

INFLUENCE OF THE LOCAL AND SYSTEMIC OXIDATIVE STRESS ON PERIODONTITIS: ROLE OF ANTIOXIDANT THERAPY

Mihail Petrovich Saulin, Sergey Bolevich, Ekaterina Silina, Aleksandra Orlova, Natalija Raičević, Sergey Vorobiev, Stefany Bolevich, Tatjana Sinelnikova
Department of Human Pathology, I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation

UTICAJ LOKALNOG I SISTEMSKOG OKSIDATIVNOG STRESA NA PERIODONTITIS: ULOGA ANTIOKSIDANTNE TERAPIJE

Mihail Petrovič Saulin, Sergej Bolevič, Ekaterina Silina, Aleksandra Orlova, Natalija Raičević, Sergej Vorobiev, Stefani Bolevič, Tatjana Sinelnikova
Odsek za humanu patologiju, I.M. Sechenov Prvi Moskovski državni medicinski univerzitet, Moskva, Ruska Federacija

Received / Priljen: 20. 11. 2018.

Accepted / Prihvaćen: 15. 12. 2018.

ABSTRACT

This study was divided into experimental part of study which was conducted on 75 Wistar rats with the modeled periodontitis and and clinical part of research which included 106 patients with the chronic generalized periodontitis (CGP). The study established an importance of the oxidative stress (both local and systemic) in development and progress of the disease. It is found out that the saliva of rats with the modeled periodontitis there is the reliable increase in the content of total protein, alkaline phosphatase (ALP) and malonic dialdehyde (MDA) in 1,2, 2,6 and 2,8 times respectively, with the reduced activity of catalase in 2,5 times (all $p < 0,05$). It is determined that the gingiva tissue of rats with the modeled periodontitis has the reduced contents of total protein, collagen, elastin and sulfated glycosaminoglycans in 2,8, 1,5, 1,6 and 1,3 times respectively (all $p < 0,05$). It is proved that the antioxidant (AO) therapy normalizes in the rat saliva the content of protein and MDA (decrease in 1,2 and 1,8 times accordingly, $p < 0,05$) and increases the activity of catalase (in 2,5 times, $p < 0,05$). Calcium D_3 normalizes the protein content and activity of ALP (decrease in 1,2 and 1,5 times, respectively, $p < 0,05$).

It is found out that the saliva of patients with CGP in the acute phase the content of protein, ALP and MDA increases in 1,9, 2,2 and 1,5 times accordingly ($p < 0,05$) with the reduced catalase activity in 1,1 times ($p < 0,05$). It is revealed that the inclusion of CGP patents in AO complex therapy results jointly with the best clinical effect in the more expressed reduction in generation of reactive oxygen species and lipid peroxidation and also the increased plasma APA.

Keywords: periodontitis, chronic generalized periodontitis, oxidative stress, free radicals

SAŽETAK

Ova studija podeljena je na eksperimentalni deo studije koji je sproveden na 75 Wistar pacova sa modelom periodontitisa i klinički deo istraživanja, koji uključuje 106 pacijenata sa hroničnim generalizovanim periodontitisom (CGP). Studija je utvrdila značaj oksidativnog stresa (kako lokalnog tako i sistemskog) u razvoju i napretku bolesti. Utvrđeno je da je pljuvačka pacova sa periodontitisom povećanog sadržaja ukupnih proteina, alkalne fosfataze (ALP) i malonil-dialdehida (MDA) i to 1,2, 2,6 i 2,8 puta postepeno, sa smanjenom aktivnošću katalaze 2,5 puta ($p < 0,05$). Utvrđeno je da tkivo gingiva pacova sa periodontitisom ima smanjeni sadržaj ukupnih proteina, kolagena, elastina i sulfatnih glikozaminoglikana 2,8, 1,5, 1,6 i 1,3 puta ($p < 0,05$).

Dokazano je da se antioksidativna terapija (AO) normalizuje u pljuvački sadržaj proteina i MDA (smanjuje se 1,2 i 1,8 puta, $p < 0,05$) i povećava aktivnost katalaze (2,5 puta, $p < 0,05$). Kalcijum D_3 normalizuje sadržaj proteina i aktivnost ALP-a (smanjenje 1,2 i 1,5 puta, respektivno, $p < 0,05$). Utvrđeno je da pluća pacijenata sa CGP u akutnoj fazi sadrže 1,9, 2,2 i 1,5 puta veći sadržaj proteina, ALP i MDA ($p < 0,05$) uz smanjenu aktivnost katalaze 1,1 puta ($p < 0,05$). Dokazano je da uključivanje antioksidativne terapije pacijentima sa CGP rezultira dobrim kliničkim efektom što se ogleda u redukciji reaktivnih kiseončnih vrsta i peroksidacije lipida, kao i povećanog APA u plazmi.

