



Pathomorphological analysis of the gum tissues in the application of the combination of Soderm®-Forte gel with Cytoflavin® in experimental periodontitis in rats

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Abstract

Introduction: Periodontitis is one of the most urgent problems of modern dentistry. The development of new paradigms and regimens of combination therapy of patients with periodontitis is a strategic task for pharmacologists and dentists. In view of this, pathomorphological examination is of high importance, since it allows us to conclude about the therapeutic effectiveness of the administered drugs with high objectivity.

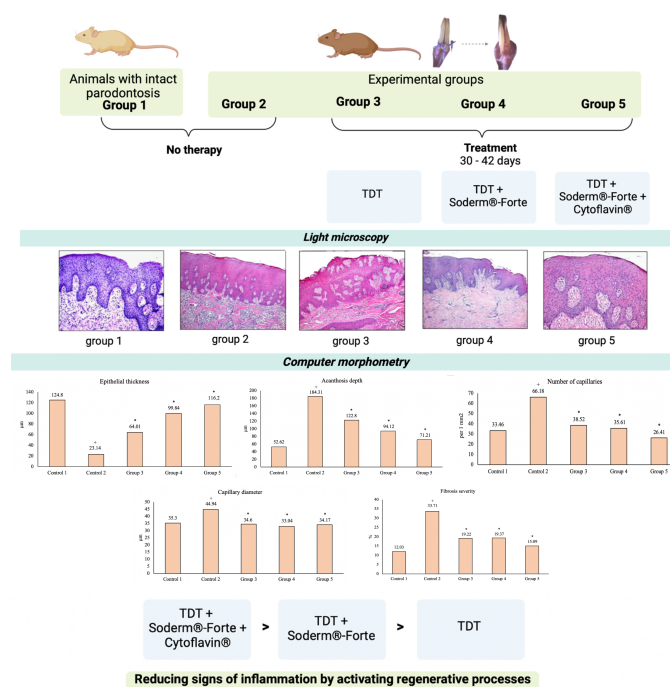
Aim of the study: to evaluate the effect of the composition of Soderm®-Forte and Cytoflavin® on the pathomorphological pattern of gum tissues of rats with experimental periodontitis (EP).

Materials and Methods: EP was simulated in rats by ligature method. Study design: animals with intact periodontium; animals with untreated EP; animals with EP treated with traditional drug therapy (TDT); animals with EP treated with the combination of TDT and Soderm®-Forte gel; and animals with EP treated with the combination of TDT, Soderm®-Forte and Cytoflavin®. For pathomorphological examination, biopsy specimen was taken from the gingival margin of the lower incisors. Slides were stained with hematoxylin and eosin, as well as by Masson. Computer morphometry was performed using the ImageJ software.

Results and Discussion: In EP, TDT has a moderate positive effect on pathomorphological changes in the gum. The combination of TDT and Soderm®-Forte and, to a greater degree, the combination of TDT, Soderm®-Forte and Cytoflavin® have high therapeutic efficacy, characterized by rapid regeneration of the gum tissues.

Conclusion: The combination of TDT, Soderm®-Forte and Cytoflavin® in EP has a more pronounced therapeutic effect, manifested by early regression of pathological changes and acceleration of tissue regeneration in the gum.

Graphical abstract:



Keywords

Soderm®-Forte gel; computer morphometry; pathomorphological changes in gum tissue; Cytoflavin®; experimental periodontitis

Introduction

Pathomorphological studies using modern light microscopy and computer morphometry, as a rule, play a pivotal role in the study of both the toxic and therapeutic effects of potential drugs in preclinical studies. This approach to the development of new drugs and regimens of their administration in periodontology, in particular in the treatment of patients with inflammatory lesions of the periodontal complex (PC), is also often preferred (Bedrova et al. 2018; Leontiev et al. 2020).

It is known that the etiological factors of periodontitis can be both violations of hydrodynamics in the PC (Kopitov and Leontiev 2022) and the microecology of the oral cavity; the presence of periodontopathogenic microorganisms of the first and second importance, co-infecting agents and opportunistic species (King et al. 2019; Ushakov and Tsarev 2019; Tsarev et al. 2020; Balmasova et al. 2021), which initiate and support the inflammatory process. Inflammatory process negatively affects not only the physiological state of the PC, but also the immunological reactivity of the body (Guerra et al. 2018; Tsepov et al. 2018).

To date, it is generally accepted in medical practice that the use of many antimicrobial drugs, in particular antibiotics, and antiseptic drugs leads to the development of resistance to them of many pathogenic microorganisms (Orehova et al. 2020; Zhuravleva et al.

2021). This encourages pharmacologists in collaboration with synthetic chemists and clinicians (including dentists) to search for and develop new highly effective drugs and their combinations capable to inhibit a wide range of pathogenic microorganisms, without causing resistance to them, as well as to possess positive pleiotropic properties (anti-inflammatory, antihypoxic, antioxidant, etc.), which would have a positive effect on the complex treatment of some diseases caused by pathogenic microorganisms, including periodontitis (Bakhit et al. 2018; Tsepov et al. 2018; Kuzmina et al. 2019; Ushakov and Tsarev 2019).

In the light of the above, application of Soderm®-Forte and Cytoflavin® in the complex treatment of periodontitis seemed promising.

