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Research Article

The effect of the composition of Soderm®-Forte gel and the new injectable form of Rexod® on pathology findings in gingival tissue in experimental periodontitis in rats

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Abstract

Introduction: Periodontitis is the most important problem of modern dentistry. The development of new medicines and treatment regimens for patients with periodontal complex lesions is a strategic direction of modern pharmacology and dentistry. In this view, pride of place goes to morphological research, which allows not only to study the effect of drugs on pathomorphological changes in periodontal tissues, but also to estimate their therapeutic effectiveness. Aim of the study: to determine the nature of the effect of the composition of Soderm®-Forte gel and the new injectable form of Rexod® on the pathology findings in gingival tissue of rats with experimental periodontitis.

Materials and methods: Experimental periodontitis (EP) was induced in rats by ligature method. The study was performed according to the following algorithm: animals with intact periodontium; animals with untreated EP; animals with EP treated with traditional drug therapy (TDT); animals with EP treated with combinations of TDT with Soderm®-Forte gel and TDT with Soderm®-Forte gel and the new injectable form (NIF) of Rexod®. For pathomorphological examination, biopsy specimen was taken from the gingival margin of the lower incisors. The ImageJ software was used for computer morphometry.

Results and discussion: Examination of the gum samples revealed moderate therapeutic effects of the TDT. The combinations of TDT with Soderm®-Forte gel and, to a greater extent, TDT with Soderm®-Forte gel and the NIF of Rexod® showed high pharmacotherapeutic efficacy, manifested in rapid regeneration of the gingival tissues.

Conclusion: The combination of TDT, Soderm®-Forte gel and the NIF of Rexod® shows the most beneficial effect on the pathological processes in the gum. The pharmacotherapeutic effect of the studied combination promotes the earliest regeneration of damaged gum tissues and reduces the risk of persistent pathology changes in them.

Keywords

Soderm®-Forte gel, computer morphometry, new injectable form of Rexod®, pathology findings, gingival tissues, experimental periodontitis.

Introduction

Morphological evaluation of the development and outcome of the inflammatory process in the tissues of the periodontal complex is of great interest due to advent of new pharmacotherapy tools for patients with chronic generalized periodontitis (CGP). In this regard, the results obtained in the experimental periodontitis (EP) are extremely important for evaluation the effectiveness of new drugs and their method of administration in patients with the corresponding pathology (Bedrosova et al. 2018; Leontyev et al. 2020).

According to modern views on the pathogenesis of CGP, an important role is played by the microecology of the oral cavity associated with the presence in it of periodontal pathogenic microorganisms of the first (Porphyromonas gingivalis, actinomicetemcomitans and Tanerella forsythia) and second (Treponema denticola, Prevotella intermedia and Prevotella nigrescens) order, co-infecting agents (viruses, chlamydia, fungi, protozoa, etc.) and opportunistic species (Staphylococcus, Pseudomonas, Mycobacterium, Bacillus, etc.) (King et al. 2019; Ushakov and Tsarev 2019; Tsarev et al. 2020; Balmasova et al. 2021). Specificity of these microorganisms is that they have a wide range of pathogenicity, specific virulence, which allows them to be aggressive, provoke and maintain the inflammation for a long time, disrupt the morphological continuity of periodontal tissues and reduce the immunological reactivity of the entire macroorganism (Guerra et al. 2018; Tsepov et al. 2018).

The administration of various antibacterial and antiseptic drugs, especially antibiotics (Orekhova et al. 2020), leads to the development of stable resistance of the pathogenic microorganisms to them. In this regard, recently more and more attention has been paid to the decrease in the antimicrobial effect of the noted groups of drugs and the increased number of various complications of their use in periodontology (Bakhit et al. 2018; Kuzmina et al. 2019). This initiates the search and development of new tools and methods of complex treatment of patients with inflammatory processes in the periodontal complex. The most important requirements for the proposed drugs and methods of their administration are their ability to reduce the titers of periodontal pathogenic microorganisms, not to cause resistance to them and not to aggravate dysbiosis. The solution to this problem should be comprehensive and targeted when using drugs with pronounced antibacterial, antiviral, antifungal, detoxifying, anti-inflammatory and wound-healing properties, as well as not causing the development of resistance of microorganisms to these drugs (Tsepov et al. 2018; Ushakov and Tsarev 2019).

To date, it is generally accepted that free radical oxidation (FRO) and violations of antioxidant defense (AOD) play an important role in the etiopathogenesis of inflammatory and destructive processes in periodontal tissues (Atabay et al. 2017). The basis of these pathochemical reactions is imbalance of the antioxidant and pro-oxidant systems, which leads to the increased formation of free radicals (FR). This creates conditions for the "oxidative stress" (OS), accompanied by hyper production of FR and cell damage (Grebenchikov et al. 2016; Menschikova and Zenkov 2016). At the level of periodontal tissues, this is manifested by disorders of oxidative homeostasis, impaired functions of the endothelial system and peripheral microcirculation, as well as an increase in vascular-tissue permeability (Chen et al. 2019), which ultimately leads to the development of morphofunctional disorders in the periodontal complex. Among the agents capable of significantly reduce the harmful effects of FR, in particular reactive oxygen species and lipid peroxidation, an important place is given to recombinant human superoxide dismutase (SOD) – Rexod®, especially its new injectable form (NIF) – NIF of Rexod® (Gulevskaya et al. 2021).

Based on the above, with respect to the resistance of periodontal pathogenic infection, drugs based on colloidal (nano) silver seem promising. It is known that these drugs have a wide range of bacteriostatic and bactericidal effects, including antibiotic-resistant gram-positive and gram-negative strains (Talapko et al. 2020; Enas et al. 2021), are able to kill various viruses (respiratory syncytial virus, hepatitis B, coronavirus, etc.) (Das et al. 2020; Dung et al. 2020; Kowalczyk et al. al. 2021), as well as have anti-inflammatory activity (Shin et al. 2018; Singh et al. 2018; Das et al. 2020; Fechaid et al. 2020; Kubyshkin et al. 2020).

As for the violation of the FRO processes in periodontitis, to increase the effectiveness of complex treatment of EP in rats, we selected Soderm®-Forte, which is a mixed-type micellar gel containing nanocluster zero valent metallic silver in the form of Ag_n^{K+} cluster 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 SOD] (LLC "Chemical-biological Corporation at the Russian Academy of Sciences "Vita Company", St. Petersburg, Russia), and NIF of Rexod® (FSUE "State Research Institute of Especially Purified Bioproducts" FMBAof Russia, St. Petersburg, Russia; registration certificate of the Ministry of Health of the Russian Federation – LP–004754), as well as their combination.

Aim of the study: to determine the nature of the effect of the composition of Soderm®-Forte gel and the new injectable form of Rexod® on the pathology findings in gingival tissues in experimental periodontitis in rats.

Materials and methods

Experimental animals

The studies were performed in the autumn-winter period (October-December), with 50 white male Wistar rats weighing 250–320 g. The animals were kept in the vivarium, got a standard food ration in accordance with the generally accepted 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). All the experiments were approved by the local independent ethical committee of Rostov State Medical University of the Ministry of Health of the Russian Federation (Minutes No.17/18 of 10/25/2018).

Prior to the study, the rats were quarantined for at least 14 days (according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes). During the period of isolation, the healthy animals with the following external indicators (inclusions) were selected: clear and shiny hair, moderate locomotor activity, no signs of lesions of the conjunctiva, mucous membranes of the nose and the mouth. Unhealthy animals (exceptions) failing to meet the above requirements were rejected.

Pharmaceutical substances

Soderm®-Forte gel and NIF of Rexod® were the studied substances. As an anesthetic, the veterinary drug "Zoletil 100" (Virbac Sante Animale, France) was used at a dose of 15–20 mg/kg intraperitoneally.

