: n — number of rodents.*

It has been established that content of glycosaminoglycans (GAG) in periodontal soft tissues in conditions of prolonged administration of omeprazole was 1.37 time higher ($p<0.05$) as compared with control. Application of «Simbiter-omega» multiprobiotic during 28 days, accompanied by omeprazole-induced hypoacidity, contributed to a significant reduce of GAG content in soft periodontal tissues, as compared with rodents without correction (Table 1).

In conditions of prolonged administration of omeprazole along with application of «Simbiter-omega» multiprobiotic free fucose content in rat periodontal soft tissues was 1.61 and 1.97 times lower ($p<0.05$) on day 28 of the experiment as compared with rats from control group and rats without correction, respectively (Table 1).

In 28-day long administration of omeprazole content of oxyproline in rat periodontal soft tissues was 1.87 time higher ($p<0.05$), as compared with control; in conditions of application of «Simbiter-omega» multiprobiotic accompanied by prolonged hypoacidity content of oxyproline was 1.49 time lower ($p<0.05$), as compared with animals without correction (Table 1).

Thus, in conditions of prolonged administration of proton pump inhibitor depolymerization of macromolecules in connective tissues occurs in rat periodontal tissues, proved by significant increase of GAG content and oxyproline, as compared with the control. Introduction of «Simbiter-omega» multistrain multiprobiotic to rodents, administered with omeprazole during 28 days, contributed to significant lowering of GAG content, free fucose and oxyproline in periodontal tissues, as compared with rats without correction.

The analysis of the total antitryptic activity in rat periodontal soft tissues in conditions of prolonged administration of omeprazole shows its significant lowering by 1.22 time ($p<0.05$), as compared with the control. 28-day long application of «Simbiter-omega» multiprobiotic accompanied by omeprazole-induced hypoacidity of gastric juice contributed to significant reduce of the mass antitryptic activity in the periodontal soft tissues by 10.8 times as compared with animals without correction (Table 1).

In 28-day long administration of omeprazole no significant change in total activity of NO-synthase in periodontal soft tissues was noted, as compared with the control, whereas the content of nitrite-anions was 1.06 time higher. Correction

with «Simbiter-omega» multiprobiotic facilitated increase of mass activity of NO-synthase by 8.64 times ($p<0.05$), as compared with rats without correction. Correction with «Simbiter-omega» multiprobiotic in conjunction with omeprazole-induced hypoacidity promoted the rise of nitrite-anions content in periodontal tissues by 3.15 times ($p<0.05$), as compared with rats without correction (Table 1).

In condition of prolonged administration of omeprazole the content of oxygen modified proteins in rat periodontal soft tissues was 3.58 times higher ($p<0.05$), as compared with the control. 28-day long application of «Simbiter-omega» multiprobiotic accompanied by omeprazole-induced hypoacidity contributed to significant reduce of oxidation-modifies proteins content in periodontal soft tissues, as compared with animas without correction (Table 1).

In condition of administration of omeprazole during 28 days the content of intermediate mass molecules in the periodontal soft tissues was 1.06 higher ($p<0.05$), as compared with the control. 28-day long application of «Simbiter-omega» multiprobiotic accompanied by the prolonged inhibition of gastric secretion contributed to its reduce by 2.61 times ($p<0.05$), as compared with animas without correction (Table 1).

The abovementioned findings made it evident that application of «Simbiter-omega» multiprobiotic reduced free radical oxidation in periodontal tissues, induced by the prolonged use of proton pump inhibitor.

In sum, 28-day long administration of «Simbiter-omega» multiprobiotic accompanied by the prolonged hypoacidity of gastric juice prevented the development of oxidative stress, the increase of catabolism of collagenous and non-collagenous proteins in periodontal connective tissues, and contributed to NO-system activation.

Objectively, application of «Simbiter-omega» multiprobiotic along with conventional treatment of patients from all groups contributed to reducing of gingival inflammation, gingival papilla inflammation and depth of parodontal recesses.

It was established that the total proteolytic activity of oral fluid of patients with generalized chronic periodontitis was significantly reduced in patients from all groups, who received treatment, as compared with the rate before treatment. It was 1.19 time higher in patients who received conventional treatment and 1.32 time higher in patients who were administered with «Simbiter-omega», as compared with the rates before treatment (Table 2). The total antitryptic activity of oral fluid was 1.81 time reliably higher in patients, who received treatment with «Simbiter-omega» (Table 2).