Soderm®-Forte is a mixed-type micellar gel containing nanocluster zero valent metallic silver in the form of Ag_n^{K+} monomers and monomer micelles, the structure of which consists of a metal core and a surface double electric layer (emulsion micelles formed by a mixture of nonionic surfactants (polyethylene glycols)), oil and an aqueous phase including superoxide dismutase (SOD), anosilver and drugs containing it, including Soderm®-Forte, have a wide spectrum of antimicrobial activity (Ahmad et al. 2020; Yunusov et al. 2020; Farheen et al. 2021; Bharkhavy et al. 2022; Galenko-Yaroshevsky et al. 2022), including antibiotic-resistant gram-positive and gram-negative strains (Talapko et al. 2020; Enas et al. 2021), antifungal activity (Novikova and Russkih 2018;

Kowalczyk et al. 2021; Galenko-Yaroshevsky et al. 2022) and antiviral activity (Das et al. 2020; Dung et al. 2020; Kowalczyk et al. 2021), as well as anti-inflammatory effect (Singh et al. 2018; Shin et al. 2018; Das et al. 2020; Fehaid et al. 2020; Kubyshevskiy et al. 2020; Farheen et al. 2021). It has been shown that nanosilver can be successfully used in the treatment of periodontitis (Kadam et al. 2020; Leontiev et al. 2020; Fernandez et al. 2021). SOD is an enzyme of the first line of the body antioxidant defense, which has a regulating effect on the antioxidant-prooxidant system, which goes out of balance due to increased production of free radicals, activation of oxidative stress and damage to cellular structures, during inflammatory and destructive processes in various tissues, including PC. It has been shown that Soderm[®]-Forte as a multi-target agent has significant therapeutic efficacy in complex pharmacotherapy of EP in rats (Galenko-Yaroshevsky et al. 2022).

Cytoflavin[®] is a combination drug (it contains metabolites – succinic acid and riboxin, and coenzymes – riboflavin and nicotinamide). Pharmacotherapeutic properties of Cytoflavin[®] are based on synergistic action of its components. Succinic acid in significantly larger concentration compared to other succinates-containing drugs phosphorylates a large number of proteins, increases the tissue oxygenation and significantly improves the cell respiration (Shapovalov et al. 2020; Bhattacharjee and Banerjee 2020). Riboxin suppresses the production of pro-inflammatory cytokines, interferon- γ , IL-1 and IL-12, tumor necrosis factor- α (TNF- α) in macrophages (activated) and spleen cells (Kondratiev et al. 2020). Riboflavin inhibits the nuclear factor-KAPPA B (NF- κ B), having an anti-inflammatory effect, also reduces TNF- α and proteasomal elimination of the ubiquitous phosphorylated inhibitor-kappa (P-I κ), and increases the production of nitric oxide (Badawy 2020). Nicotinamide plays the role of a highly active modulator of pro-inflammatory cytokines IL-1, IL-6 and IL-8, TNF- α (Ungerstedt et al. 2003). A variety of pharmacological effects of Cytoflavin[®] components result in its antihypoxant, antioxidant, anti-inflammatory, anti-dystrophic and regenerative activities, activating effect on the redox enzymes of the mitochondrial respiratory chain and resynthesis of high-energy compounds, as well as a positive effect on energy formation in tissues and a decrease in the production of free radicals and restore the activity of antioxidant defence enzymes (Marashly and Bohlega 2017; Olenskaya 2021; Orlov et al. 2021; Maksimova et al. 2022).

According to Leontiev et al. (2012) additional application of Cytoflavin[®] to TDT significantly increases the treatment success in patients with chronic generalized periodontitis and has a beneficial effect on PC tissues.

To date, it has been established that the combination of the TDT with Soderm[®]-Forte and Cytoflavin[®] in the EP in rats can significantly increase the pharmacotherapy effectiveness (Popkov et al. 2022).

Based on the above, it seemed important to conduct comparative studies of the effect of TDT, combinations of TDT with Soderm[®]-Forte and TDT with Soderm[®]-Forte

and Cytoflavin[®] on the pathomorphological pattern of gum tissues in EP in rats.

Aim of the study was to evaluate the effect of the combination of Soderm[®]-Forte and Cytoflavin[®] on the pathomorphological pattern of the gum tissues in EP in rats.

Materials and Methods

Experimental animals

The studies were performed from September to December. The experiments were performed in 50 white male Wistar rats weighing 230-250 g. The animals were kept in the vivarium in the standard lighting and food ration in accordance with the requirements of the Law of the Russian Federation "On the Animal Protection from Animal Cruelty" dated 06/24/1998, Good Laboratory Practice in Preclinical Studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), the provisions of the World Medical Association Declaration of Helsinki (Report of the AVMA Panel on Euthanasia, JAVMA, 2001), Council Directive 86/609/EEC, the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1997), as well as the rules of the Good Laboratory Practice passed in the Russian Federation (Order of the Ministry of Health of the Russian Federation No.708 of 29.08.2010). The experimental protocols were approved by the local independent Ethical committee of Rostov State Medical University of the Ministry of Health of the Russian Federation (Minutes No. 16/21 of 21.10.2021).

Prior to the study, the rats were quarantined (for at least 15 days), according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. After the quarantine was completed, healthy animals with clear and shiny hair, moderate locomotor activity, no signs of lesions of the conjunctiva, mucous membranes of the nose and the mouth (inclusions) were selected. Unhealthy rats with signs of lesions from the above mentioned tissues were rejected (exclusions).

Pharmaceutical substances

Soderm[®]-Forte (Chemical and Biological Association "Firm VITA" Ltd) and Cytoflavin[®] ("Polysan" Ltd) were the studied substances in the EP in rats. As an anesthetic, "Zoletil 100" (Virbac Sante Animale, France) was used at a dose of 15–20 mg/kg intraperitoneally.

Study design

The rats were randomized into 5 groups of 10 individuals each: group 1 – the animals with intact periodontium (control-1); group 2 – the animals with EP (control-2); group 3 – the animals with EP treated with TDT, including irrigation of the oral cavity with a solution of chlorhexidine bigluconate (0.05%), Septo-Pack dentogingival dressing (Septodont, France); group 4 – the animals with EP treated with the combination of TDT with Soderm[®]-Forte gel, which was injected into parodontal recess of the lower incisors (as well as in the 5th group of animals) using a syringe and disposable syringe tips cannulae (Bisco Inc., USA); group 5 – the animals with EP treated

with the combination of TDT with Soderm®-Forte gel and Cytoflavin®, which was administered intraperitoneally at a dose of 100 mg/kg (in terms of succinic acid). The animals with EP (groups 3-5) were treated for 12 days. The period of observation for groups 1-5 of rats was 42 days (Fig. 1).