Experimental design

The rats were divided into five groups of 10 individuals each: group 1 included animals with intact periodontium (IP) (control-1); 2nd – with EP, which was simulated within 30 days after ligation of the crevicular area of the gum [this group of animals was untreated (control-2)]; 3rd – with EP treated with TDT, including irrigation of the oral cavity with a solution of chlorhexidine bigluconate (0.05%), application of Septo-Pack dentogingival dressing (Septodont, France); 4th – 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 (Cisco Inc., USA); 5th – with EP, where TDT was combined with Soderm®-Forte gel and the NIF of Rexod®, which was used intraperitoneally at a dose of 8000 units/kg. The animals with EP (groups 3–5) were treated for 12 days. The observation period for all groups of animals was 42 days (Fig. 1).

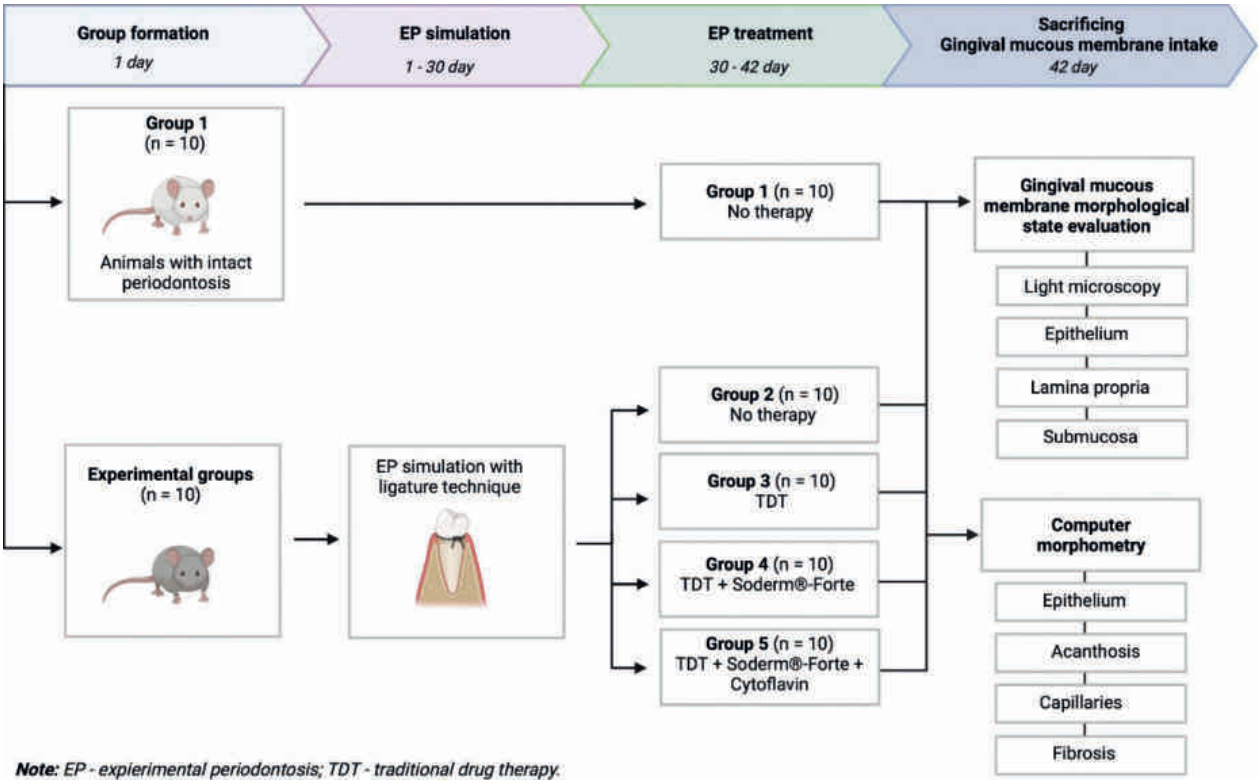

Figure 1. Experimental protocol and group division.

Research methods

The EP was simulated according to the method described by Leontev et al. (2020), by ligation of the necks of the lower incisors with EVROLON 4/0 material (LLC "MZ-KRS Suture materials", Russia). The ligature was a mechanical irritant of the crevicular part of the gum and a retention point of bacterial plaque, which led to calcification of the dental plaque and an inflammatory reaction in periodontal tissues. For secure fixation of the ligature, a light-activated flowable composite resin – Versa Flo (Cetntrics Inc., USA) was used, fixing the ligature to the neck region of the teeth. The pathology induced in this way is an adequate model for simulation of the inflammatory reaction in periodontal tissues and studying the effectiveness of pharmacological agents (Zhulev et al. 2015).

For pathomorphological examination, biopsy samples were taken from the marginal part of the gum of the cervical region of the lower central incisors of rats on the 42nd day of observation, i.e. 18 days after the start of pharmacotherapy. Samples of the animals' gum were fixed in a 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; by Masson – for collagen fibers; and by Mallory – for multicolored staining of fibrous structures of a connective tissue. In addition, methods of combined staining with alcian blue – neutral red, as well as a histochemical periodic acid Schiff reaction according to McManus-Hotchkiss, were used. 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) at magnification ×100, ×200, and ×400.

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 ×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.

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 the 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 p-value less than 0.05.

Results and discussion

Group 1 – the animals with intact periodontium (control-1)

Morphological examination of the gingival mucosa in animals with intact periodontium (IP) showed the absence of any pathological changes in the marginal part of the gum. Both the epithelial lining and the connective tissue corresponded to the norm (Fig. 2). Keratinized stratified squamous epithelium of the non-fixed part of the gum (gingival margin) in most cases had a linear orientation, a typical layering and cellular composition. The cells were of the usual size and shape. The thickness of the stratified squamous epithelium corresponded to the normal parameters. In the most superficial horny layer, the uniform keratinization was observed, characterizing the normal picture of orthokeratosis.

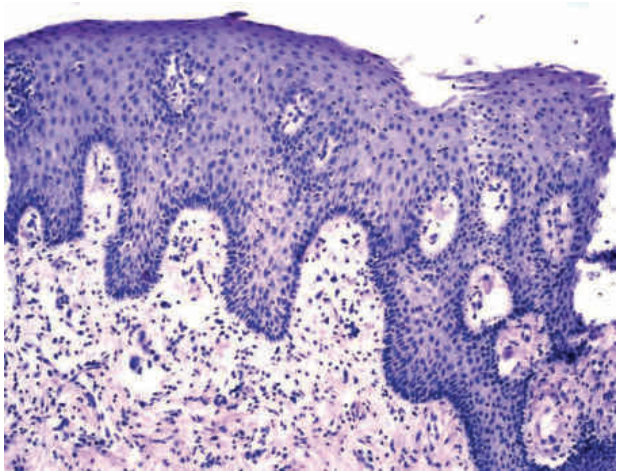

Figure 2. Histological structure of the gingival mucosa in the area of the interdental papillae. Stained with hematoxylin and eosin. Magnification ×100.

The basal layer of the epithelium in most samples was represented by a single row of cells with well-visualized borders. The cells of this layer formed numerous outgrowths (epithelial papillae), which were uniformly immersed in the underlying connective tissue of the proper mucous plate. The acanthosis was absent (Fig. 3) or had minimal severity.

In the gingival epithelium (Fig. 4), in addition to epithelial cells, an insignificant number of various cellular elements (segmented neutrophils, lymphocytes, etc.) were detected, quantities of which corresponded to the average normal values.