Ključne reči: periodontitis, hronični generalizovani periodontitis, oksidativni stres, slobodni radikali



UDK: 616.314.7:542.943'78; 616-008.9:[577.334:546.21

Ser J Exp Clin Res 2018; 19 (4): 365-372

DOI: 10.2478/SJECR-2018-0078

Corresponding author: Prof. dr. Bolevich Sergey,

Department of Human Pathology, I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation

E-mail: boleovich2011@yandex.ru



INTRODUCTION

To date, one of the most pressing problems of dentistry is inflammatory periodontal diseases (1, 2). According to the WHO, these diseases are very aggressive and almost not treatable (3). Their incidence between the ages of 35 to 44 ranges from 55% to 95%, while for people over 45 years it makes from 95% to 99% (4, 5). Currently, there is large bank of accumulated extensive studies on periodontitis. However, despite the improvement of surgical, therapeutic and orthopedic techniques, periodontal diseases are steadily progressing (6, 7). In addition, it is still a controversial issue on the priority of etiology and pathogenesis. The researchers pay more and more attention to studying endogenous causes of periodontitis, as one of the most common forms of periodontal inflammatory diseases (8, 9). However, the impact of exogenous factors on the development of such diseases, in our view, is insufficiently viewed in the literature.

The research relevance is determined by such factors such as the growth of morbidity (10), difficulty of early diagnostics, difficulty in reaching the stable remission, relationship of the general body condition and the condition of periodontal tissues (11).

One of the first hypotheses for the development of periodontitis was the hypothesis of nonspecific infection bloom (1960-1973) which implied that inflammatory periodontal diseases developed due to nonspecific infection of dental plaques microorganisms. It was assumed that periodontitis develops due to the increased number of dental plaque bacteria. However, experimentally it was discovered that not all experimental dogs evolved periodontitis, while there was the increasing dental plaques biomass (3-5).

In 1975 leadership the leadership was captured by the plaque specific hypothesis. It was discovered that on the tooth plaque there is the specific micro flora, the so-called periodontal pathogenic bacteria (2, 6). Thus, in 1985 year a new theory of periodontitis development was introduced, the theory of opportunistic infections.

To date, the generally accepted view is that under the influence of exogenous or endogenous factors there is an activation of microorganisms in the dental plaque replacing other bacteria (12, 13). Thus the changed body defenses, local changes in the acid-base equilibrium, hypoxia, anaerobic niche etc. form the environment which is convenient for reproduction of pathogenic bacteria causing the opportunistic infection activity and development of inflammatory periodontal diseases. The pathogenesis of periodontitis, as an inflammatory periodontal disease involves free-radical processes (FRP), oxygen (generation of active oxygen forms by leukocytes (GAROFL) and lipid (peroxidation of lipids) (POL) (7). However, the number of researches devoted to FRP study for the patients with periodontal diseases is few and conflicting (14-16).

Due to that it is reasonable and actual to have a complex study of GAROFL, the content of malonic dialdehyde

(MDA) in the saliva, the gum tissues and blood plasma and also the anti-peroxide activity (APA) in the rat plasma with the modeled periodontitis and the patients with the chronic generalized periodontitis (CGP) and to correct their disturbances by the antioxidant (AO) therapy.

MATERIALS AND METHODS

Experimental part of study

The experiments were carried out on 75 Wistar male rats with the weight of 180-200 g at the age of 16-18 weeks. The animals were divided into five groups: 1. Control (n=15); 2. Comparison group (n=15); 3. Group of animals treated with AO therapy (citoflavin dose of 130,0 mg/kg/day, mexidol dose of 100 mg/kg/day and the ascorbic acid dose of 4 mg/kg/day (n=15); 4. Group of animals treated with calcium D₃ dose of 130,0 mg/kg/day by calcium, 51,4 ME/kg/day by vitamin D₃ (n=15); 5. Group of animals treated with AO therapy and calcium D₃ (n=15).

For animals of 2-5 groups the periodontitis was modeled by adding to the fodder mix of experimental animals the oxidized sunflower (10 % of the ration weight, approximately 2 ml/day per rat) (5). It is proved that this atherogenic diet causes periodontal changes, which according to the morphological manifestations correspond to periodontitis of the human. The sunflower oxidation was made by its heating within 40 minutes at the temperature of 130–150°C with the air blowing under the presenting catalyst - oxidizer (0,1% CuSO₄).

The animals were observed for 45 days, after which 60 rats receiving the oxidized diet were divided into 2, 3, 4 and 5 groups and marked. 1st group of rats (n=15) received the common sunflower and was a group of the negative control. Further on animals from 3-5 groups were treated according to their marking by the intra-ventral introduction of researched preparations each day for 14 days.

