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COMPARATIVE EFFICIENCY OF PARODONTOPROTECTIVE ACTIONS OF ANTIDISBIOTIC PREPARATIONS "ARGAKOL" AND "CM-2" IN RATS AFTER ORTHODONTIC SURGERY ON A BACKGROUND OF TYPE 2 DIABETES MELLITUS

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Abstract

Comparison of the periodontal protective effect, two antidisbiotic agents were tested on rats after orthodontic surgery on the background of type 2 diabetes mellitus:"Argakol" (contained antimicrobial agents, alginate, collagen hydrolyzate) and "CM-2" (contained extracts of medicinal plants, glycerophosphate Ca, vitamins, trace elements, gelatin). Type 2 diabetes mellitus was induced in rats by protamine. Orthodontic surgery included fixing of the spring. The condition of the gums was assessed by the level of urease, lysozyme, elastase and hyaluronic acid. The state of the periodontal bony tissue - by the activity of alkaline, and acid phosphatases, and the content of calcium and protein. The mineralizing index was calculated by the ratio of phosphatase activity, and the degree of mineralization by the ratio of the content of Ca and protein. The degree of dysbiosis was determined by the ratio of the relative activities of urease and lysozyme.

It was noted an increase in the gum level of urease, elastase and a decrease in the level of lysozyme and hyaluronic acid, more expressed in the combination of type 2 diabetes mellitus and orthodontic surgery. In the bony tissue with type 2 diabetes mellitus, alkaline phosphatase activity decreased, calcium content and acid phosphatase activity increased, more significant in the combination of type 2 diabetes mellitus and orthodontic surgery. Anti-disbiotic applications had the periodontal protective effect (reduced inflammation, dysbiosis, increased mineralization index and the degree of mineralization. "CM-2" turned out to be more effective.

Antidisbiotic agents had a periodontal protective effect during orthodontic operations on the background of type 2 diabetes mellitus.

Keywords: *periodontal, diabetes mellitus, orthodontics, anti-disbiotic agents.*

Introduction

Almost 90 % of patients with diabetes mellitus suffer from type 2 insulin-dependent diabetes mellitus, in the pathogenesis of which lipid metabolism disorders play a crucial role, leading to a significant increase in free fatty acid (FFA) blood levels causing insulin resistance [*Titov V, 2003; Titov V 2012; Tikhomirova Iu et al., 2013; Sears B, Perry M,, 2015*].

One of the most significant complications of type 2 diabetes mellitus is the development of generalized dysbiosis, characterized by bacteremia, endotoxemia, and systemic inflammation [*Savchenko I, 2015; Stupak E et al., 2015; Velikaia M, Sheveleva N,, 2015*].

One of the vulnerabilities in diabetes mellitus is the oral cavity and, especially, periodontal disease, in which, as a rule, inflammation of the soft tissues and atrophy of the periodontal bone tissue develops [*Savchenko I et al., 2012; Moroz B et al., 2013; Basov A et al., 2014*].

Pathological changes in the periodontium in peo-

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ple with diabetes mellitus are aggravated after orthodontic operations [*Andriushechkina T et al., 2015; Blashkov S et al., 2015*], although there is evidence of the positive impact of orthodontic surgery on the condition of the oral cavity in rats with experimental type 1 diabetes [*Demianenko S et al., 2018*].

Earlier, we showed a periodontal protective effect in rats with type 1 diabetes who underwent orthodontic surgery of the multifunctional antidisbiotic agent Quertulin [*Demianenko S et al., 2018*].

The purpose of this study was to compare the periodontal protective effect of two domestic antidisbiotic preparations: "Argakol", containing a large amount of antimicrobial compounds, and the "CM-2" plate, containing plant extracts, vitamins, calcium and phosphorus salts.

Material and methods

For the study we used the drug "Argakol" produced by LLC Sirena (St. Petersburg, RF), registration certificate of the Ministry of Health of the Russian Federation No. FSO 1262005 / 1878-05 dated July 05, 2005. The composition of the drug: collagen hydrolyzate, sodium alginate, Ag-containing component, antiseptics, glycerin [*Sapronova O et al., 2010*].