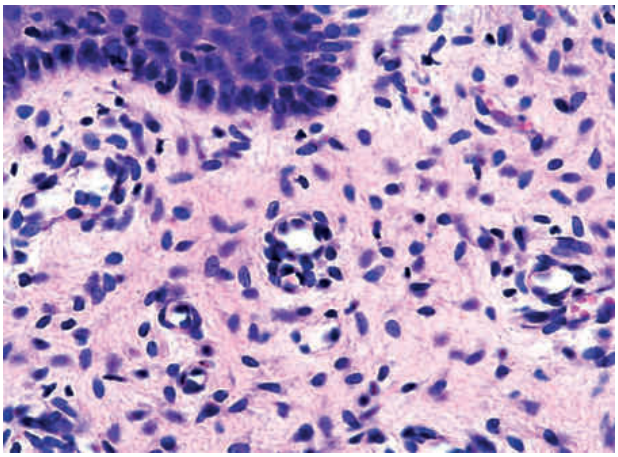

Figure 3. The basal layer of the epithelium is represented by a single row of cells with clear boundaries. Stained with hematoxylin and eosin. Magnification ×400.

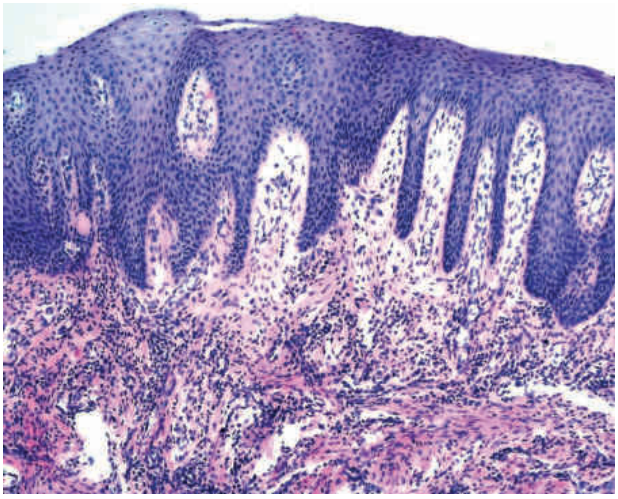

Figure 4. There is a small number of cellular elements in the gingival epithelium, mainly leukocytes. Stained with hematoxylin and eosin. Magnification ×200.

The proper mucous plate of the non-fixed part of the gum was represented by two layers: the superficial papillary, formed by loose connective tissue, and the deeper reticular, formed by dense connective tissue. The differentiation of these layers was unapparent. A small number of different cellular elements (fibroblasts, macrophages, leukocytes, mast cells, plasma cells, etc.) were determined in the both layers of the connective tissue. The presence of fibrous structures was determined in the intercellular substance. There were also single capillaries and postcapillary venules, as well as arterioles with single erythrocytes, indicating their moderate blood filling (Fig. 5).

Thus, the histologic pattern of the non-fixed part of the gum in the animals of the first group (control-1) corresponded to the physiologically normal state (Gushchin and Kvanchiani 2020).

The results of the computer image analysis of the epithelial lining and connective tissue vascular structures of the proper mucous plate of the rat's gum in the control group without experimental periodontitis are presented in Table 1.

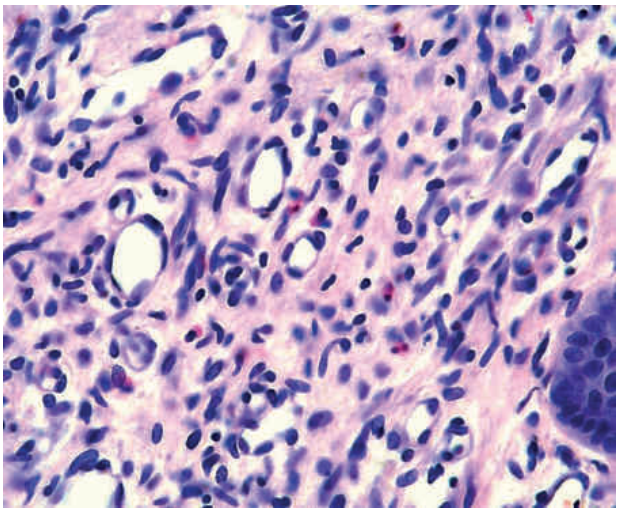

Figure 5. The proper mucous plate of the non-fixed part of the rat's gum. Stained with hematoxylin and eosin. Magnification ×400.

Table 1. Computer morphometry of the gingival mucosa in the animals of group 1 with IP

Group	Me [Q1; Q3]			
	Epithelial thickness, µm	Acanthosis depth, µm	Number of capillaries per 1 mm²	Capillary diameter, µm
1 (IP)	124.80 [110.19; 141.86]	52.62 [31.17; 94.74]	33.46 [24.04; 47.39]	22.30 [20.61; 35.32]

Group 2 – the animals with untreated EP (control-2)

In all samples of the marginal part of the gingival mucosa of the animals with EP, pronounced purulent inflammation was detected, represented as an extensive inflammatory cell infiltrate, which occupied the area of the altered tissues of the gingival mucosa and consisted of various cell elements (neutrophils, lymphocytes, macrophages, and plasma cells). In some places, along with the suppuration, the areas of histolysis were observed, which was evidenced by ulceration of the keratinized stratified squamous epithelium of the gum with pronounced neutrophil infiltration in its subjacent connective tissue (Fig. 6).

Evaluation of the stratified squamous epithelium of the gum samples revealed the diffuse thickening of its layers and the presence of elongation of epithelial papillae. Signs of dyskeratosis and acanthosis with growth down of acanthotic rete ridges were noted. Under the epithelium, at the level of the basal layer, there was a pronounced neutrophil infiltration with presence of lymphocytes, which is characteristic of leukodiapedesis due to increased permeability of the microvascular endothelium. In some cases, fosci of suppuration with histolysis and ulceration were determined in the presence of intensive diapedesis of leukocytes. Locally, the granulations were noted. Morphological characteristics indicate the presence of purulent inflammation in course of formation of granulation tissue (Fig. 7).

Additional staining of the histological specimens made it possible to assess the degree of development of young connective tissue in the gingival mucosa.

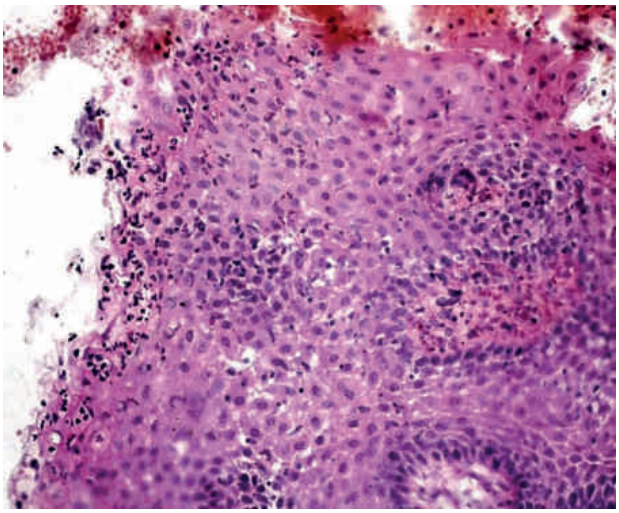

Figure 6. Pronounced purulent inflammation: extensive inflammatory cell infiltration. Areas of ulceration and histolysis. Stained with hematoxylin and eosin. Magnification ×400.

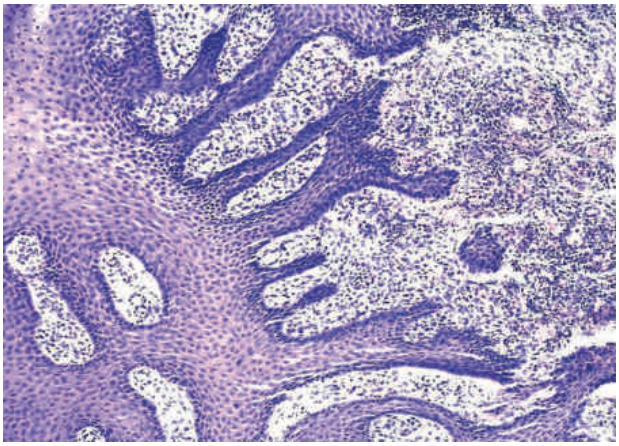

Figure 7. Severe purulent inflammation with acanthosis. Inflammatory cell infiltration and granulation tissue formation. Stained with hematoxylin and eosin. Magnification ×200.