To evaluate the therapy we assessed the local (gum biochemistry) and system action of the drugs (saliva biochemistry).

At the end of the research protocol the animals were subjected to euthanasia under the ethereal anesthesia by the instant decapitation. 24 hours before that the rat saliva stimulated by pilocarpin was sampled (0,1% pilocarpin hydrochloride subbualno). The collected samples were centrifuged at 6000 rpm for 20 minutes to obtain supernatants, with the determined content of protein (by biuret method), alkaline phosphatase (ALP, by Bessei-Lowry-Brock method), MDA (reaction with 2-thiobarbituric acid) and catalase (by reaction with the molybdenum reagent) (8,9,10,15). After euthanasia, the animal tissues were sampled for the biochemical analysis. In the gum tissue the protein content was evaluated by the biuret method and also components of the connective tissue matrix: collagen, elastin and sulfated glycosaminoglycans spectrophotometrically with using the readymade sets of "BIOCOLOR" firm (UK).



Clinical part of study

The clinical research included 106 patients (51 men (48,1%) and 55 women (51,9%) with CGP at the age from 17 to 77 years (the average age made $49,81 \pm 9,04$).

When included in the study the patients were randomized by the random sampling method into three groups: 1 comparison group was 32 (30,2%) patients at the age from 30 to 73 (the average age made $45,76 \pm 9,13$), receiving the traditional therapy including the standard complex of surgical, therapeutic and orthopedic measures. 2 main group included 39 (36,8%) patients at the age from 28 to 76 (the average age made $50,33 \pm 10,62$). All patients in this group in the traditional therapy structure received additionally AO therapy (cytoflavin of 1–2 pills 2 times per day for 25 days, mexidol of 1 pill (125 mg) 3 times per day and ascorbic acid of 1 bean (100 mg) 3 times per day). 18 (16,7%) patients received additionally calcium (calcium D₃) of 1 chewing pill 2 times per day for 30 days. 3rd group included 35 (33%) patients at the age from 28 to 76 (the average age made $49,76 \pm 9,20$), receiving the traditional therapy (10 persons; comparison group) and additionally the treatment - prophylactic paste (LPP) based on medicinal herbs (15 persons). They cleaned their teeth with it at home in the usual way 2-4 times per day for 2-3 weeks. The patient groups were homogeneous and statistically not different from each other.

The patient treatment tactics corresponded to the standards and included a complex of surgical, therapeutic and orthopedic measures. Dental deposits of all patients were removed, with smoothing and polishing the root naked part and immobilization of movable teeth by splinting. As the local therapy they used antiseptic rinsing, applications on the gingival edge mucous membrane and introduction of anti-inflammatory agents in the periodontal pocket.

The patient condition was made in dynamics (at admission, on the 4th and 30th day of the therapy) based on the complex of clinical, laboratory, instrumental research methods: studying the anamnesis and complaints, examination of the dental edge on the mucous membrane with evaluating the recession degree of the dental edge and teeth mobility, researching the content of total protein, ALT, MDA and catalase in the saliva, examining FRP in the blood plasma by GAROFL indices - basal rate of the chemiluminescence intensity (PICLb) and the chemiluminescence intensity rate stimulated by zymosan (PICLs), by indicators of APA plasma, MDA, the roentgen imaging based on the orthopantomogram with analysis of the reduced intra-alveolar septum height and the level of teeth root exposure, measuring the depth of periodontal pockets by the periodontal probe, periodontal index (PI), Federov-Volodkin index of hygiene (IH) and expression index of the inflammatory phenomena, gingivitis, papillary-marginal-alveolar index (PMA).

Chemiluminescence (CL) indexes of GAROFL were researched on the chemiluminescencemeter LCB "Wallac" (Sweden) adapted for the chemiluminescencemetry at the

standard temperature of $36,9^{\circ}\text{C}$. The detected parameters were the level of basal (spontaneous) CL (PICLb) in the standard mist volume of leucocytes with their standard concentration (2500 in $1 \mu\text{l}$). After adding a non-specific activator (0,1 ml of 1% zymosan solution) PICLs were detected. MDA - is the secondary product of POL, determined with using the methods described by Douest J.C. (1983). The research method of plasma APA is based on measuring and comparing indices of the CL plasma induced by the hydrogen peroxide and its spontaneous CL (Ind/Sp CL). The calculated ratio is the value which is inversely proportionally to plasma APA. The less is this ratio, the more is APA and vice versa.