The second drug was "plates CM-2" produced by the Russian research and production enterprise "Saluta-M" (Moscow, RF), registration certificate of the Roszdravnadzor (Federal Service for Surveillance in Healthcare) No. FSR 2010 / 07797 dated May 21, 2010. The composition of the drug: herbal extracts (St. John's wort, yarrow, sage), vitamin C and B1, glycerophosphate Ca, trace elements, gelatin.

Type 2 diabetes mellitus (T2DM) was coused in rats using the protamine method [*Ulianov A, Tarasov Iu,, 2000]. Orthodontic surgery was performed in rats in accordance with the recommendations [Gorokhivskii V et al., 2017*].

Investigated antibiotics were applied to the gum daily at a dose of 0.3 *ml (g)* for each rat.

In total, the experiment involved 35 white Wistar rats (males, 5 months, all body weight 180 ± 12 *g*), divided into 5 groups of 7 animals in each: group 1 - intact rats, served as controls; the in rats of 2 and 3rd, 4th and 5th groups were modeled type 2 diabetes mellitus [*Ulianov A, Tarasov Iu,, 2000*]. Rats of the 3rd, 4th and 5th groups were after orthodontic surgery (OS) - spring fixation [*Gorokhivskii V et al., 2017*]. Rats of the 4th group from the 1st day of the experiment received daily applications of the Argakol gel (0.3 *ml* per rat) on the gum. From the 1st day, rats of the 5th group received daily applications of the drug "CM-2" (0.3 g per rat) on the gum.

Euthanasia of animals was performed on the 32nd day of the experiment under thiopental anesthesia (20 *mg/kg*). Then we isolated the gums, and the alveolar proceses of the mandibles.

Urease activity (a marker for microbial contamination) [*Gavrikova L, Segen I,, 1996*], lysozyme (an indicator of nonspecific immunity) [*Levitsky A, 2005*], elastase (a marker of inflammation) [*Levitsky A, Stefanov A,, 2002*] and hyaluronic acid (intercellular "cement") [*Asatiani V, 1965*] were determined in the homogenate of the gums. The ratio of the relative activities of urease and lysozyme was calculated by the degree of dysbiosis by A. P. Levitsky [*Levitsky A et al., 2007*].

In bone homogenate, alkaline phosphatase (AlP) and acid phosphatase (AP) activity were determined by hydrolysis of p-nitrophenyl phosphate [*Levitsky A et al., 2005*], as well as calcium [*Levitsky A et al., 2005*] and soluble protein [*Lowry O et al., 1951*]. The mineralization index (MI) was calculated by the ratio of alkaline phosphatase / acid phosphatase [*Levitsky A et al., 2006*], and the degree of mineralization (DM) was calculated by the ratio of calcium (*g/kg*) and protein (*g/kg*) [*Levitsky A et al., 2005*].

The research results were subjected to standard statistical processing [*Truhacheva N, 2012*].

Results and discussion

Table 1 presents the results of determining the biochemical parameters of the gums, reflecting the state of soft periodontal tissues. It is evident that in all groups there was a significant increase in the level of urease, indicating the growth of bacterial contamination of this tissue, and the most significant for the state of T2DM and OS. Both anti-disbiotic drugs reducd urease activity (by 37-38%) approximately equally. On the contrary, the activ-

Table 1.

The effect of antidisbiotic agents on the biochemical parameters of the gums of rats with type 2 diabetes mellitus (T2DM) after orthodontic surgery (OS)
(M ± m, n = 7 in all groups)