Consequently, the findings of proteinase-inhibitory potential of the oral fluid of patients with generalized chronic periodontitis showed that application of «Simbiter-omega» agent in dentoalveolar trays, contributed to normalization of proteolytic processes.

It has been found that before treatment the activity of ornithinedecarboxylase was reliably being reduced in the oral fluid of all patients with

## Table 2

Biochemical indices of oral fluid of patients with generalized chronic periodontitis of I-II degree before and after conventional treatment and adjunctive therapy with «Simbiter-omega» multiprobiotic (M±м)

| No | Index | 1. Control (n=20) | 2.Conventional treatment (n=15) | | 3. Application of «Simbiter-omega» (n=36) | | Statistical indicator | |
|---|---|---|---|---|---|---|---|---|
| | | | Before | After | Before | After | Before | After |
| 1. | Total proteolytic activity, mkg/ml*min | 11.4±0.44 | 15.24±0.64 | 12.76±0.60* | 18.03±0.13 | 13.7±0.49* | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}>0.05$ |
| 2. | Total antitryptic activity, g/l | 5.03±0.03 | 24.07±0.48 | 26.07±0.68* | 2.44±0.16 | 4.42±0.08* | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 3. | Ornithine decar-boxylase activity, nmol/ml*min | 34.47±0.53 | 17.89±0.46 | 19.50±0.36* | 23.64±1.15 | 30.9±1.11* | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 4. | Content of oxygen modified proteins, a.u. | 0.05±0.003 | 0.127±0.007 | 0.083±0.021 | 0.120±0.005 | 0.06±0.002* | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}>0.05$ | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 5. | Content of intermediate mass molecules, a.u. | 0.10±0.004 | 0.263±0.015 | 0.183±0.019* | 0.200±0.006 | 0.110±0.005* | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ | $p_{1-2}>0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ |
| 6. | Catalase activity, mccat/l | 0.32±0.004 | 0.40±0.07 | 1.08±0.05* | 0.13±0.0067 | 0.29±0.0099* | $p_{1-2}<0.05$<br>$p_{1-3}<0.05$<br>$p_{2-3}<0.05$ | $p_{1-2}<0.05$<br>$p_{1-3}>0.05$<br>$p_{2-3}<0.05$ |

** — p<0.05 between the groups before and after treatment.*

generalized chronic periodontitis, as compared with the control (Table 2). In patients with generalized chronic periodontitis treated with «Simbiter-omega» multiprobiotic, applied under custom-made dentoalveolar trays overnight the activity of ornithinedecarboxylase in the oral fluid after treatment significantly increased, as compared with the activity of the enzyme before treatment (Table 2).

Activation of free radical oxidation in the oral fluid in all patients with chronic generalized periodontitis have been noted, indicated by the significant increase of the content of oxygen modified proteins, as compared with the control. Content of oxygen modified proteins in oral fluid of patients with generalized chronic periodontitis, treated with «Simbiter-omega» multiprobiotic was 2 times lower (Table 2).

The significant lowering of endogenous intoxication degree was noted in the oral fluid of patients with chronic generalized periodontitis who received conventional treatment along with «Simbiter-omega» multistrain multiprobiotic, indicated by the reduction of the content of intermediate mass molecules by 1.44 and 1.82 times after treatment and before treatment, respectively (Table 2).

Furthermore, before treatment a significant reduction in catalase activity has been noted in the oral fluid of patients with generalized chronic periodontitis of all the studied groups, as compared with the control. After treatment catalase activity increased significantly in patients with generalized chronic periodontitis, who received conventional treatment and adjunctive therapy with «Simbiter-omega» by 2.7 and 2.23 times, respectively (Table 2).

Another evidence of intensification of free radical oxidation in the oral fluid of all patients with generalized chronic periodontitis is the significant increase of the TBA-reactants content. Application of «Simbiter-omega» multiprobiotic for patients with generalized chronic periodontitis contributes to significant decrease of content of TBA-reactants in oral fluid as compared to the rates before treatment and the control (Table 2).

Therefore, application of «Simbiter-omega» multiprobiotic for all patients with generalized chronic periodontitis contributes to oxidative stress inhibition along with increase of antiradical protection of the oral fluid.

In conclusion, the study of proteinase-inhibitory potential, activity of ornithinedecarboxylase and enzymes of antioxidant protection, as well as the degree of endotoxicosis and activity of free radical processes of oral fluid of patients with generalized chronic periodontitis has proven the clinical efficacy of «Simbiter- omega» multiprobiotic application.

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