STATISTICAL ANALYSES

The statistical processing of the results was made by using the programs of Microsoft Excel and the data statistic analysis package of Statistica 8.0 for Windows (StatSoft Inc., USA) and SPSS 15.0. The differences were considered statistically important at the error level of $p < 0,05$. For the quantitative variables the test was made on the normality of distribution by means of Shapiro- Wilk criterion. To assess the factual results the statistic analysis methods were used: χ^2 - Pearson criterion (analysis of mating tables), t-criterion of Student, Newman - Keils criterion for multiple comparisons. For independent non-parametric samples we used Mann-Whitney criterion, for the multiple comparison - Kraskel - Wallis criterion. For the dependent non-parametric samples we used the Wilcoson criterion, for the multiple comparisons - Friedman.

RESULTS

We have detected that in the experiment the rat saliva with the modeled periodontitis is known to have the increased total protein for 1,2 times ($p < 0,05$) due to inflammation and ALP in 2,6 times ($p < 0,05$) due to cytolysis with the simultaneous increased content of MDA in 2,8 times ($p < 0,05$) and less catalase activity in 2,5 times ($p < 0,05$), which proves activation of FRP and less AO protection (Table 1).

In the gum tissues of rates with the modeled periodontitis there is the less content of total protein, collagen, elastin and sulfated glycosaminoglycans in 2,8, 1,5, 1,6 and 1,3 times accordingly (all $p < 0,05$), which proves the predominance of catabolic processes in development of periodontal disease (Table 2).

The similar data were obtained for the patients with the CGP. In the saliva there was the protein increased in 2,2 times ($p < 0,05$), ALP in 3,3 times ($p < 0,05$) and MDA content in 1,7 times ($p < 0,05$) at the simultaneously reduced catalase in 3,1 times ($p < 0,05$ times; Table 3).

For the same patients in the blood there was the increased PICLb in 1,6 times ($p < 0,05$) and PICLs in 3,9 times



Table 1. The content of protein, MDA and ALP activity and rat saliva catalase in the control, comparison group and after treatment of AO therapy, calcium D₃ and combination of AO therapy with calcium D₃.

Researched groups	Protein (g/l)	ALP (ME/l)	MDA (μ/l)	Catalase (μcat/l)
Control (n=15)	6,28±0,29	522,60±4,26	1,21±0,14	192,7±8,1
Comparison (n=15)	7,79±0,34*	1308,6±125,0*	3,27±0,22*	72,8±6,2*
AO (n=15)	6,42±0,38**	1199,8±176,5* ##	1,72±0,36**	179,3±12,8** #
Calcium D ₃ (n=15)	6,47±0,53**	872,4±44,5* **^	2,32±0,19* **^	114,3±11,0* **#^
AO+ Calcium D ₃ (n=15)	6,35±0,38**	558,3±81,3**##^	1,36±0,38**^	191,0±9,3**^

Note: * - $p \leq 0,05$ as compared with the Control group; ** - $p \leq 0,05$ as compared with the comparison group; # - $p \leq 0,05$ the difference of rat groups receiving AO therapy and Calcium -D₃; ## - $p \leq 0,05$ the difference of rat groups receiving AO therapy and AO therapy + Calcium -D₃; ^ - $p \leq 0,05$ the difference of rat groups receiving Calcium -D₃ and AO therapy + Calcium -D₃

Table 2. The content of protein, collagen, elastin and sulfated glycosaminoglycans (GAG) in the gum tissues of rats in the control, comparison group and after treatment of AO therapy, calcium D₃ and in combination of AO therapy with calcium D₃.

Researched groups	Protein (g/l)	Collagen (mcg/g)	Elastin (mcg/g)	Sulfated GAG (mcg/g)
Control (n=15)	201,0±4=5,1	65,7±5,1	33,8±2,6	107,0±7,9
Comparison (n=15)	71,8±2,9*	42,7±1,9*	23,1±2,3*	81,1,8±1,9*
AO (n=15)	161,2±8,8* ** ##	56,7±2,6** ##	31,3±2,1** #	89,1±2,9* ** ##
Calcium D ₃ (n=15)	148,4±6,8* **^	61,4±2,0**^	24,2±1,6* #^	82,4±3,8*^
AO+Calcium D ₃ (n=15)	215,7±10,4** #^	71,5±1,4** #^	36,1±2,5** ^	108,9±3,9** #^

Note: * - $p \leq 0,05$ as compared with the Control group; ** - $p \leq 0,05$ as compared with the comparison group; # - $p \leq 0,05$ the difference of rat groups receiving AO therapy and Calcium -D₃; ## - $p \leq 0,05$ the difference of rat groups receiving AO therapy and AO therapy + Calcium -D₃; ^ - $p \leq 0,05$ the difference of rat groups receiving Calcium -D₃ and AO therapy + Calcium -D₃