No	Groups	Urease, *μkat / kg*	Lysozyme *unit / kg*	Elastase *μkat / kg*	Hyaluronic acid, *g / kg*
1	Control	1.12±0.25	215±19	25.4±1.6	3.59±0.41
2	T2DM	2.49±0.33 p<0.01	131±15 p<0.05	33.9±3.0 p<0.01	2.72±0.37 p>0.05
3	T2DM+ OS	3.05±0.35 p<0.01 p_1>0.1	110±14 p<0.01 p_1>0.3	42.3±3.5 p<0.01 p_1>0.3	2.40±0.33 p<0.05 p_1>0.3
4	T2DM +OS+"Argakol"	1.87±0.20 p<0.05 p_1>0.05 p_2<0.05	105±16 p<0.01 p_1>0.2 p_2>0.5	33.2±3.0 p<0.05 p_1>0.05 p_2>0.05	2.94±0.36 p>0.05 p_1>0.3 p_2>0.1
5	T2DM +OS+"CM-2"	1.91±0.23 p<0.05 p_1>0.05 p_2<0.05	182±21 p>0.1 p_1>0.05 p_2<0.05	30.0±2.2 p>0.05 p_1<0.05 p_2<0.05	3.23±0.27 p>0.3 p_1>0.1 p_2>0.05

Notes: *p - in comparison with gr. one; p1 - in comparison with gr. 2; p2 - in comparison with gr. 3.*

Table 2.

The effect of antidisbiotic agents on the biochemical parameters of periodontal bone tissue of rats with type 2 diabetes mellitus (T2DM) after orthodontic surgery (OS)
(M ± m, n = 7 in all groups)

No	Groups	ALP, *mk-cat / kg*	AP, *mk-cat / kg*	Ca, *mol / kg*	Protein, *g/kg*
1	Control	228±16	12.9±1.4	1.95±0.18	26.8±2.3
2	T2DM	152±15 p<0.05	17.6±1.6 p<0.05	1.63±0.15 p>0.05	25.7±2.4 p>0.3
3	T2DM+ OS	138±15 p<0.05 p_1>0.3	21.3±1.9 p<0.05 p_1>0.05	1.38±0.14 p<0.05 p_1>0.1	26.9±2.7 p>0.5 p_1>0.3
4	T2DM +OS+"Argakol+	182±17 p>0.05 p_1>0.05 p_2>0.05	18.4±1.6 p<0.05 p_1>0.3 p_2>0.05	1.59±0.16 p>0.05 p_1>0.5 p_2>0.3	25.6±2.4 p>0.3 p_1>0.8 p_2>0.3
5	T2DM +OS+"CM-2"	217±18 p>0.5 p_1<0.05 p_2<0.05	14.0±1.5 p>0.3 p_1>0.05 p_2>0.05	1.88±0.18 p>0.5 p_1>0.2 p_2<0.05	26.3±2.5 p>0.5 p_1>0.5 p_2>0.5

Notes: *see tab. one.*

ity of lysozyme in the gums was significantly reduced (by 48.8%) in the combination of T2DM and OS, and under the action of antidisbiotic agents, only the “CM-2” lysozyme restores its activity. On figure 1 it is shown that in the combination of T2DM and OS the degree of dysbiosis in the gum increased 5.3 times. The applications of “Argakol” reducd the degree of dysbiosis by 36%, and “CM-2” - by 63%.

Table 2 presents the results determining the biochemical parameters of bony tissue of the alveolar process in the lower jaw of rats. These data showed, that the activity of alkaline phosphatase, which is a marker of osteoblasts, significantly decreased

[Levitsky A. et al., 2006] in T2DM and, especially, in combination of T2DM and SO. Applications of anti-disbiotic agents increased the activity of alkaline phosphatase "Argakol" by 35.5% and "CM-2"

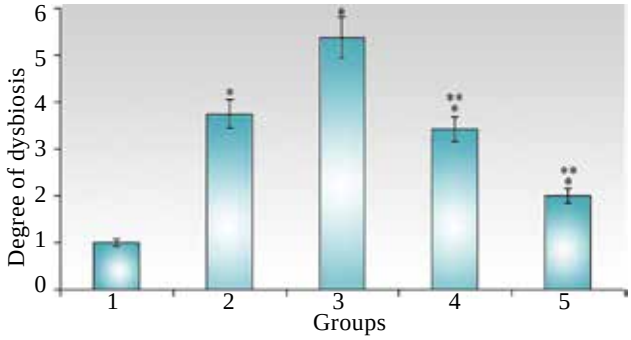

Figure 1. *Influence of drugs "Argakol" (4) and "CM-2" (5) on the degree of dysbiosis in the gums of rats with type 2 diabetes (2) and in the combination of type 2 diabetes and OS (3)*
Notes: ** in comparison with group 1;*
** * - in comparison with group 3)*

