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EXPERIMENTAL SUBSTANTIATION OF POLYVALENT ORAL GEL USAGE FOR PREVENTION AND TREATMENT OF PERIODONTAL INFLAMMATION

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Key words: *prednisolone, periodontitis, alveolar bone tissue, oral gel.*

Now at prosthetic treatment of edentulous patients exist a number of problems. Conditions of complete denture fixation and servicing in edentulous patients may be improved of alveolar bone reshaping, the denture base mastication forces allocation on the prosthetic bed tissue. The same can also be achieved through the use of implants, which is an effective method of prosthetic treatment of patients with significant loss of teeth [1, 2].

However, numerous investigations have shown a significant number of inflammatory complications arising in various (surgery, prosthetic) stages of implantation. Recent years, a significant increase in the number of inflammatory (mucositis, periimplantitis) complications occur after implantation. Further development of inflammation around the implant leads to a rejection of implants almost in 22.2% of clinical cases [3, 11, 15, 19-21].

For the prevention and treatment of periimplantitis was proposed an oral gel consisting of osteovit, propolis tinctures, metrogyl-dent, chlorhexidine digluconate, silicon dioxin (model for application № u201411415). As the comparator drug was used mefenat.

The aim of this investigation was determination of anti-inflammatory and osteostimulate action of proposed drug composition (oral gel) in experimental condition under modeling of prednisolone periodontitis.

Material and methods. This experiments were performed on 32 Wistar rats that were divided into 4 groups: 1 – standard, groups 2, 3 and 4 received prednisolone first two days, 10 mg / kg, and 12 days at 5 mg / kg. Prednisolone was given to rats with food.

Administration of prednisolone to experimental animals causes the development of serious injuries of alveolar bone tissues [10, 24], alteration in carbohydrates and fats metabolism [8], immune system [6, 12], leading to the development of experimental osteoporosis [24] and periodontitis [10] .

Rats of 3rd group received daily an oral gel in the form of applications on the oral mucosa at a dose of 0.7 ml per rat (0.75 ml / kg). Rats of 4th group received sodium mefenat in a dose of 0.1 grams per rat (0.75 g / kg). This group served as a control (group) to the 3rd group.

Rats were subjected to euthanasia on the 15th day of the experiment by total heart bleeding under thiopentalum anesthesia (20 mg / kg). In gingival homogenate the markers of inflammation were determined [5]: the elastase activity [13], the content of malondialdehyde (MDA) [22], a biochemical indicator of microbial contamination – urease activity [7], a biochemical indicator of nonspecific immunity – lysozyme activity [14] catalase activity [9] and the content of hyaluronic acid [4].

The ratio of catalase activity and MDA contents counted antioxidant-prooxidant index API [5] and the ratio of the relative activities of urease and lysozym expected degree of dysbiosis by Levitsky [18]. Atrophy of alveolar bone was determined by the A.V. Nykolayeva method [16]. The results were processing by statistical methods [12].

For histology, gingiva was kept in 10% neutral formalin. Later it was stained with hematoxylin and eosin [23]. Subsequently they investigated an optical microscope and photographed (digital camera Canon 5D).

Results and discussion. After prednisolone administration, in experimental animals develop atrophy of the jaws alveolar bone. In the case of applications of oral gel and comparator sodium mefenat these changes were returned practically to normal. Oral gel revealed better results than mefenat.

Table 1 shows the results of determination of biochemical markers of inflammation in the rat gingiva – elastase activity and MDA content. These data show that prednisolone significantly injures the level in the gingival inflammatory markers, and used drugs significantly reduce it, and somewhat better – oral gel.

Table 2 shows the results of determination of urease and lysozyme activity in rat gingiva. According to the presented data urease activity in the gingiva of rats with prednisolone periodontitis increased by 2 times, and under the influence of proposed drugs composition it was significantly reduced (almost to normal). The oral gel revealed somewhat better effect on this index.

On the contrary, under the influence of prednisolone the lysozyme activity in the gingiva is reduced almost three times, and under oral gel it was return almost to normal. Unlike the gel, mefenat sodium had a reduced impact on the activity of lysozyme.