Table 3. The content of protein, MDA and ALP activity and saliva catalase for healthy donors and patients with CGP before treatment and on the 14th and 30th day after treatment

Researched indicator	1 day	14 day	30 day
Protein (N: 2,00±0,2 g/l)			
CGP comparison group (n=10)	4,38±0,56*	3,41±0,33*	2,30±0,21**
CGP of LPP group (n=15)	3,78±0,20*	2,70±0,27**	2,10±0,22** #
ALP (N: 1,0±0,1ME/L)			
CGP comparison group (n=10)	3,27±0,3*1	2,91±0,29*	2,31±0,21* **
CGP of LPP group (n=15)	2,21±0,38*	1,30±0,14**	1,10±0,12**
MDA(N: 7,0±0,7 mmol/l)			
CGP comparison group (n=10)	11,73±0,67*	10,87±0,62*	9,39±0,56* **
CGP of LPP group (n=15)	10,21±0,48*	8,80±0,90*	7,70±0,80**
Catalase (N: 250,0±20,0ME/l)			
CGP comparison group (n=10)	80,24±5,41*	89,05±5,52*	91,86±5,15*
CGP of LPP group (n=15)	219,98±9,30*	231,00±11,37*	245,00±12,55**

Note: * - $p \leq 0,05$ – as compared with the norm; ** - $p \leq 0,05$ – as compared with 1st day; # $p \leq 0,05$ – as compared with 14 day;

($p < 0,05$), and also MDA content in the plasma in 1,4 times ($p < 0,05$) and the reduced plasma APA in 2,1 times ($p < 0,05$).

The revealed changes of FRP objectify the relevance of prescribing antioxidant, energy-correcting drugs to the patients suffering periodontitis which is mostly efficient in the early stages of the disease. The additional prescription of pathophysiological treatment will prevent FRP transfer from the deficient stage of O₂ and energy into the stage of

necrosis. To restore the cellular energy deficit the amber acid was used, stimulating the Krebs cycle and production of ATP additional amount, citoflavin in combination with mexidol and ascorbic acid. To improve the results treatment was supplemented with calcium and vitamin D₃ (calcium D₃).

According to data obtained when researching the rat saliva it is clear (Table 1) that AO therapy normalizes the



content of protein and MDA (it is reduced in 1,2 and 1,8 times accordingly; $p < 0,05$ and $p < 0,05$ accordingly) and increases the catalase activity (in 2,5 times; $p < 0,05$). Calcium D_3 normalizes the protein content and ALP activity (they are decreased in 1,2 and 1,5 times accordingly; $p < 0,05$ and $p < 0,05$ accordingly). The combined treatment of AO therapy and calcium D_3 completely normalized all researched parameters of the rat saliva. The research of the rat gum tissue demonstrated (Table 2), that AO therapy increases the content of protein, collagen and elastin (in 2,3, 1,2 and 1.4 times; all $p < 0,05$) but they remained significantly lower than the norm. Calcium D_3 caused the further increase in the content of protein and collagen (in 2,1 and 1,4 times accordingly; $p < 0,05$ и $p < 0,05$ accordingly). The combined introduction of AO therapy and calcium D_3 completely normalized all researched parameters of the rat gum tissue. Thus the antioxidant therapy resulted in the regressed inflammation and reduced FRP with the simultaneous improvement of AO protection in the saliva and rat gum tissues with the modeled periodontitis.

Based on the saliva indicators of CGP patients (Table 3) LPP resulted in less content of pro-inflammatory substances in the saliva (protein, MDA and ALP in 1,8, 1,3 and 2,0 times accordingly; all $p < 0,05$) and increased the anti-inflammatory agents (catalase in 1,1 times; $p < 0,05$), which was more expressed than in the comparison group. LPP caused both improved biochemical parameters and better clinical picture of the disease (index of RMA, PI and IG reduced in 3,4, 1,6 and 1,5 times accordingly; all $p < 0,05$).

The impact results of the traditional therapy, AO therapy and AO therapy in combination with calcium D_3 on PICLb are given on Fig.1.

For CGP patients receiving the traditional therapy ($n=32$) PICLb reduced in 1,2 times ($p > 0,05$), and for CGP patients, receiving AO therapy ($n=39$), and AO therapy in combination with calcium D_3 ($n=18$) in 1,4 and 1,6 times accordingly ($p < 0,05$ and $p < 0,05$ accordingly). Thus, PICLb reduced reliably only under the impact of AO and AO therapy in combination with calcium D_3 .

The impact results of the traditional therapy, AO therapy and AO therapy in combination with calcium D_3 on PICLs are given on Fig.2.