In the deep mucosa, there were the layers of loose connective tissue under the epithelium with signs of acanthosis, along with cellular inflammatory infiltrate, among the newly formed granulation tissue with newly formed thin-walled capillary vessels (Fig. 8). Such pathomorphological findings indicated the development of a purulent process in the mucous membrane of the gum with signs of restructuring not only the epithelium, but also its lamina propria (papillary and reticular layers) with the areas of organization and neoangiogenesis in it.

Epithelial projections in the form of acanthotic structures ("spikes") were found in the proper mucous plate, which grew down into the papillary and reticular layers, sometimes intertwined and anastomosed with each other. This morphological pattern of the samples corresponded to the pseudoepitheliomatous hyperplasia against the background of pronounced inflammatory cell infiltration with lymphocytes and neutrophils (Fig. 9).

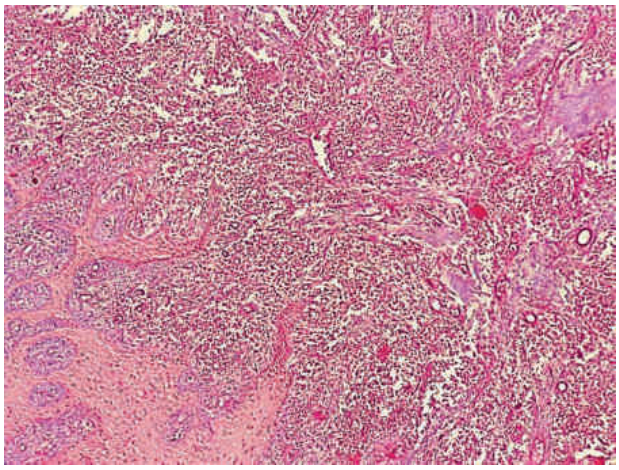

Figure 8. Purulent inflammation; incipient formation of loose connective tissue with angiomatosis. Stained with alcian blue and neutral red. Magnification ×200.

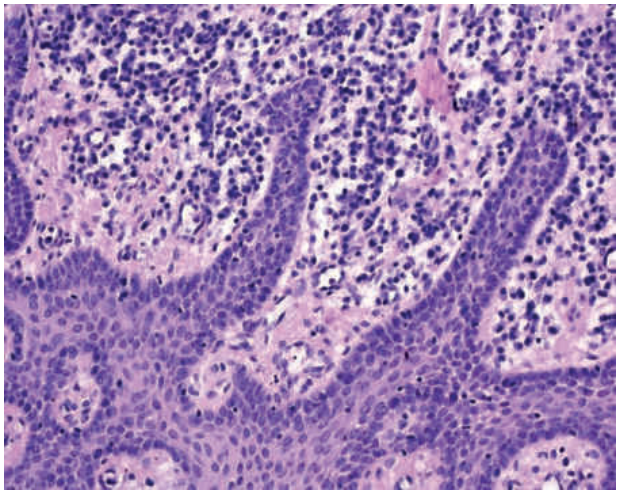

Figure 9. Purulent inflammation in the gum with the restructuring of the epithelial layer: acanthosis with rete ridges. Pronounced infiltration of the stroma with neutrophils or lymphocytes. Stained with hematoxylin and eosin. Magnification ×400.

Thus, the presented morphological findings in the samples of the gingival mucosa of the second experimental group of the animals show the prevalence of the exudative proliferative inflammatory process. These structural changes were realized due to the restructuring of the cells of the stratified squamous epithelium – acanthosis and dyskeratosis (Fig. 10), the proper mucous plate (acanthotic rete ridges, leukodiapedesis, angiomatosis) and the submucous layer of the gum – purulent inflammation, the formation of granulation and loose connective tissue, neoangiogenesis (Fig. 11).

Computer morphometry showed that the studied parameters in rats with experimental periodontitis (Table 2) had statistically significant differences from the animals with intact periodontium ($p<0.05$). The thickness of the epithelial layer of the gum decreased by an average of 2.2 times, the depth of acanthosis increased 5.7 times, the number of blood capillaries in the proper mucous plate increased 2.1 times, and their diameter was 1.5 times larger.

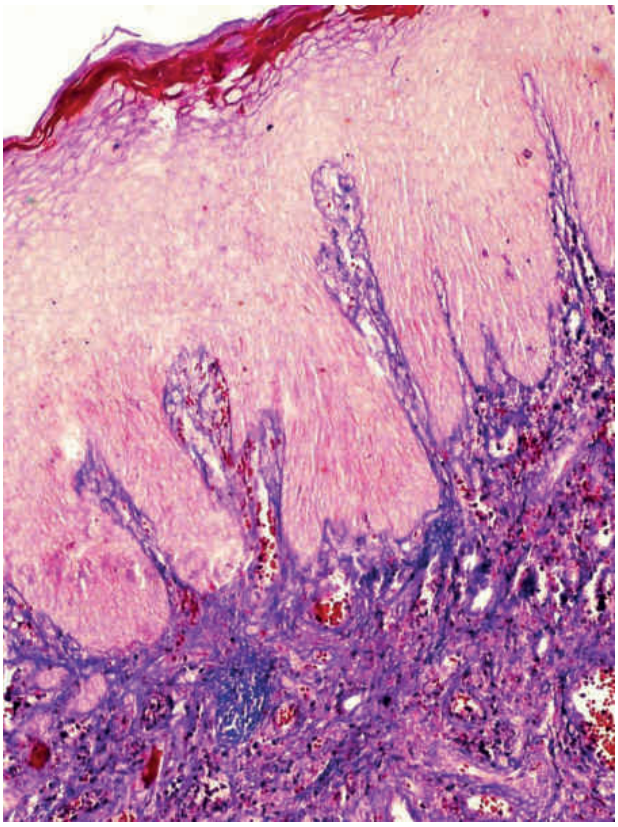

Figure 10. Hyperkeratinization of the surface layer of the epithelium (keratin is colored red). Stained with aldehyde fuchsin, chromotrope aniline blue. Magnification ×400.

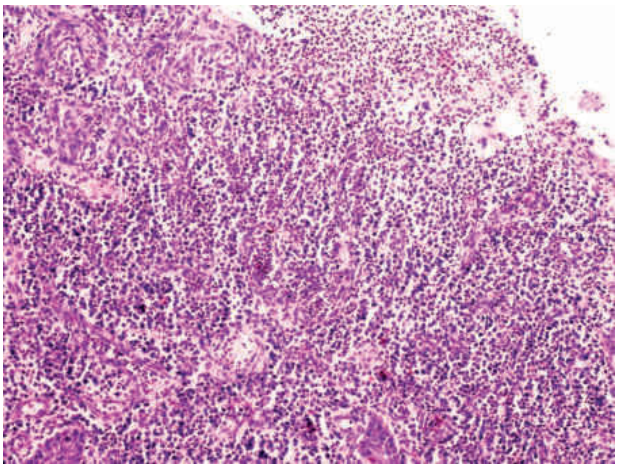

Figure 11. Polymorphic inflammatory infiltrate of lymphocytes and neutrophils in the reticular layer of the gum. Stained with hematoxylin and eosin Magnification ×200.

Table 2. Computer morphometry of the gingival mucosa in the animals of group 2 with untreated EP

Group	Me [Q1; Q3]			
	Epithelial thickness, µm	Acanthosis depth, µm	The number of capillaries per 1 mm^2	Capillary diameter, µm
1 (IP)	124.80 [110.19; 141.86]	52.62 [31.17; 94.74]	33.46 [24.04; 47.39]	22.30 [20.61; 35.32]
2	57.45 [17.75; 82.5]*	297.71 [167.08; 361.9]*	69.90 [41.16; 82.07]*	33.20 [31.30; 42.25]*

Note: *– the difference is significant in comparison with group 1 (IP), $p<0.05$.