Research methods

EP in rats was simulated by the ligature method described by Leontiev et al. (2020). A ligature with EVROLON 4/0 material (LLC “MZKRS Suture Materials”, Russia) was applied to the necks of the lower incisors, followed by their immersion to the dento-gingival groove for 30 days. For secure fixation of the ligature, a light-activated flowable composite resin – Versa Flo (Cetnrics Inc., USA) was used. Biopsy material for pathomorphological examination was taken from the marginal part of the gum of the cervical region of the lower incisors on the 42nd day of observation, i.e. 12 days after the start of pharmacotherapy.

Samples of the animals' gum were fixed in 10% neutral buffered formalin for 24 hours. After that, histological processing of the obtained samples was carried out using isopropyl alcohol and xylene in a Logos microwave tissue processor (Milestone, Italy). Then the samples of the gum were embedded in paraffin according to the generally accepted method; the sections were made 3–5 microns thick, using a rotary microtome Leica RM 2255 (Leica, Germany). Histological sections were stained with hematoxylin-eosin to obtain general pattern; Masson's trichrome was used for collagen fibers.

The obtained slides were studied using a Leica DM1000 microscope (Leica, Germany), and microphotography was performed with a Leica ICC50 E digital camera with Leica LAS Core software (Leica, Germany).

Morphometry was performed by computer image analysis using a Zeiss AxioLab.A1 microscope (Carl Zeiss Micro Imaging GmbH, Germany) with a personal computer and open source software “ImageJ” (US National Institutes of Health, USA). The epithelial thickness, acanthosis depth and diameter of blood vessels were measured using the “Straight Selection” software at the magnification of $\times 40$. The number of capillaries was calculated using a fine tuned “Analyze particles” tool. At the same time, the slice area required for calculation was determined manually using the “Free hand selection” or “Polygon selection” tools. To quantify the fibrosis severity, the staining area coefficient (SAC) was calculated, which is the percentage ratio of the total area of collagen fibers detected by Masson to the total area of the biopsy section.

Statistical data processing

Statistical analysis of the morphometric study was carried out using “MedCalc Statistical Software” (Belgium). All samples were tested for the type of distribution using the Shapiro-Wilk W-test. Due to the absence of a normal distribution in most ordered samples, the results were described as a median (Me), lower and upper quartiles [Q1 and Q3, respectively]. Pearson's chi-squared test was used to determine the significance of the differences. The null hypothesis was rejected at the level of statistical significance $p < 0.05$.

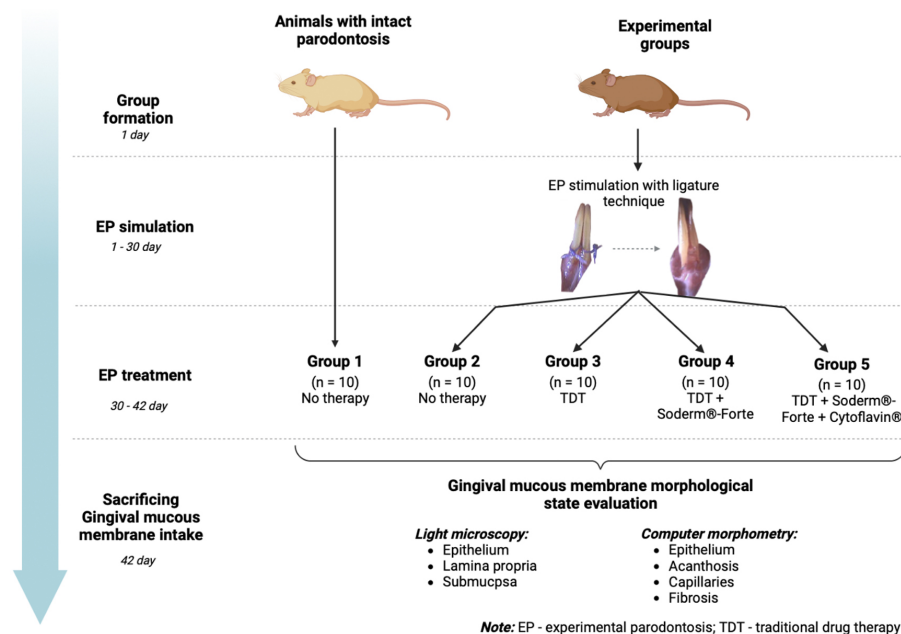


Figure 1. Flow diagram of the study design. **Note:** EP – experimental periodontitis; TDT– traditional drug therapy.

Results and Discussion

Results of pathomorphological examination using light microscopy found that in the samples of the mucous membrane of the marginal part of the gum in group 1 of the rats with intact periodontium (control-1), the epithelial layer and submucous tissue did not have any pathological changes. Stratified squamous epithelium had a typical layering and cellular composition, as well as a pattern of uniform keratinization. The cells of the basal layer formed numerous outgrowths (rete ridges) in the underlying connective tissue. The proper mucous plate is represented by a superficial papillary layer formed by loose connective tissue and the deeper reticular layer, consisting of dense connective tissue.

The acanthosis had minimal severity (Fig. 2A).

The gum sections in the 2nd group of the animals with EP (control-2) revealed changes in the epithelial layer and the proper mucous plate specific to proliferative inflammation. Stratified squamous epithelium had keratinization disorder and hydropic degeneration. The thickening of the epithelial layer, hyperplasia of the basal layer, elongation of the rete ridges with the formation of acanthosis were observed. Focal or diffuse lymphocyte and macrophage infiltration with an admixture of single neutrophils and plasma cells was present in the proper mucous plate. Infiltrating cells reached the alveolar processes, destruction of the collagen fibers, areas of neoangiogenesis were observed (Figs 2B and 2C).