For CGP patients receiving the traditional therapy ($n=32$) PICLs reduced in 2,8 times ($p < 0,05$), and for CGP patients, receiving AO therapy ($n=39$) and AO therapy in combination with calcium D_3 ($n=18$) in 3,1 and 3,7 times accordingly ($p < 0,001$ and $p < 0,001$ accordingly). Thus, PICLs was reliably reduced for CGP patients, receiving AO therapy and AO therapy in combination with calcium D_3 as compared with CGP patients receiving only the traditional therapy.

The impact results of the traditional therapy, AO and AO therapy in combination with calcium D_3 on MDA content in the plasma are given on Fig.3.

For CGP patients receiving the traditional therapy ($n=32$) MDA content in the blood plasma was reduced in 1,2 times ($p > 0,05$), and CGP patients receiving AO therapy ($n=39$) and AO therapy in combination with calcium D_3 ($n=18$) in 1,3 and 1,3 times paza accordingly ($p < 0,05$ and $p < 0,05$ accordingly). Thus, MDA content in the blood plasma was reliably reduced only under the influence of AO therapy and AO therapy in combination with calcium D_3 .

The impact results of the traditional therapy, AO therapy and AO therapy in combination with calcium D_3 on the plasma APA are given on Fig.4.

For CGP patients receiving the traditional therapy ($n=32$) the ratio of the induced to spontaneous plasma CL reduced in 2,0 times ($p < 0,05$). Similar results were obtained for CGP patients receiving AO therapy ($n=39$) and AO therapy in combination with calcium D_3 ($n=18$; in 2,0 and 2,0 times accordingly; $p < 0,05$ and $p < 0,05$ accordingly). Thus, the plasma APA was increased for all patients regardless the method of treatment.

The positive dynamics of FRP parameters was accompanied with the between clinical picture of the disease and treatment results. The patients receiving AO therapy to a greater degree demonstrated the reduced featured of inflammation, the mucous became pink, with its more density. Already in 14 days of the therapy the periodontal pocket depth decreased significantly - for 0,8—2,3 mm, on average for 24%. The patients treated with the traditional therapy had the average reduced depth of the periodontal pocket of 16% (for 0,2—1,3 mm). Differences between the groups are reliable ($p < 0,05$). But for that the positive dynamics at the additionally received AO therapy was more expressed with the better image of orthopantogram. These positive changes were accompanied by the dental scale dynamics of the group which is more relevant and surpassing the comparison group (index of RMA, PI and IG reduced in 4,9, 1,5 and 1,5 times; all $p < 0,05$).

DISCUSSION

Periodontitis is an inflammation of periodontal tissues characterized by the progressive destruction of periodontal ligament and bone. Etiology and pathogenesis of inflammatory periodontal diseases has not been established. It is proved that periodontitis can't be without the plaque. It is now believed that the primary etiological factor in the development of periodontal diseases is bacteria and their toxins. The main pathogenetic factor - is the inflammation that occurs in response to the invading periodontal pathogenic micro flora in the periodontal tissue, with the expression depending on a number of systemic and local factors (17).

Today it is believed that under the certain forms of periodontitis the specificity of bacteria is stimulated by the fact that dental plaque microorganisms evolve



under the exogenous or endogenous influence and crowd out other bacteria. Therefore the inflammatory periodontal diseases are now viewed as an opportunistic infection, depending on both available pathogenic bacteria and the environment promoting their reproduction (pH local changes, anaerobic niche, resistance changes, etc.) (18).

Causes of periodontitis can be the following: malocclusion, violated teeth shapes, poor oral hygiene, violated diet (lack of protein, vitamins). In the development of periodontitis the structure of food is also important - too soft food, not promoting the teeth cleaning and normal load during the chewing which can also be a cause of periodontitis. Bad habits such as chewing on one side of the jaws, i.e. the functional overload of some jaw areas, can also contribute to the development of periodontitis. It develops often after the gum inflammation - gingivitis or periodontal disease. The special role is played by chronic illnesses, poor ecology, occupational hazards and metabolic disorders (19).

An important etiological factor in the development of periodontal disease is periodontal pathogenic bacteria. According to WHO recommendations their representatives are such species which along with the predominantly anaerobic respiration differ by high adhesives, invasive and toxic properties in relation to periodontal tissues (20).

The dental plaque, as one of the main instruments for microorganisms to affect the paradontium accumulates in the margin areas and inter-dental spaces.

When there is inflammation in periodontal tissues due to the increased permeability of blood vessels, it is to be the increased flow of the dental liquid, with the increased migration of polymorphic nuclear leucocytes, which are an essential element of nonspecific blood protective system. Periodontitis results in hyper activation of leucocytes, macrophages, and platelets. Process of accumulating hyper activated leucocytes and platelets in areas of inflammation is the base to develop the tissue destruction (21).