Group 3 – the animals with EP treated with TDT

Significant changes in the tissue structures of the affected gingival mucosa of the experimental animals of the third group occurred after 12 days of treatment with TDT. Some areas of impaired keratin synthesis were found in the superficial epithelial layer of the gum. Cell differentiation was detected in the acanthotic rete ridges of the epithelial layer; a decrease in the neutrophilic diapedesis into surrounding tissues was noted (Fig. 12).

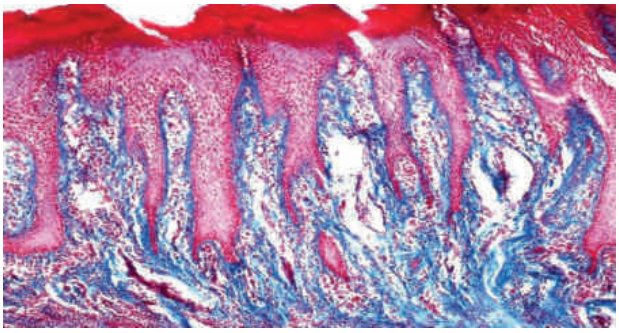

Figure 12. Cell differentiation and a decrease in the severity of inflammatory cell infiltration are in the acanthotic rete ridges of the epithelial layer. Stained with aldehyde fuchsin, chromotrope aniline blue. Magnification ×100.

There were areas of ulceration with the ribbon-like acanthotic restructuring of the surface epithelium, penetrating deep into the lower connective tissue and forming granulation tissue. At the same time, the inflammatory process was noted in the samples, represented as a mild cellular infiltration with lymphocytes and neutrophils, which formed nests of cells in a number of structures – "lacunae" surrounding capillary vessels with the penetration of these cellular elements into the lower connective tissue and elements of the surface epithelium (Fig. 13). This morphological pattern indicated the start of the organization of inflammatory process and the circulatory system reorganization for its further differentiation and functioning.

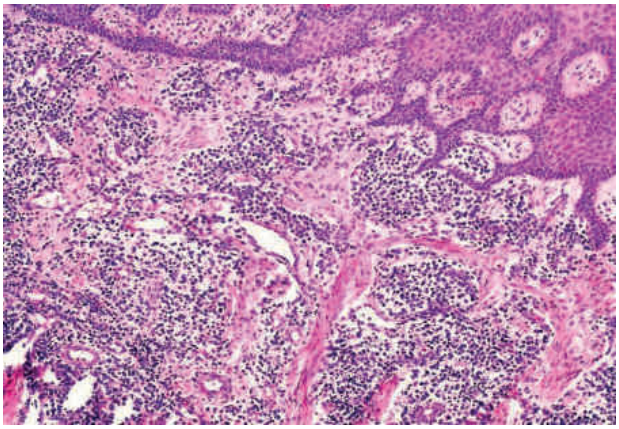

Figure 13. Moderate inflammatory cell infiltrate. Ribbon-like acanthosis grows down into the forming granulation tissue; the start of the organization of inflammatory process. Stained with hematoxylin and eosin. Magnification ×100.

Some areas of an ulcerous-necrotic alteration with destruction of the surface epithelial cells surrounded by inflammatory cell infiltrate, which contained lymphocyte-like cells, macrophages and fibroblasts, were determined in the samples of the gingival mucosa. The number of lymphocytes and neutrophils decreased. There was no pronounced diapedesis of neutrophils in the epithelial lining (Fig. 14).

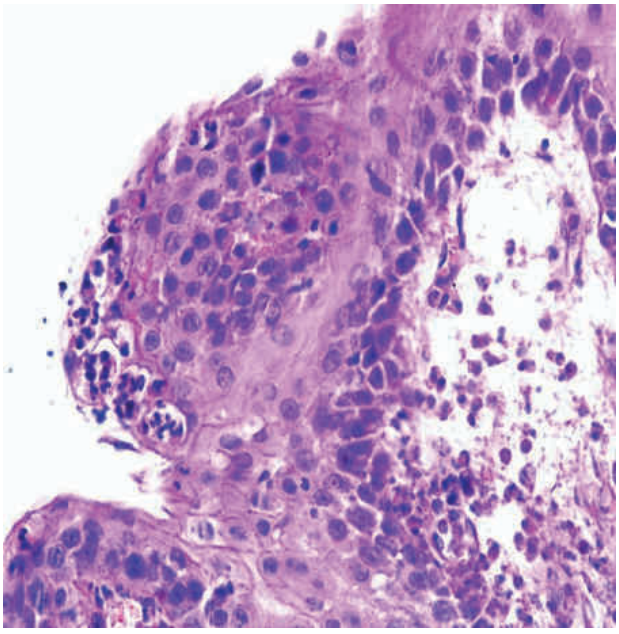

Figure 14. Moderate inflammation. Ulcerous defect of the epithelium with the granulations. Stained with hematoxylin and eosin. Magnification ×400.

In some cases, inflammatory cell infiltrates of the subepithelial layers of the gingival mucosa contained areas of granulations as well as strands of multicellular developing and fibrous connective tissue (Fig. 15).

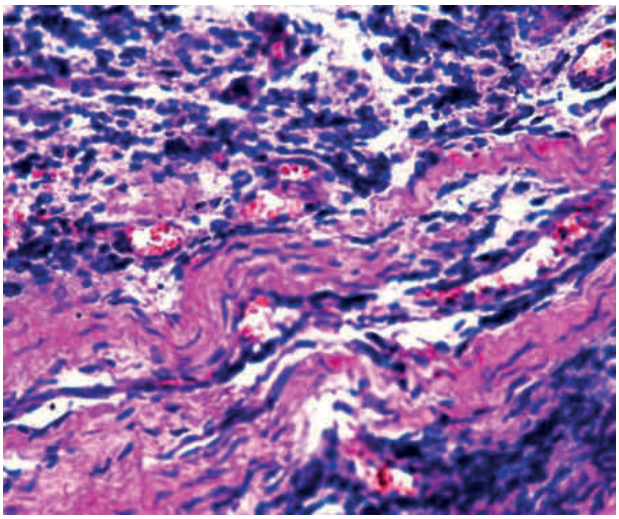

Figure 15. Inflammatory cell infiltrates of the subepithelial layers contained areas of granulations as well as strands of multicellular developing and fibrous connective tissue. Stained with hematoxylin and eosin. Magnification ×400.

Morphological findings in the samples of the gingival mucosa of the third experimental group of animals showed the prevalence of the alterative exudative inflammatory process. The exudative inflammatory reaction was evidenced as the areas of impaired keratin synthesis in the surface epithelium with moderate neutrophilic and lymphocytic infiltration of the papillary and reticular layers of the proper mucous plate and its epithelial lining (Figs 16, 17).

The proliferative inflammatory reaction was based on the strands-like acanthotic restructuring of the cells of the stratified squamous epithelium, the presence of granulations, multicellular developing and fibrous connective tissue. The strands were surrounded by the "nests" of neutrophils – "lacunae" in their proper mucous plate and epithelium. These findings can be considered as a compensatory adaptive reaction to damage.

Computer morphometry revealed a tendency to normalize 3 out of 4 quantitative parameters as a result of the administration of traditional drug therapy (Table 3).