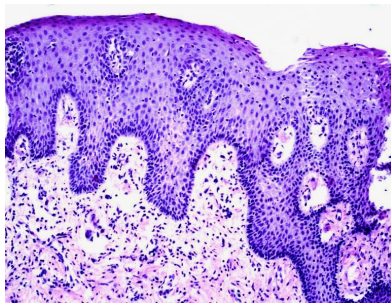


Figure 2A. Gum mucosa of the rat with intact periodontium. Stained with hematoxylin and eosin. Magnification $\times 50$

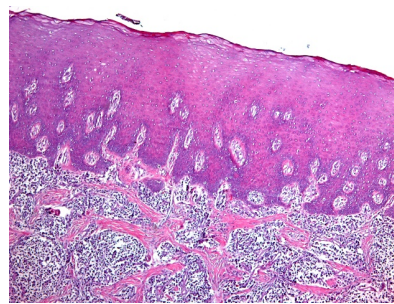


Figure 2B. Stratified squamous epithelium with hyperkeratosis. Severe acanthosis. Diffuse leukocyte and macrophage infiltration of the subepithelial layer of the gum. Stained with hematoxylin and eosin. Magnification $\times 50$

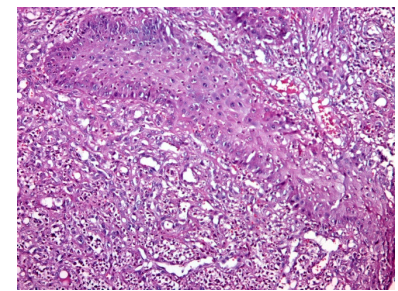


Figure 2C. Acanthotic epithelial strands surrounded by a lot of thin-walled small vessels, diffuse lymphocyte and macrophage infiltration of the gingival mucosa. Stained with hematoxylin and eosin. Magnification $\times 100$

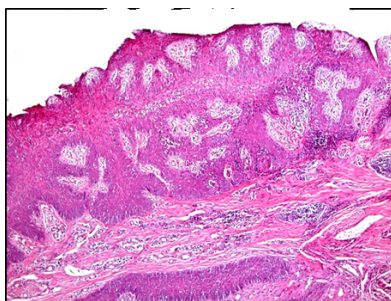


Figure 2D. Stratified squamous epithelium with parakeratosis, acanthosis. Fibrosis, perivascular focal lymphocyte and macrophage infiltration in the proper mucous plate. Stained with hematoxylin and eosin. Magnification $\times 50$

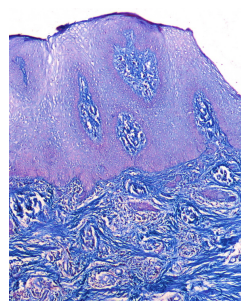


Figure 2E. Severe fibrosis of the proper mucous plate and rete ridges. Masson's trichrome. Magnification $\times 100$

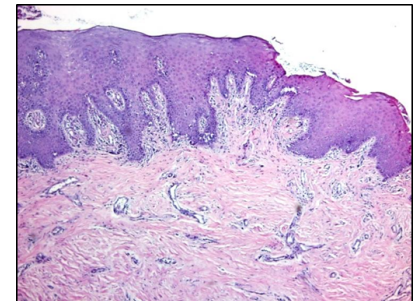


Figure 2F. Stratified squamous epithelium with parakeratosis and acanthosis, lymphocyte and macrophage infiltration in the rete ridges, fibrosis of the proper mucous plate. Stained with hematoxylin and eosin. Magnification $\times 50$

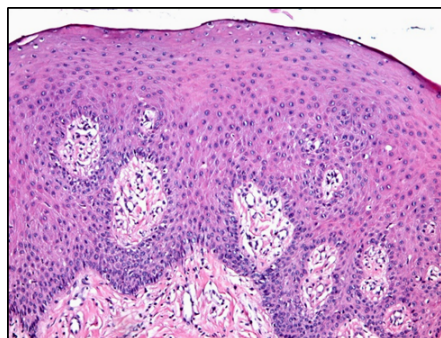


Figure 2G. Normal histological structure of the gingival epithelium. Absence of acanthosis in the stratified squamous epithelium. Scanty lymphocyte and macrophage infiltration of the proper mucous plate.

Figure 2. Histological samples of the rat's gum

In two cases (20%), the inflammation had signs of a severe exudative component with areas of epithelial destruction and the formation of erosions, the presence of a large number of intraepithelial leukocytes.

In the 3rd group of the rats with EP treated with TDT epithelial changes of the gingival mucosa in the form of hyperkeratosis and acanthosis, hyperplasia of the spinous layer, basal cell hyperplasia were less frequently observed compared with the 2nd group (control-2). Inflammatory cellular infiltration was perivascular predominantly (Figs 2D and 2E).

In one case (10%), necrotic changes of the mucous membrane persisted in the form of acute erosions. Fibrosis of the proper mucous plate was noted in one case (10%) – the formation of fibro-papillary overgrowths on the gum surface.

In the gingival samples of the 4th group of the animals with EP treated with the combination of TDT with Soderm[®]-Forte gel, epithelial acanthosis was low-grade, hyperplasia of the cells of the spinous layer, basal cell hyperplasia were observed. Inflammatory cell infiltration was reduced in comparison with the control group to focal mild and moderate, with a predominant presence in the rete ridges. In some cases, intraepithelial leukocytes and moderate stroma fibrosis were present (Fig. 2F).

In the samples of the 5th group of the rats with EP treated with the combination of TDT with Soderm[®]-Forte gel and Cytoflavin[®], in most cases there were no changes in the epithelial layer, fibrosis of the proper mucous plate was not pronounced; in rare cases, mild inflammatory cell infiltration persisted (Fig. 2G).