Leucocytes phagocytize bacteria, decomposition products of tissues and destroy them by their lysosome enzymes (such as peptidase, proteases, oxidases, deoxyribonuclease and lipase). The activated cellular membranes of polymorphic nuclear leucocytes excrete the arachidonic acid - an unsaturated fatty acid, which serves as a precursor of leucotrienes, thromboxanes and prostaglandins. This group of substances plays an important role in the launch of inflammation, regulation of the lumen and permeability of blood vessels (20, 21).

Degraded trophy of periodontal tissues leads to changes in the energy process that ensures the viability of cells. It activates primitive ways to generate energy using peroxide and free radical oxidation with formation of large amounts of highly toxic products: reactive oxygen species, MDA, etc. The change environmental acidity disrupts maturation of osteoblasts with the active formation of osteoclasts (19, 20).

We have found experimentally that in the saliva of rats with modeled periodontitis there is an increase in total protein due to inflammation and ALP due to cytolysis with simultaneous increase of MDA content and lower catalase activity which proves FRP activation and less AO protection. Along with that in the saliva of rats with modeled periodontitis there is the decreased content of protein, collagen, elastin and sulfated glycosaminoglycans demonstrating the predominance of catabolism in inflammation.

The similar results were obtained for patients with CGP. In the saliva there is the increased protein, ALP and MDA content in the plasma with the simultaneous reduction of catalase. In the blood of the same patients there are increased indicators of PICLb and PICLs, and also the reduced plasma MDA content and less plasma APA.

Thus, CGP pathogenesis is a very complex process. With the low level of oral hygiene, insufficient teeth self-cleaning, changes in the qualitative and quantitative composition and increased pathogenic micro flora there is the stronger pathogenic potential of the "dental" plaque. Microorganisms can affect the paradontium through extraction of toxins: exotoxins, endotoxins, metabolites and enzymes. The released enzymes can provide a lytic effect on the connective tissue fiber carcass of the paradontium (collagenase, protease), epithelial structures (cearatase), and surface structure of cells (neuraminidase). This especially occurs when common protective factors are poor (atherosclerotic damage of vessels, disturbed neurohumoral regulation, changed immunological activity, diseases of internal organs, chronic psycho - emotional stress, intoxications, hypo - and vitamin deficiency, genetic predisposition) and when local protective paradontium factors are weakened (local traumatic factors, functional overload or insufficiency of periodontal tissues, development abnormalities of teeth-maxilla system, qualitative and quantitative changes in saliva and oral fluid).

These substances activate leucocytes that begin to generate intensively reactive oxygen species. They themselves cause damage and inflammation of paradontium, as well as through POL initiation. These processes are taking place under the declining AO protection.

The revealed changes in FRP objectify the relevance of prescribing AO, energy correction drugs for the patients with periodontitis that is most effective in the early stages of the disease.

The use of AO therapy contributed to reversing the oxidative stress and activating the catalase of saliva, as well as restoration of the protein level. Preparation of calcium and vitamin D₃ increasingly reduced ALP activity enhanced in pathology, but poorly compensated the system disorders of antioxidants — pro-oxidants. The combined introduction of mixed AO and calcium D₃ fully normalized biochemical parameters of the rat saliva with periodontitis: protein, ALP activity, catalase and MDA levels. AO therapy provid-



ed the increased levels of total protein, collagen and elastin. For rats with periodontitis the introduction of calcium D3 also contributed to the increase in the level of protein and collagen in tissues, however, revealed positive changes were significantly higher than such only for animals from the comparison group, but differed from those in the control group of rats. Calcium D₃ did not influence the level of elastin and sulfated glycosaminoglycans. The combined introduction of calcium D₃ and AO therapy fully normalized the revealed pathological changes in gingival tissues of rats with periodontitis.

CONCLUSION

When evaluating the influence of AO therapy in patients with CGP the results were obtained positively evaluating the dynamics of both oxygen and lipid spectrum of FRP. It is important to note that the existing imbalance of FRP oxygen cascade is tracked up to 30 days of observation for the patients with CGP, which proves the long-term persistence of oxidative stress ongoing processes in these patients.

This positive dynamics in the group of AO therapy is combined with a more positive indicator of plasma APA than in the comparison group by the 30 day of treatment. Positive dynamics of oxidative stress parameters correlated with an improvement of the clinical picture and treatment results.

Thus, the identified violations of the local and systemic oxidant stress in periodontitis required the immediate correction of AO preparations.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

ACKNOWLEDGMENTS

This work was supported by the "Russian Academic Excellence Project 5-100".