The degree of dysbiosis in the gingiva was calculated on the urease activity indicators and lysozyme influenced prednisolone increased almost 8 times, and under the influence of proposed our gel almost at the normal level. In contrast, the drug comparison – mefenat, although it reduced the degree of dysbiosis, but did not return it to normal.

Table 3 shows the results of determination catalase activity and antioxidant-prooxidant index (API). These data show that prednisolone significantly reduced the activity of catalase in the gingiva and an even greater extent. The use of oral gel restores the activity of catalase and API index. In contrast, mefenat had much less impact on these indices.

Administration of prednisolone significantly reduced the content of hyaluronic acid in the gingiva, and applied both drugs (oral gel and the comparator mefenat) significantly increase his.

Thus, the present investigation demonstrated that prednisolone really plays a role in periodontitis, as evidenced by: increasing alveolar bone atrophy, development of inflammation in the gingiva, the development of dysbiosis in the gingiva and reduce protection systems (lysozyme, catalase activity, hyaluronic acid content).

Table 1.

Effect of oral gel and mefenat on the level of markers of inflammation in the gingiva of rats with prednizolone periodontitis (in all groups n = 8)

№№ п/п	Groups	Elastase, mkkat / kg	MDA, mmol / kg
1	Normal	0,043±0,002	12,6±1,4
2	Prednizolone periodontitis (PP)	0,058±0,003 p<0,01	I8,2±1,1 p<0,05
3	PP + oral gel	0,048±0,002 p>0,05 p,<0,05	14,1±1,0 P>0,3 p,<0,05
4	PP + mefenat sodium	0,050±0,001 p<0,05 pi<0,05	16,2±1,0 p<0,05 Pi>0,1

Notes: p – compared to group № 1; p_1 – compared with group № 2.

Table 2.

Effect of oral gel and mefenat on urease and lysozyme activity lizotsym in the gingiva of rats with prednizolone periodontitis (in all groups n = 8)

№№ п/п	Groups	Urease mkkat / kg	Lysozyme, U / kg
1	Normal	0,47±0,08	384±29
2	Prednizolone periodontitis (PP)	0,93±0,05 p<0,001	132±19 p<0,001
3	PP + oral gel	0,50±0,09 p>0,5 pi<0,01	325±30 p>0,1 pi<0,001
4	PP + mefenat sodium	0,55±0,08 p>0,3 Pi<0,01	190±28 p<0,001 pi>0,05

Notes: p – compared to group № 1; p_1 – compared with group № 2.

Table 3.

Effect of oral gel and mefenat on catalase activity and API index in the gingiva of rats with prednizolone periodontitis (in all groups n = 8)

№№ п/п	Groups	Catalase, mkat/ kg	API, units
1	Normal	8,54±0,51	6,78±0,50
2	Prednizolone periodontitis (PP)	6,94±0,45 p<0,05	3,76±0,38 p<0,01
3	PP + oral gel	7,58±0,45 p>0,05 pi>0,05	5,38±0,55 p>0,05 pi<0,05
4	PP + mefenat sodium	7,23±0,48 p>0,05 pi>0,3	4,49±0,46 p<0,05 Pi>0,3

Notes: p – compared to group № 1; p_1 – compared with group № 2.

Proposed oral gel significantly eliminates all abnormal phenomena that develop in conditions of action of prednisolone.

Administration of prednisolone causes the periodontitis in experimental animals. In gingival tissues revealed focal epithelium acanthosis, which is accompanied by epithelial keratinization processes in all layers. There are areas of liquefaction necrosis of the epithelium with symptoms of inflammatory infiltration in subepithelial layer. There is of uneven keratinization of epithelial cells, which applies to all layers of the epithelium. This process is accompanied by significant edema and focal fibrocytes proliferates subepitelial tissue, among which there are lymphocytes (fig. 1, 2, 3).

After oral gel applications pathomorphological changes in the gingiva can be reduced only to medium degree edema of the oral mucosa, slight swelling of submucosal layer, violation of epithelial cells differentiation and abuse uneven hyperkeratosis (fig. 4, 5).

Using the drug of comparison, the histologic changes did not differ from gingiva of rats that received application of proposed oral gel (fig. 6, 7).