Computer morphometry revealed that in the 1st group of the animals with intact periodontium, the median thickness of the epithelium was 124.80 [110.19; 141.86] μm ; the acanthosis depth was 52.62 [31.17; 94.74] μm ; the number of capillaries per 1 mm^2 was 33.46 [24.04; 47.39]; the diameter of capillaries was 35.30 [28.61; 37,31] μm , and the fibrosis severity (SAC) – 12.03 [10.84; 14.09] % (Table, Figs 3-7).

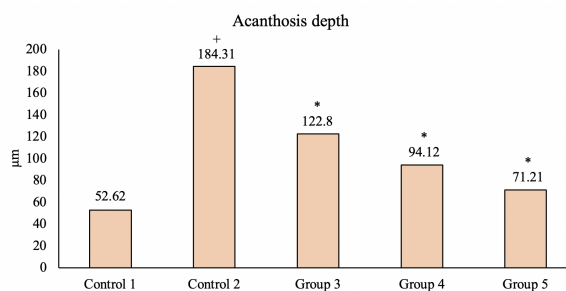


Figure 4. Acanthosis depth in the rat's gingival epithelium (microns). **Note:** + – difference from group 1, $p < 0.05$; * – difference from group 2, $p < 0.05$

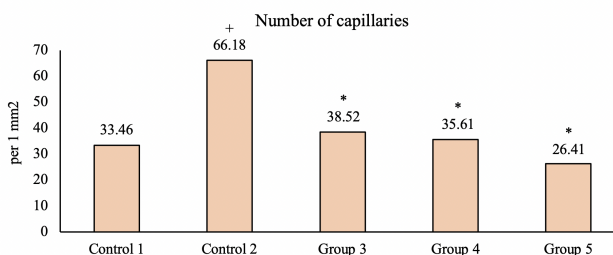


Figure 5. Number of capillaries in the rat's gingival epithelium (per 1 mm^2). **Note:** + – difference from group 1, $p < 0.05$; * – difference from group 2, $p < 0.05$.

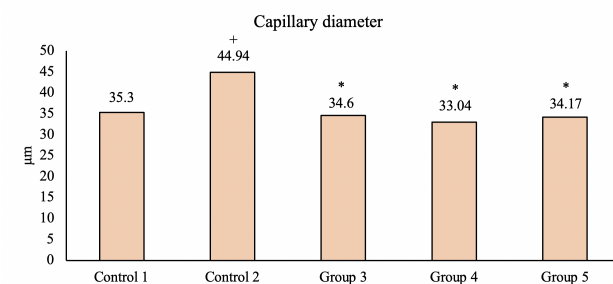


Figure 6. Capillary diameter in the rat's gingival epithelium (microns). **Note:** + – difference from group 1, $p < 0.05$; * – difference from group 2, $p < 0.05$.

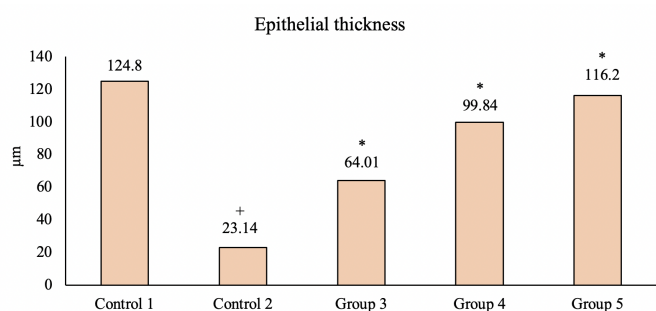


Figure 3. Thickness of the rat's gingival epithelium (microns). **Note:** + – difference from group 1, $p < 0.05$; * – difference from group 2, $p < 0.05$

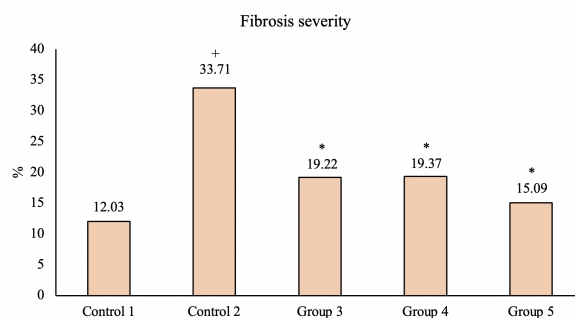


Figure 7. Fibrosis severity in the rat's gingival epithelium (SAC, %). **Note:** + – difference from group 1, $p < 0.05$; * – difference from group 2, $p < 0.05$.

Table. Computer morphometry of the rat's gingival mucosa

Group	Me [Q1; Q3]				
	Epithelial thickness, μm	Acanthosis depth, μm	Number of capillaries per 1 mm ²	Capillary diameter, μm	SAC of fibrosis, %
1	124.80 [110.19; 141.86]	52.62 [31.17; 94.74]	33.46 [24.04; 47.39]	35.30 [28.61; 37.31]	12.03 [10.84; 14.09]
2	23.14 [19.56; 30.51]*	184.31 [160.12; 202.15]*	66.18 [51.79; 83.18]*	44.94 [41.61; 47.05]*	33.71 [28.29; 39.24]*
3	64.01 [57.24; 72.03]**	122.80 [105.10; 137.79]**	38.52 [35.93; 41.88]**	34.60 [29.98; 38.63]**	19.22 [15.72; 22.35]**
4	99.84 [85.44; 107.76]**	94.12 [78.63; 110.20]**	35.61 [30.95; 38.67]**	33.04 [30.03; 35.66]**	19.37 [17.61; 21.82]**
5	116.20 [105.23; 133.51]**	71.21 [46.52; 90.66]**	26.41 [25.58; 29.08]**	34.17 [32.28; 37.00]**	15.09 [13.54; 16.77]**

Note: SAC – the staining area coefficient; * – significant difference from the group 1, $p < 0.05$; ** – significant difference from the group 2 (untreated EP), $p < 0.05$.