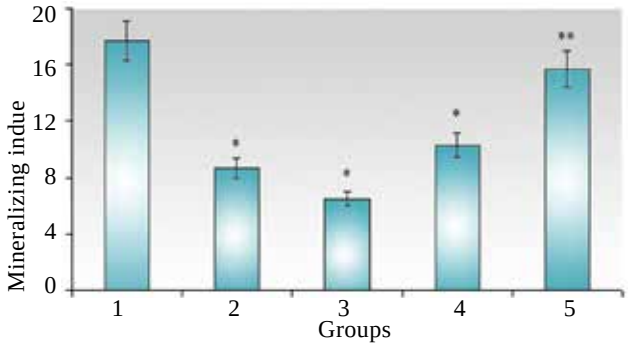

Figure 2. *The effect of the preparations "Argakol" (4) and "CM-2" (5) on the mineralizing index of periodontal bony tissue of rats in T2DM (2) and in the combination of T2D and OO (3)*
Notes: ** - in comparison with group 1;*
*** - in comparison with group 3*

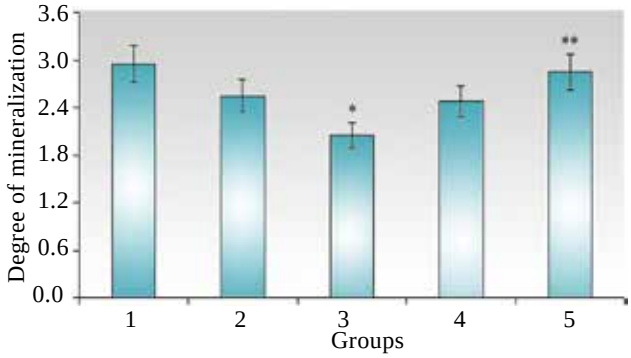

Figure 3. *The effect of the preparations "Argakol" (4) and "CM-2" (5) on the degree of bony tissue mineralization in periodontal rats in T2DM (2) and in the combination of T2DM and OS (3)*
Notes: ** - in comparison with group 1;*
*** - in comparison with group 3)*

by 57.2%. On the contrary, AP activity significantly increased in T2DM and, especially, in the combination of T2DM and SO. This increase in AP level reflected the activation of osteoclasts [*Levitsky A et al., 2006*]. Cut of the two tested products, Argakol reduced AP activity by 13.6%, and CM-2 by 34.2%, practically returning this figure to the control level. The mineralizing index (MI), which was calculated by the alkaline phosphate / AP ratio when combined with T2DM and SO decreased by 2.7 times, the application of "Argacol" increased by 1.6 times, and the application of "CM-2" - 2.4 times (Fig. 2).

Calcium content in bony tissue decreases significantly (by 29%) in the combination of T2DM and SO, and after the action of the studied agents, the calcium content increased: after "Argacol" by 15% and after "CM-2" by 36%. The content of soluble protein in bony tissue had not significant changes in any group.

The degree of mineralization calculated by the Ca / protein ratio is shown in figure 3 and reflects that in the combination of T2DM and SO, this figure decreases by almost 30%. The applications of "Argakol" increased it by 21%, and the applications "CM-2" - by 40%.

Conclusion

1. Diabetes mellitus type 2 (T2DM) causes a decrease in the level of nonspecific immunity in the gums, an increase in microbial contamination, the degree of dysbiosis and the development of the inflammatory-dystrophic process.
2. Orthodontic surgery on the background of type 2 diabetes aggravated pathological processes in the gums.
3. Oral applications of anti-disbiotic drugs ("Argakol" and "CM-2") had a periodontal protective effect, more expressed after "CM-2".
4. In the periodontal bony tissue, T2DM caused a decrease in the mineralizing index and the degree of mineralization, more expressed in the combination of T2DM and OS.
5. Oral applications of anti-disbiotic drugs had the osteoprotective effect, more evident after CM-**2**

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