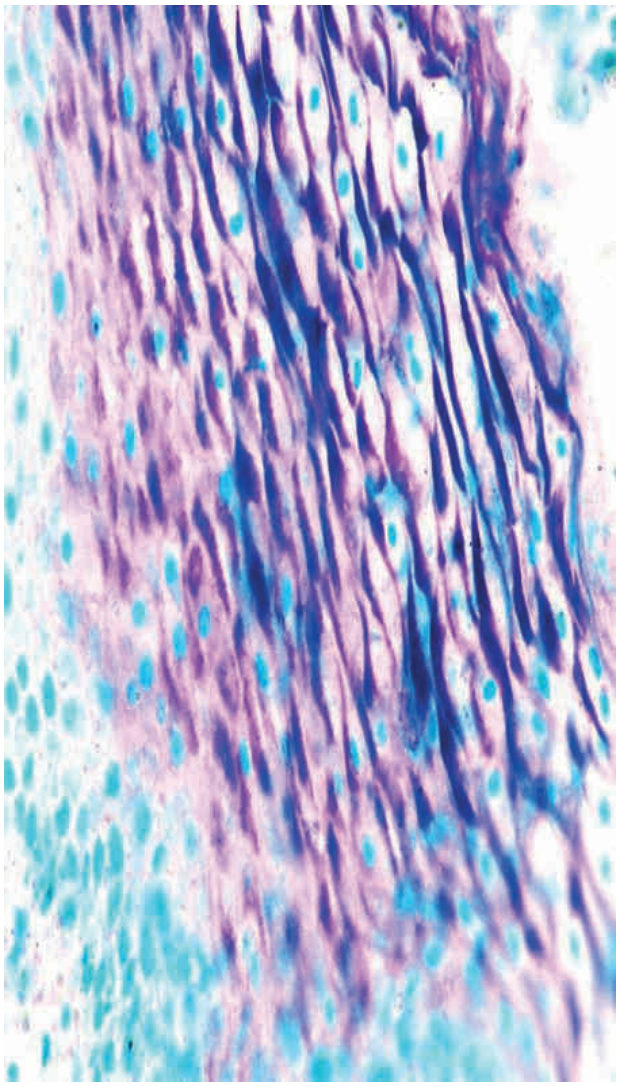

Figure 16. Formed areas of the impaired keratin synthesis in the surface epithelium. Stained with the Hotchkiss-McManus procedure and methylene blue. Magnification ×400.

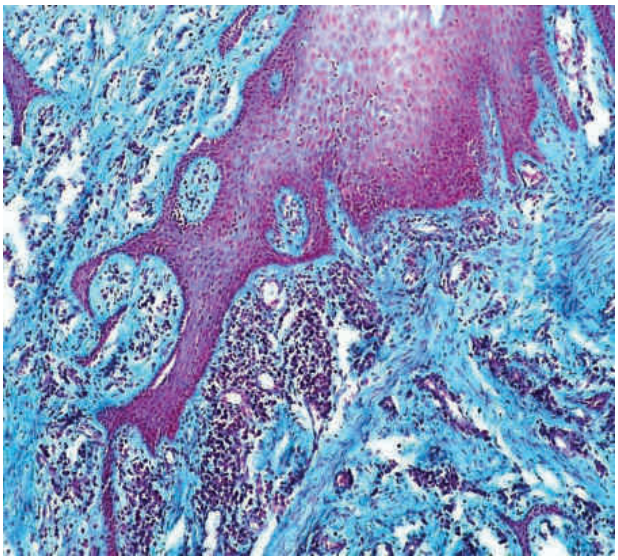

Figure 17. The alterative exudative inflammatory process, expressed as moderate cellular infiltration of the tissues under the stratified squamous epithelium. Stained with alcian blue and neutral red. Magnification ×100.

Table 3. Computer morphometry of the gingival mucosa in the 3rd group of animals with EP treated with the traditional drug therapy

Group	Me [Q1; Q3]			
	Epithelial thickness, µm	Acanthosis depth, µm	The number of capillaries per 1 mm²	Capillary diameter, µm
2	57.45 [17.75; 82.5]	297.71 [167.08; 361.9]	69.90 [41.16; 82.07]	33.20 [31.30; 42.25]
3	83.66 [73.81; 87.53]*	95.23 [30.30; 149.70]*	57.15 [36.10; 90.94]*	39.20 [24.48; 50.48]*

Note: *– the difference is significant in comparison with the group 2 (untreated EP), $p<0.05$.

Due to the treatment, the thickness of the epithelial layer increased 1.5 times, the depth of acanthosis sharply decreased 3.1 times, and the number of capillaries decreased 1.2 times. However, the average diameter of the blood capillaries increased 1.2 times, which cannot be considered a sign of the inflammation reduction in the gingival mucosa. All these changes are statistically significant ($p<0.05$).

Group 4 – the animals with EP, treated with the combination of TDT with Soderm®-Forte gel

The performed pharmacotherapy for the animals of the fourth group with EP using the combination of drugs including TDT + Soderm®-Forte gel changed the morphological pattern of the invaded tissues of the gingival mucosa. First of all, this change related to the epithelial lining, in which the inflammatory and destructive processes were replaced by the processes of reparative regeneration (Fig. 18).

A decrease in the severity of the epithelial cell damage was noted in the samples of the gingival mucosa. Rod-shaped nuclei in cells were sometimes visualized closer to the surface layers of the epithelium. However, the general picture spoke for the normalization of keratinization (Fig. 19).

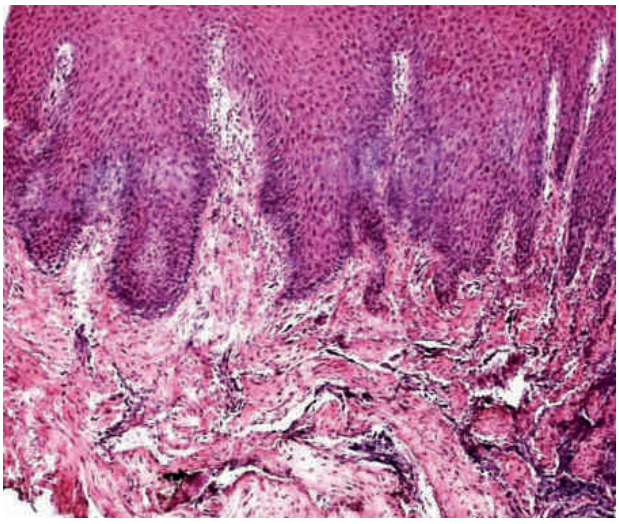

Figure 18. Moderate inflammation: mild cellular infiltration and stroma fibrosis under the epithelium. Stained with hematoxylin and eosin. Magnification ×200.

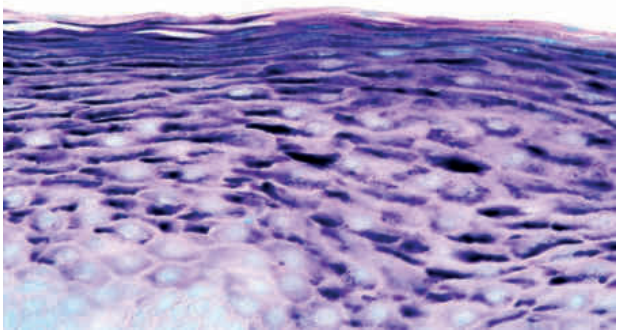

Figure 19. Normalization of keratinization in the surface layer of the epithelium. Periodic acid Schiff reaction according to McManus - Hotchkiss. Magnification ×400.

Diapedesis of neutrophils, monocytes and other blood cell elements to the epithelium was not observed. The severity of acanthosis decreased. Reparative regeneration processes in the stratified squamous epithelium of the gingival mucosa led to a 2-time decrease in its average thickness, compared with the similar indicators in the animals with EP of the second group and a 1.4-timedecrease compared with the animals of the third group. The papillary layer of the gum looked ordinary. Its numerous elongations became like “church spires” and evenly penetrated into the epithelium (Fig. 20).

The cellular composition of the papillary layer was represented by a small number of macrophages, neutrophil granulocytes; sometimes lymphocytes were found. The additional inclusion of Soderm®-Forte gel to the complex pharmacotherapy apparently influenced the regenerative activity of the microcirculation. Neither sclerotic changes in the blood vessels nor hyperelastosis was observed. The processes of capillary growth – neoangiogenesis - were noted (Fig. 21). The vessels and their anastomoses, as well as the capillary loops, acquired a normal oval shape.