In the 2nd group of the rats with EP, the thickness of the gingival epithelium decreased 5.4 times ($p < 0.05$). The values of the other measured parameters increased as follows: the acanthosis depth – 3.5 times ($p < 0.05$), the number of capillaries per 1 mm² of the slice – 2.0 times ($p < 0.05$), the capillary diameter – 1.3 times ($p < 0.05$), and the fibrosis severity – 2.8 times ($p < 0.05$) (Table, Figs 3-7).

In the 3rd group of the animals with EP treated with TDT, the thickness of the gingival epithelium increased 2.8 times ($p < 0.05$) when compared with the 2nd group (control-2). On the contrary, the acanthosis depth decreased 1.5 times ($p < 0.05$); the number of capillaries per 1 mm² reduced 1.7 times ($p < 0.05$); the capillary diameter decreased 1.3 times ($p < 0.05$), and the fibrosis severity decreased 1.8 times ($p < 0.05$) (Table, Figs 3-7).

In the 4th group of the rats with EP treated with the combination of TDT with Soderm[®]-Forte, the gingival epithelium was 4.3 times thicker ($p < 0.05$); the acanthosis depth decreased twofold ($p < 0.05$); the number of capillaries per 1 mm² reduced 1.9 times ($p < 0.05$); the capillary diameter decreased 1.4 times ($p < 0.05$), and the fibrosis severity decreased 1.7 times ($p < 0.05$) compared with the 2nd group (control-2) (Table, Figs 3-7).

In the 5th group of the animals with EP, treated with the combination of TDT with Soderm[®]-Forte and Cytoflavin[®], the thickness of the gingival epithelium increased 5.0 times ($p < 0.05$); the acanthosis depth decreased 2.6 times ($p < 0.05$); the number of capillaries per 1 mm² reduced 2.5 times ($p < 0.05$); the capillary diameter reduced 1.3 times ($p < 0.05$), fibrosis severity decreased 2.2 times ($p < 0.05$) compared with the 2nd group (control-2) (Table, Figs 3-7).

Thus, the performed pathomorphological studies show that the TDT has a moderate positive effect on pathomorphological changes in the gum in EP in rats. The combination of the TDT with Soderm[®]-Forte and, more significantly, the combination of the TDT with Soderm[®]-Forte and Cytoflavin[®] have high therapeutic efficacy, characterized by rapid regeneration of the gum tissues.

Conclusion

The administration of the combination of the TDT with Soderm[®]-Forte and Cytoflavin[®] for 12 days in EP in rats has a pronounced regulating effect on pathomorphological changes in the gum tissues, characterized by resolution of inflammation due to the activation of regenerative processes.

According to the significance of the regenerative effect, the studied combinations and TDT can be arranged in the following decreasing range: TDT + Soderm[®]-Forte + Cytoflavin[®] > TDT + Soderm[®]-Forte > TDT.

The data obtained in this study, as well as the results of the previous studies (Popkov et al. 2022) indicate the promising future outlook of clinical studies of the combination of the TDT with Soderm[®]-Forte and Cytoflavin[®] to improve the treatment effectiveness in patients with periodontitis.

Conflict of interest

The authors declare no conflict of interests.