REFERENCES

1. Fentoğlu Ö, Kirzioğlu FY, Bulut MT et al. Evaluation of lipid peroxidation and oxidative DNA damage in patients with periodontitis and hyperlipidemia. *J Periodontol.* 2015;86(5):682-8.
2. Muniz FW, Nogueira SB, Mendes FL et al. The impact of antioxidant agents complimentary to periodontal therapy on oxidative stress and periodontal outcomes: A systematic review. *Arch Oral Biol.* 2015;60(9):1203-14.
3. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. *J Clin Periodontol.* 2017;44(5):456-462. doi: 10.1111/jcpe.12732.
4. Al-Mubarak S, Ciancio S, Baskaradoss JK. Epidemiology and diagnosis of periodontal diseases: recent advances and emerging trends. *nt J Dent.* 2014;2014:953646. doi: 10.1155/2014/953646.
5. Chandra A, Yadav OP, Narula S, Dutta A. Epidemiology of periodontal diseases in Indian population since last decade. *J Int Soc Prev Community Dent.* 2016;6(2):91-6. doi: 10.4103/2231-0762.178741.
6. Varela-Centelles P, Diz-Iglesias P, Estany-Gestal A et al. Periodontitis Awareness Amongst the General Public: A Critical Systematic Review to Identify Gaps of Knowledge. *J Periodontol.* 2016;87(4):403-15. doi: 10.1902/jop.2015.150458.
7. Slots J. Periodontitis: facts, fallacies and the future. *Periodontol 2000.* 2017;75(1):7-23. doi: 10.1111/prd.12221.
8. Górski B, Jalowski S, Górski R, Zaremba M. Treatment of intrabony defects with modified perforated membranes in aggressive periodontitis: subtraction radiography outcomes, prognostic variables, and patient morbidity. *Clin Oral Investig.* 2018; doi: 10.1007/s00784-018-2712-7.
9. Kornman KS. [Contemporary approaches for identifying individual risk for periodontitis.](#) *Periodontol 2000.* 2018;78(1):12-29. doi: 10.1111/prd.12234.
10. Feng X, Zhu L, Xu L et al. Distribution of 8 periodontal microorganisms in family members of Chinese patients with aggressive periodontitis. *Arch Oral Biol.* 2015;60(3):400-7. doi: 10.1016/j.archoral-bio.2014.11.015.
11. Narita M, Shibahara T, Takano N et al. Antimicrobial Susceptibility of Microorganisms Isolated from Periapical Periodontitis Lesions. *Bull Tokyo Dent Coll.* 2016;57(3):133-42. doi: 10.2209/tdcpublication.2015-0018.
12. Pinelis YI, Malezhik MS, Malezhik LP. The role of free-radical oxidation for patogenesis of chronic generalization periodontitis in elderly age patients. *Adv Gerontol.* 2017;30(1):109-113.
13. Shen YX, Guo SJ, Wu YF. Oxidative stress and antioxidant therapy of chronic periodontitis. *Zhonghua Kou Qiang Yi Xue Za Zhi.* 2016;51(7):442-6. doi: 10.3760/cma.j.issn.1002-0098.2016.07.013.
14. Wang Y, Andrukhov O, Rausch-Fan X. Oxidative Stress and Antioxidant System in Periodontitis. *Front Physiol.* 2017;8:910. doi: 10.3389/fphys.2017.00910.
15. Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends Immunol.* 2014;35(1):3-11. doi: 10.1016/j.it.2013.09.001.
16. Ivaniushko TP, Tumblinskaia LV, Donnikov AE. PCR study of opportunistic species composition in periodontitis patients. *Stomatologiya (Mosk).* 2011;90(5):22-6.



17. Gomes BPFA, Herrera DR. Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology. *Braz Oral Res.* 2018 Oct 18;32(suppl 1):e69. doi: 10.1590/1807-3107bor-2018.vol32.0069.
18. Ji S, Choi YS, Choi Y. Bacterial invasion and persistence: critical events in the pathogenesis of periodontitis? *J Periodontal Res.* 2015 Oct;50(5):570-85. doi: 10.1111/jre.12248.
19. Márton IJ, Kiss C. Overlapping protective and destructive regulatory pathways in apical periodontitis. *J Endod.* 2014;40(2):155-63. doi: 10.1016/j.joen.2013.10.036.
20. Gümüş P, Nizam N, Nalbantsoy A et al. Saliva, Serum Levels of Interleukin-21, -33 and Prostaglandin E2 in Patients with Generalised Aggressive or Chronic Periodontitis. *Oral Health Prev Dent.* 2017;15(4):385-390. doi: 10.3290/j.ohpd.a38740.
21. Liu C, Mo L, Niu Y et al. The Role of Reactive Oxygen Species and Autophagy in Periodontitis and Their Potential Linkage. *Front Physiol.* 2017;8:439. doi: 10.3389/fphys.2017.00439.