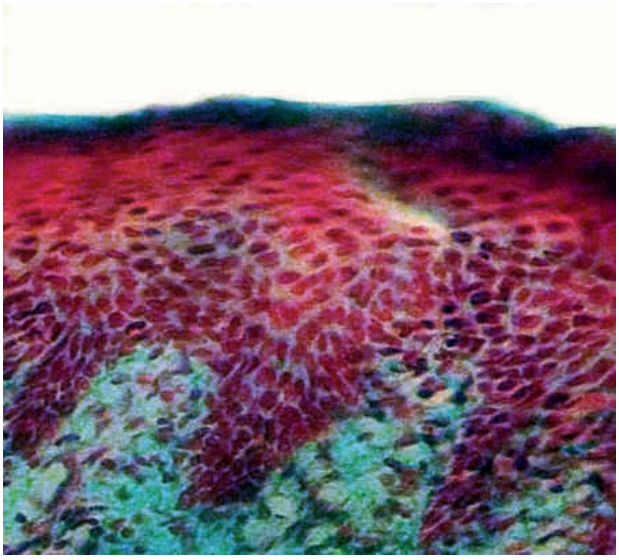

Figure 20. Numerous conical papillae are evenly penetrated into the epithelium. Stained with aldehyde fuchsin, chromotrope aniline blue. Magnification ×200.

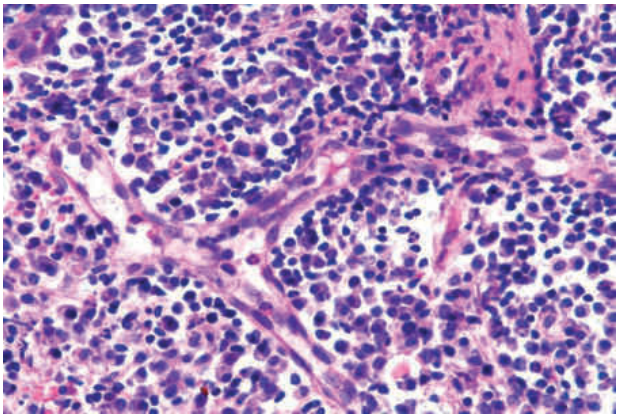

Figure 21. Sclerotic changes in the blood vessels are reduced. Neoangiogenesis occurs. Stained with hematoxylin and eosin. Magnification ×400.

The inflammatory cell infiltrates in the reticular layer of the gum decreased in the number and volume and, to a greater extent, were represented by lymphocytes and macrophages. They were replaced by the areas of the forming connective tissue, which included confined cell nests of lymphocytes and macrophages (Fig. 22).

Most commonly the described cell infiltrates were located in the deep areas of the sclerosed reticular layer and the severity of sclerotic changes decreased as it went to the superficial areas. The newly formed connective tissue, coming close to the mucous membrane surface, became looser, less stranding, and the amount of intercellular matrix increased (Fig. 23).

Microcirculation in the areas of the newly formed connective tissue was not reduced. Microcirculatory vessels were sometimes located in rare cellular infiltrates (Fig. 24). The areas of fibrosis were rare. Sclerotic areas were found in the deep parts of the reticular layer.

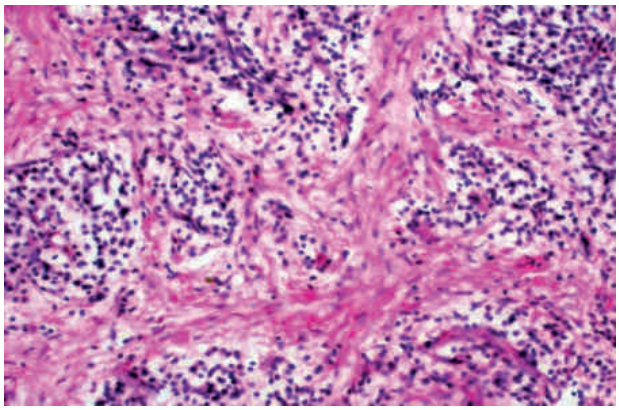

Figure 22. Lymphocyte and macrophage infiltration and the forming loose connective tissue in the reticular layer. Stained with hematoxylin and eosin. Magnification ×400.

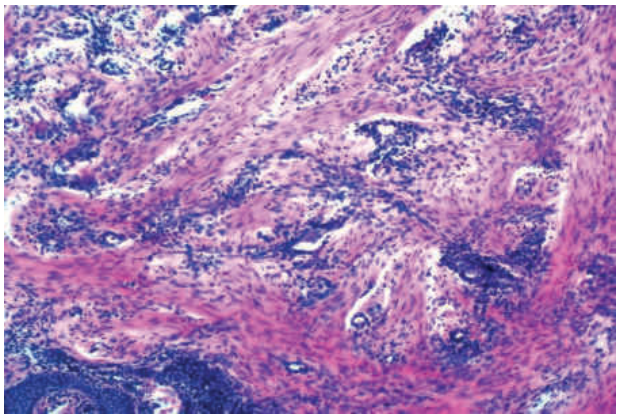

Figure 23. The newly formed connective tissue of the reticular layer. Stained with hematoxylin and eosin. Magnification ×100.

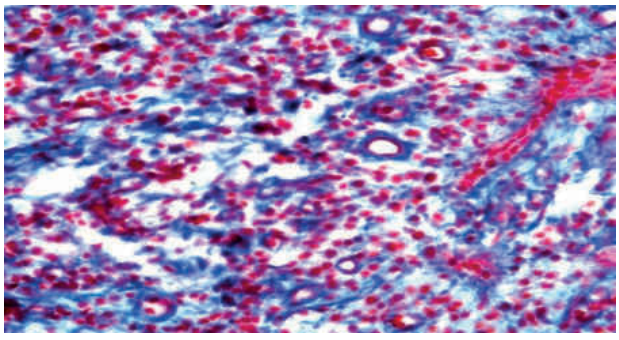

Figure 24. The newly formed capillaries are located in rare cell infiltrates of the reticular layer. Stained with aldehyde fuchsin, chromotrope aniline blue. Magnification x400.

According to the computer morphometry, the performed treatment had a positive effect on the normalization of the pathological processes in the gums of the experimental animals (Table 4). The epithelium of the mucous membrane became 2.1 times thicker, the severity of acanthosis decreased 3.2 times, and the number of capillaries in the mucous membrane decreased 1.8 times. The significance of these changes is $p<0.05$. As for the 1.2-time reduction of the capillary diameter, this indicator has no statistically significant difference from the untreated animals ($p>0.05$).

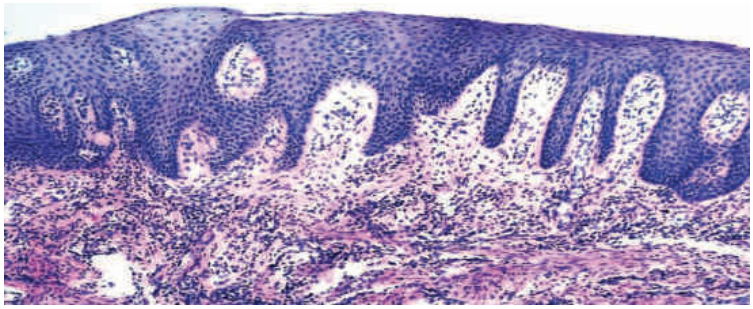

Figure 25. Normalization of the epithelial structure of the gingival mucosa. Stained with hematoxylin and eosin. Magnification ×200.