References

- Ahmad SA, Das SS, Khatoun A, Ansari MT, Afzal M, Hasnain MS, Nayak AK (2020) Bactericidal activity of silver nanoparticles: A mechanistic review. *Materials Science for Energy Technologies* 3: 756–769. <https://doi.org/10.1016/j.mset.2020.09.002>
- Badawy AA (2020) Immunotherapy of COVID-19 with poly (ADP-ribose) polymerase inhibitors: Starting with nicotinamide. *Bioscience Reports* 40(10): BSR20202856. <https://doi.org/10.1042/BSR20202856> [PubMed] [PMC]
- Bakhit M, Hoffmann T, Scott AM, Beller E, Rathbone J, Del Mar C (2018) Resistance decay in individuals after antibiotic exposure in primary care: a systematic review and meta-analysis. *BMC Medicine* 16(1): 26. <https://doi.org/10.1186/s12916-018-1109-4> [PubMed] [PMC]
- Balmasova PE, Tsarev VN, Yanushevich OO, Maev IV, Mkrtumyan AM, Arutyunov SD (2021) Periodontal Microecology. The Relationship of Local and System Effects: monograph. Practical Medicine, Moscow, 264 pp. [in Russian]
- Bedrosova KA, Faustov LA, Zadorozhnyi AV, Sycheva NL, Stepanyuk GI, Sukoyan GW, Galenko-Yaroshevsky PA, Popkov VL, Leontiev VK (2018) Benzofurocaine in the Complex Treatment of Periodontitis. Education-South, Krasnodar, 176 pp. [in Russian]
- Bharkhavy KV, Pushpalatha C, Anandakrishna L (2022) Silver, the magic bullet in dentistry – A review. *Materials Today: Proceedings* 50(2): 181–186. <https://doi.org/10.1016/j.matpr.2021.12.200>
- Bhattarjee S, Banerjee M (2020) Immune thrombocytopenia secondary to COVID-19: a systematic review. *SN Comprehensive Clinical Medicine* 2(11): 2048–2058. <https://doi.org/10.1007/s42399-020-00521-8> [PubMed] [PMC]
- Das C, Paul SS, Saha A, Singh T, Saha A, Im J, Biswas G (2020) Silver-based nanomaterials as therapeutic agents against coronaviruses: A review. *International Journal of Nanomedicine* 15: 9301–9315. <https://doi.org/10.2147/IJN.S280976> [PubMed] [PMC]
- Dung TTN, Nam VN, Nhan TT, Ngoc TTB, Minh LQ, Nga BTT, Le VP, Quang DV (2020) Silver nanoparticles as potential antiviral agents against African swine fever virus. *Materials Research Express* 6(12): 1250g9. <https://iopscience.iop.org/article/10.1088/2053-1591/ab6ad8>
- Enas TE, Amal AA, Sakeenabi B, Nayef HF, Sanaa MF, Gad El-Rab (2021) Antimicrobial activity of biosynthesized silver nanoparticles, amoxicillin, and glass-ionomer cement against *Streptococcus* mutants and *Staphylococcus aureus*. *Nanotechnology* 32(21): 215101. <https://doi.org/10.1088/1361-6528/abe577> [PubMed]
- Farheen S, Oanz AM, Khan N, Umar MS, Jamal F, Kashif M, Alshameri AW, Khan S, Owais M (2021) Antimicrobial effect of nano-silver against oral streptococci: Implications in containment of bacterial biofilm on orthodontal appliances. Preprints. 2021: 2021020436. <https://doi.org/10.20944/preprints202102.0436.v1>
- Fehaid A, Fujii R, Sato T, Taniguchi A (2020) Silver nanoparticles affect the inflammatory response in a lung epithelial cell line. *The Open Biotechnology Journal* 14: 113–123. <https://doi.org/10.2174/1874070702014010113>
- Fernandez CC, Sokolonski AR, Fonseca MS, Stanisic D, Araújo DB, Azevedo V, Portela RD, Tasic L (2021) Applications of silver nanoparticles in dentistry: Advances and technological innovation. *International Journal of Molecular Sciences* 22(5): 2485. <https://doi.org/10.3390/ijms22052485> [PubMed] [PMC]
- Galenko-Yaroshevsky PA, Tseluiko KV, Leontev VK, Zadorozhnyi MA, Popkov VL, Zelenskaya AV, Babichev SA, Zadorozhnyi AV, Meladze SV (2022) The effectiveness of Soderm®-Forte gel and a new injectable dosage form of Rexod® in the complex treatment of experimental periodontitis in rats. *Research Results in Pharmacology* 8(1): 51–58. <https://doi.org/10.3897/rppharmacology.8.79641>
- Galenko-Yaroshevsky PA, Tseluiko KV, Pavlyuchenko II, Leontiev VK, Zadorozhnyi AV, Popkov VL, Lebedeva SA, Zelenskaya AV, Zadorozhnyi MA, Zobenko VY (2022) Effect of traditional drug therapy and its combination with Soderm®-Forte and new injectable form of Rexod® on the antioxidant-prooxidant red blood cell system in rats with experimental periodontitis. *Kuban Scientific Medical Bulletin [Kubanskii Nauchnyi Meditsinskii Vestnik]* 29(4): 32–52. <https://doi.org/10.25207/1608-6228-2022-29-4-32-52> [in Russian]
- Guerra F, Mazur M, Ndokaj A, Corridore D, La Torre G, Polimeni A, Ottolenghi L (2018) Periodontitis and the microbiome: A systematic review and meta-analysis. *Minerva Stomatologica* 67(6): 250–258. <https://doi.org/10.23736/S0026-4970.18.04198-5> [PubMed]
- Kadam P, Mahale S, Sonar P, Chaudhari D, Shimpi S, Kathurwar A (2020) Efficacy of silver nanoparticles in chronic periodontitis patients: A clinico-microbiological study. *Iberoamerican Journal of Medicine* 2(3): 142–147. <http://dx.doi.org/10.5281/zenodo.3749621>
- King G, Jefferson M, Thomas EL, Stein SH, Jefferia JH, Babu JP (2019) Inhibition of porphyromonas gingivalis gingipain activity by prenylated flavonoid, Sanggenol A. *Online Journal of Dentistry & Oral Health* 2(3): 2019. <https://doi.org/10.33552/OJDOH.2019.02.000536>
- Kondratiev AN, Alexandrovich Yu, Dryagina NV (2021) Method of two-component model of neurovegetative and metabolic stabilization with complicated course of coronavirus infection COVID-19. Handbook for doctors. Association of North-West Anesthesiologists, Saint-Petersburg, pp. 128 [in Russian]
- Kopitov AA, Leontiev VK (2022) Violation of hydrodynamics as an etiological factor of parodontitis and paradontosis. BelSU Publishing House, Belgorod, 128 pp. [in Russian]
- Kowalczyk P, Szymczak M, Maciejewska M, Laskowski Ł, Laskowska M, Ostaszewski R, Skiba G, Franiak-Pietryga I (2021) All that glitters is not silver – a new look at microbiological and medical applications of silver nanoparticles. *International Journal of Molecular Sciences* 22(2): 854. <https://doi.org/10.3390/ijms22020854> [PubMed] [PMC]
- Kubyshekin A, Pisareva O, Bessalova Y, Fomochkina I (2020) The prospects of using the silver nanoparticles composition in sodium alginate matrix. *MATEC Web of Conferences* 315. <https://doi.org/10.1051/mateconf/202031509001>
- Kuzmina AB, Asetskaia IL, Polivanov VA, Zyryanov SK (2019) Medical errors when applying beta-lactam antibiotics: analysis of the Russian base of spontaneous messages. *Antibiotics and Chemotherapy [Antibiotiki i Khimioterapiya]* 11-12(64): 48–53. <https://doi.org/10.37489/0235-2990-2019-64-11-12-48-53> [in Russian]
- Leontiev VK, Faustov LA, Galenko-Yaroshevsky PA, Popkov VL, Sycheva NL, Popkov LV, Ordian LL (2012) Chronic Generalized Periodontitis: Clinical and Experimental Pharmacotherapy with Metabolic Correctors. Education-South, Krasnodar, 403 pp. [in Russian]
- Leontiev VK, Zelujko KV, Zadorozhnyi AV, Popkov VL, Galenko-Yaroshevsky PA (2020) The influence of nano-silver and new injection form of Rexod on the condition of periodontal tissues in experimental periodontitis in rats. *Dentistry for Everyone [Stomatologiya Dlya Vsekh]* 2(91): 12–16. [https://doi.org/10.5556/idr-2020-2\(91\)12-16](https://doi.org/10.5556/idr-2020-2(91)12-16) [in Russian]
- Maksimova AI, Valamina II, Polushina LG, Tsvirenko SV, Bazarniy VV (2022) Modification of reparative liver regeneration of laboratory animals after partial hepatectomy. *Perm Medical Journal [Permskii Meditsinskii Zhurnal]* 39(1): 124–132. <https://doi.org/10.17816/pmj391124-132>
- Marashly ET, Bohlega SA (2017) Riboflavin has neuroprotective potential: Focus on Parkinson's disease and migraine. *Frontiers of Neurology* 8: 333–340. <https://doi.org/10.3389/fneur.2017.00333> [PubMed] [PMC]
- Novikova VV, Russkih AA (2018) The investigation of antifungal activity of new silver salt of Pyrazol-3-carboxamides in vitro. *Drug Development & Registration [Razrabotka i Registratsiya Lekarstvennykh Sredstv]* (2): 92–95. [in Russian]
- Olenskaya TL (2021) Innovative methods of rehabilitation at the outpatient and home stages in patients after pneumonia COVID-19. *Medical Council [Meditsinskii Sovet]* 4: 220–229. <https://doi.org/10.21518/2079-701X-2021-4-220-229> [in Russian]
- Orekhova LYu, Loboda ES, Kosova EV, Vashneva VYu, Petrov AA (2020) Topical antibiotic therapy in periodontology. *Periodontology [Parodontologiya]* 25(3): 217–223. <https://doi.org/10.33925/1683-3759-2020-25-3-217-223> [in Russian]