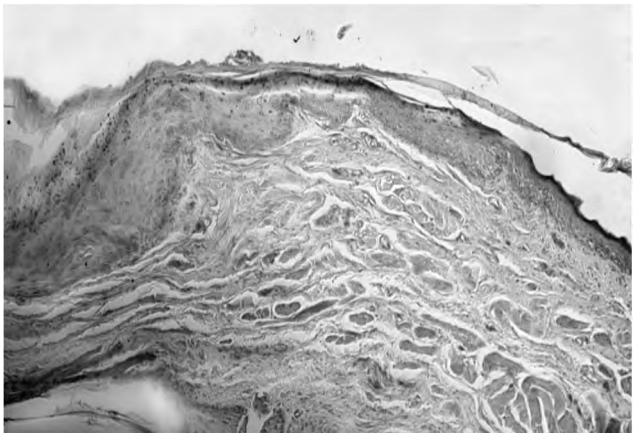

Fig. 1. Pathomorphological changes in rat gingiva after prednisolone action. It is noted uneven thickening of the gingival epithelium, violation of cell differentiation, hyperkeratosis, peeling of the stratum corneum. Hematoxylin-eosin x 120

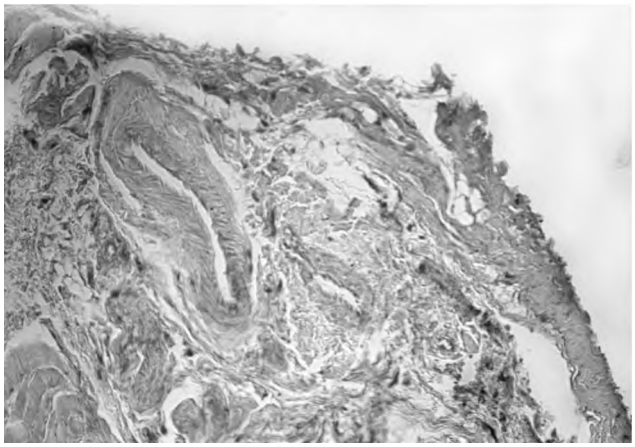

Fig. 2. Pathomorphological changes in rat gingiva after prednisolone action. It is noted liquefaction epithelial necrosis, the formation of ulcers, edema of subepithelial layer and hemorrhage. Hematoxylin-eosin x 120

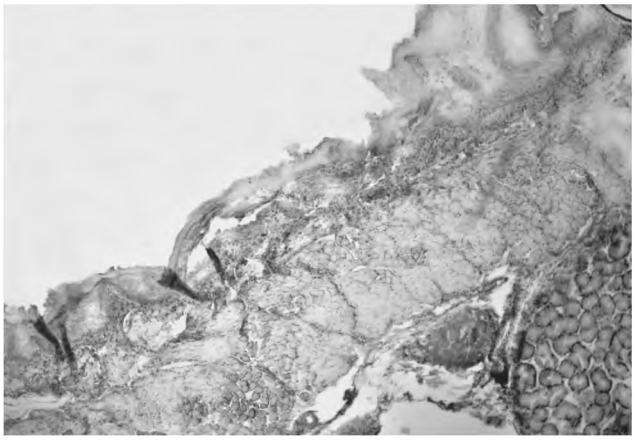

Fig. 3. Pathomorphological changes in rat gingiva after prednisolone action. It is noted focal liquefaction epithelial necrosis, hyper- and dyskeratosis of epithelial cells. There are medium degree innflammatory infiltration in the lamina propria of gingival connective tissue. Hematoxylin-eosin x 120