- Orlov YuP, Govorova NV, Korpacheva OV, Afanasiev VV, Khilenko IA (2021) On the possibility of using drugs of succinate group under hypoxia conditions at COVID-19. *General Reanimatology [Obtshaya Reanimatologiya]* 17(3): 78–98. <https://doi.org/10.15360/1813-9779-2021-3-78-98> [in Russian]
- Popkov VL, Zadorozhny MA, Heigetyan AV, Galenko-Yaroshevsky PA (2022) Influence of Soderm®-Forte composition and Cytoflavin metabolic energy corrector during inflammatory process under conditions of experimental periodontitis. *Dentistry for Everyone [Stomatologiya Dlya Vsekh]* 2(99): 46–53. [https://doi.org/10.35556/idr-2022-2\(99\)46-53](https://doi.org/10.35556/idr-2022-2(99)46-53) [in Russian]
- Shapovalov KG, Tsydenpilov GA, Lukyanov SA, Trusova YuS, Konnoav VA (2020) Prospects for the application of succinates in the severe course of a new coronavirus infection. *Experimental and Clinical Pharmacology [Eksperimental'naya i Klinicheskaya Farmakologiya]* 83(10): 40–43. <https://doi.org/10.30906/0869-2092-2020-83-10-40-43> [in Russian]
- Singh P, Sungeun A, Jong-Pyo K, Soshnikova V, Huo Y, Singh H, Chokkaligam M, El-AgamyFarh M, Aceituno VC, Kim YJ, Deok-Chun Y (2018) In vitro anti-inflammatory activity of spherical silver nanoparticles and monodisperse hexagonal gold nanoparticles by fruit extract of *Prunus serrulata*: a green synthetic approach. *Artificial Cells, Nanomedicine, and Biotechnology* 8(46): 2022–2032. <https://doi.org/10.1080/21691401.2017.1408117> [PubMed]
- Shin HS, Ye MK, Che MH, Lee DW (2018) Anti-inflammatory effect of nano-silver in chronic rhinosinusitis mouse model. *Biomedical Journal of Scientific and Technical Research* 11(1): 8287–8292. <https://doi.org/10.26717/bjstr.2018.11.002050>
- Talapko J, Matijevic T, Antolovic-pozgan A (2020) Antibacterial activity of silver and its application in dentistry, cardiology and dermatology. *Microorganisms* 8(9): 1400. <https://doi.org/10.3390/microorganisms8091400> [PubMed] [PMC]
- Tsarev VN, Yagodina EA, Tsareva TV, Nikolaeva EN (2020) The impact of the viral-bacterial consortium on occurrence and development of chronic periodontitis. *Periodontology [Parodontologiya]* 25(2): 84–89. <https://doi.org/10.33925/1683-3759-2020-25-2-84-88> [in Russian]
- Tsepov LM, Nikolaev AI, Petrova EV, Nesterova MM (2018) Pathogenetic substantiation of clinical use of medicines in complex therapy for inflammatory diseases of parodontium (literature review). *Periodontology [Parodontologiya]* 23(2): 4–9. <https://doi.org/10.25636/PMPI.2018.2.1> [in Russian]
- Ungerstedt JS, Blomback M, Soderstrom T (2003) Nicotinamide is a potent inhibitor proinflammatory cytokines. *Clinical and Experimental Immunology* 131(1): 48–52. <https://doi.org/10.1046/j.1365-2249.2003.02031.x> [PubMed] [PMC]
- Ushakov RV, Tsarev BH (2019) Antimicrobial therapy in dentistry. Principles and algorithms. Practical medicine, Moscow, 240 pp. [in Russian]
- Yunusov KE, Sarymsakov AA, Mullajonova SV, Turakulov FM (2020) Bactericidal effect of cotton fabric treated with polymer solution containing silver nanoparticles of different sizes and shapes. *Asian Journal of Chemistry* 32(6): 1335–1342. <https://doi.org/10.14233/ajchem.2020.22266>
- Zhuravleva MV, Rodionov BA, Lysenko MA, Yakovlev SV, Andreev SS, Ilyukhina NN, Prokofiev AB (2021) Study of cases of bacteremia with gram-negative pathogens with multiple and extreme antibiotic resistance in real clinical practice. *Antibiotics and Chemotherapy [Antibiotiki i Khimioterapiya]* 66(3-4): 27–34. <https://doi.org/10.37489/0235-2990-2021-66-3-4-27-34> [in Russian]

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