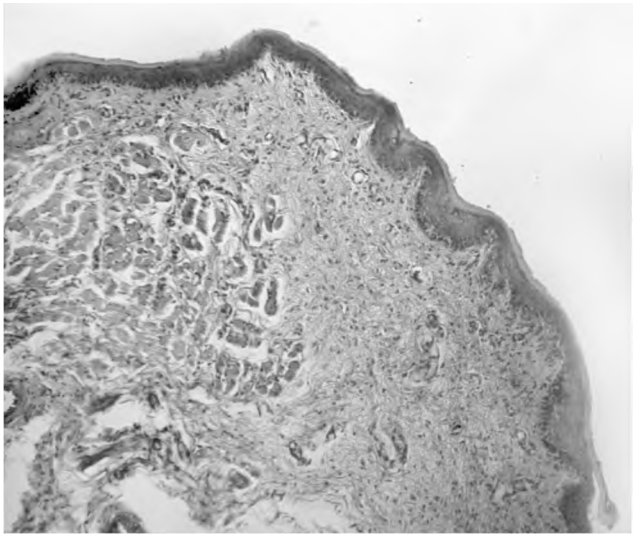

Fig. 4. Pathomorphological changes in rat gingiva after prednisolone action and oral gel application. It is noted only minor violations of epithelial cells differentiation with focal symptoms of hyperkeratosis. Hematoxylin-eosin x 120

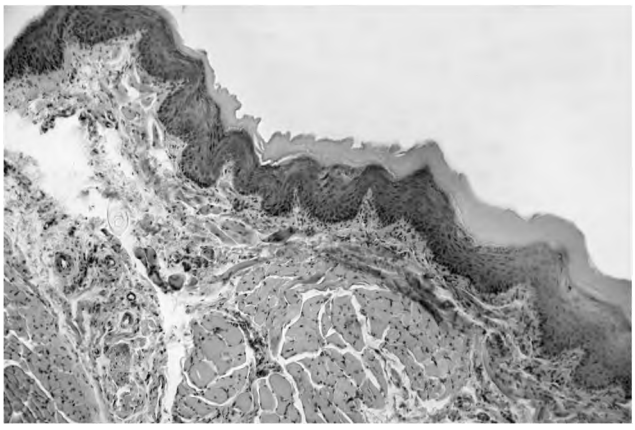

Fig. 5. Pathomorphological changes in rat gingiva after prednisolone action and oral gel application. It is noted practically normal structure of epithelium. A little lymphoid infiltration of the gingival connective tissue lamina propria. Hematoxylin-eosin x 120

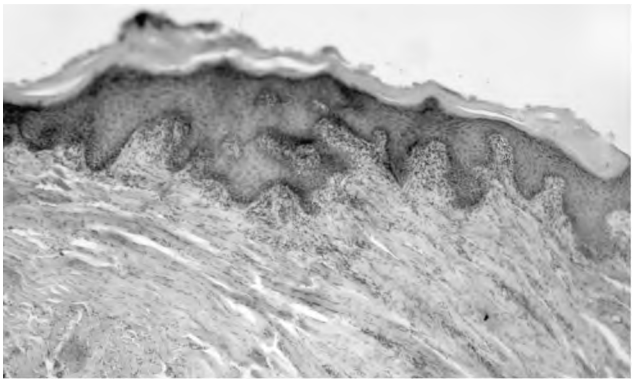

Fig. 6. Pathomorphological changes in rat gingiva after prednisolone action and mefenat application. It is noted violation of epithelial cells differentiation, hyperkeratosis, insignificant inflammatory infiltration in subpitelial layer. Hematoxylin-eosin x 120

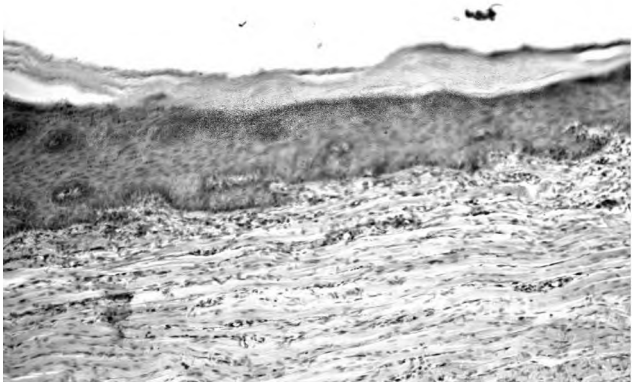

Figure 7. Pathomorphological changes in rat gingiva after prednisolone action and mefenat application. It is noted violation of epithelial cells differentiation, hyperkeratosis, insignificant inflammatory infiltration in subpitelial layer. Hematoxylin-eosin x 120

These histological investigations revealed the marked anti-inflammatory action of the proposed drug composition – oral gel.

Conclusions.

1. Prednisolone caused the development of periodontitis in experimental animals.
2. In the mechanism of prednisolone action there is its ability to cause the development of dysbiosis, reduce the hyaluronic acid content and the level of protective systems.
3. Oral gel prevents the development of prednisolone induced periodontitis and has anti-inflammatory action.

Declaration of interest.

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this article.

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ЭКСПЕРИМЕНТАЛЬНОЕ ОБОСНОВАНИЕ ПРИМЕНЕНИЯ ПОЛИВАЛЕНТНОГО ОРАЛЬНОГО ГЕЛЯ ДЛЯ ПРОФИЛАКТИКИ И ЛЕЧЕНИЯ ВОСПАЛЕНИЯ ПАРОДОНТА

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Резюме. При проведении имплантации довольно часто возникает воспалительный процесс в костной ткани челюсти вокруг имплантата. Для профилактики и лечения такого воспалительного процесса предложен оральный гель. Он состоит из остеовита, настойки прополиса, метрогил-дента, хлоргексидина и диоксида кремния. Эта композиция оказывает антибактериальное и противовоспалительное действие. В экспериментальных условиях было изучено остеостимулирующее действие данной композиции на воспалительный процесс в пародонте. Эксперимент был проведен на 32 белых крысах, которые получали преднизолон: 5мг/кг в течение 14 дней. У них возникал экспериментальный пародонтит. Затем им вводили данную медикаментозную композицию. В качестве препарата сравнения использовали мазь мефенат. Результаты изучали при использовании гистологических и биохимических методов. Были определены следующие параметры: степень атрофии альвеолярного отростка, уровень маркеров воспаления десны, МДА, эластаза, микробное обсеменение (уреаза), неспецифический иммунитет (лизоцим), антиоксидантную защиту (каталаза), гиалуроновую кислоту. Введение преднизолона вызывает развитие воспалительного процесса в пародонте крыс. После введения медикаментозной композиции патологоанатомическое исследование выявило наличие у композиции противовоспалительного действия. Данная медикаментозная композиция у крыс с преднизолоновым пародонтитом снижает активность кислой фосфатазы, увеличивает соотношение щелочная фосфатаза/кислая фосфатаза и степень минерализации, снижает степень атрофии альвеолярного отростка. Проведенные экспериментальные патогистологические и биохимические исследования выявили наличие у предложенной медикаментозной композиции противовоспалительного действия.

Ключевые слова: преднизолон, пародонтит, костная ткань, оральный гель.

EXPERIMENTAL SUBSTANTIATION OF POLYVALENT ORAL GEL USAGE FOR PREVENTION AND TREATMENT OF PERIODONTAL INFLAMMATION

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Summary. During the dental implantation quite often develope an inflammatory process in the bone tissue around the implant. For the prevention and treatment of the inflammatory process proposed oral gel. It consists of osteovit, propolis tincture, Metrogil-Denta, chlorhexidine and silicon dioxide. This composition has antibacterial and anti-inflammatory effect. At experimental conditions anti-inflammatory effect of this composition on the inflammatory process in the periodontium were studied. Experiments were performed on 32 Wistar rats which were administered with food prednisolone at 5 mg/kg for 14 days. Part of the rats received daily applications of oral gel containing propolis, osteovit, metrogil, chlorhexidine, silicon dioxide. As a comparison, the drug ointment Mefenat was used. The results were studied using histologic and biochemical techniques. The following parameters were investigated: the degree of atrophy of the alveolar bone, the level of markers of inflammation in the gingiva (MDA, elastase), microbial contamination (urease), nonspecific immunity (lysozyme), antioxidant defenses (catalase), permeability of histohematic barriers (hyaluronic acid). Prednisolone administration with food causes the development of inflammation in periodontal tissue of rats. After the application of drug compositions pathohistological examination revealed the presence of anti-inflammatory effect of the composition. This medicamental composition in rats with prednisolone periodontitis reduces the activity of acid phosphatase, increases the ratio of alkaline phosphatase/acid phosphatase and mineralization, reduces the degree of atrophy of the alveolar bone. The experimental pathohistological and biochemical investigations revealed the presence of anti-inflammatory drug action in the proposed medicamental composition.

Key words: prednisolone, periodontitis, alveolar bone tissue, oral gel.