Table 4. Computer morphometry of the gingival mucosa in the 4rd group of animals with EP treated with the combination of TDT with Soderm®-Forte gel

Group	Me [Q1; Q3]			
	Epithelial thickness, µm	Acanthosis depth, µm	The number of capillaries per 1 mm²	Capillary diameter, µm
2	57.45 [17.75; 82.5]	297.71 [167.08; 361.9]	69.90 [41.16; 82.07]	33.20 [31.30; 42.25]
4	119.4 [70.61; 126.5]*	92.2 [80.2; 106.5]*	37.78 [22.64; 43.56]*	28.40 [24.20; 43.70]

Note: *– the difference is significant in comparison with group 2 (untreated EP), $p<0.05$.

Group 5 – the animals with EP, treated by the combination of TDT with Soderm®-Forte gel and the NIF of Rexod®

A morphological examination of the gingival mucosa of rats revealed that the additional inclusion of the NIF of Rexod® to the complex pharmacotherapy induced the active elimination of acute inflammation, both in the epithelial lining and in the lower connective tissue. Active processes of the reparative regeneration were noted in almost all samples. The condition of the mucous membrane of the marginal part of the rats' gum was like that of full and healthy tissue. The integrity of the surface epithelium of the gingival mucosa was determined throughout its entire length, and there was neither ulceration nor newly formed granulations (Fig. 25).

It should be noted that minimal inflammatory dystrophic changes in epithelial layers were detected in all samples of the animals of this group. Acanthosis became less pronounced compared to groups 3 and 4, and decreased significantly compared to group 2. There were no signs of cell damage and necrosis. The epithelial cells did not contain vacuoles. There were no signs of keratin formation disorders. The epithelial layers assumed a typical structure and cell composition of stratified squamous epithelium (Fig. 26).

Inflammatory edema was resolved in the papillary layer; there were no signs of sclerotic changes. Along with these processes, active neoangiogenesis was observed. The number of vascular anastomoses in the proper mucous plate increased, which contributed to the formation of extensive capillary plexuses (Fig. 27).

The loops of these plexuses were located along the collagen fibers and were not changed. The histological structure of the connective tissue papillae was normal (Fig. 28). The number of macrophages and fibroblasts decreased.

In the reticular layer of the mucous membrane, the number of cell infiltrates, represented by lymphocytes and macrophages, significantly decreased, which indicates a low intensity of the inflammatory response of tissues to damaging agents and a start of regenerative processes (Fig. 29).

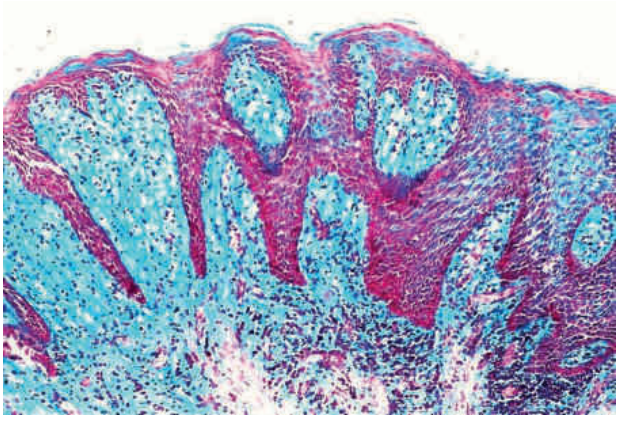

Figure 26. Normalization of the cell composition of the epithelial lining of the gum. Stained with alcian blue and neutral red. Magnification ×100.

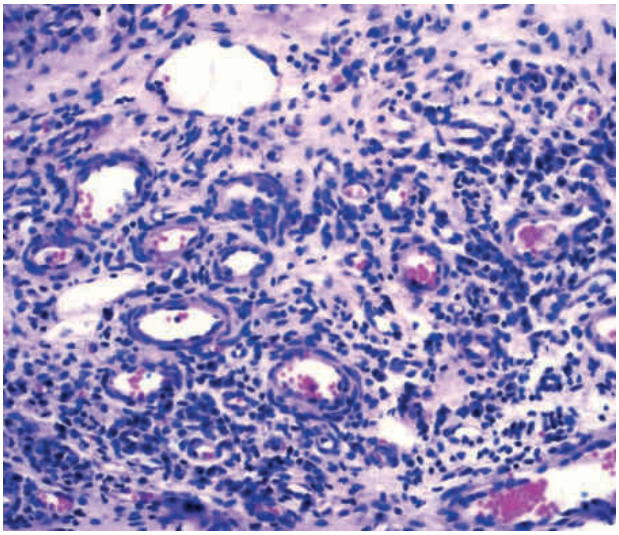

Figure 27. Newly formed vascular tree. Capillary plexuses in the proper mucous plate. Stained with hematoxylin and eosin. Magnification ×400.

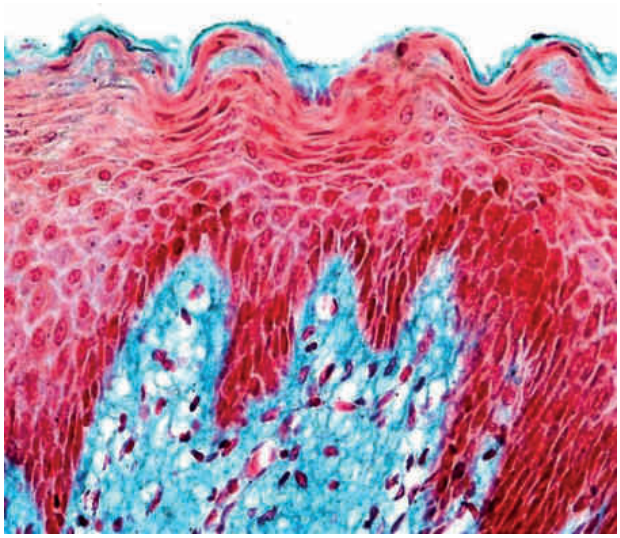

Figure 28. Usual structure of the connective tissue papillae. Stained with alcian blue and neutral red. Magnification ×400.

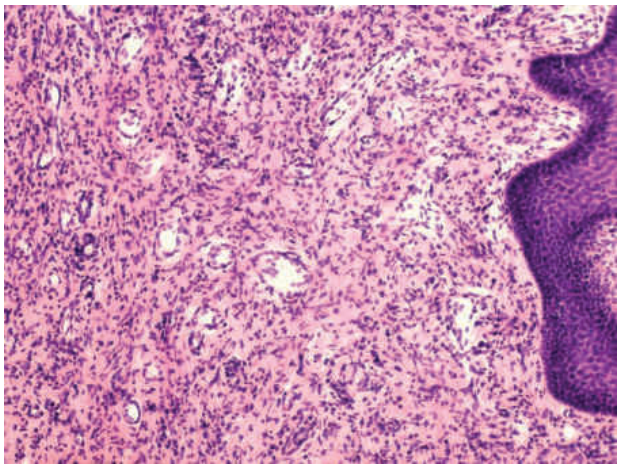

Figure 29. Lymphocyte and macrophage infiltration of the reticular layer of the gingival mucosa. Stained with hematoxylin and eosin. Magnification ×200.

Sometimes small isolated foci of infiltrates were located in the deep sections of the restored reticular layer along small blood vessels. No neutrophils were noted in these infiltrates. The intensity of sclerotic changes was low; the areas of loose connective tissue were determined, in which peripheral microcirculatory vessels (capillaries, venules, lymphatic vessels), cell elements (single lymphocytes, fibroblasts, mast cells) and a loose network of thin collagen fibers were noted. There were no areas of fibrosis (Figs 30, 31). To a greater extent, areas of restoration of the tissue complex structure of the epithelial layer by the type of restitution were visualized in the samples.

Thus, the morphological pattern of the stratified squamous epithelium of the marginal part of the gingival mucosa in the 5th experimental group of rats came close to the state of the same structures in the 1st group of animals (intact, control-1). It became obvious that a complete restoration of the normal structure of the proper mucous plate took place on a significant area of the tissue complex due to the process of active reparative regeneration of inflammatory cell infiltrates described earlier.

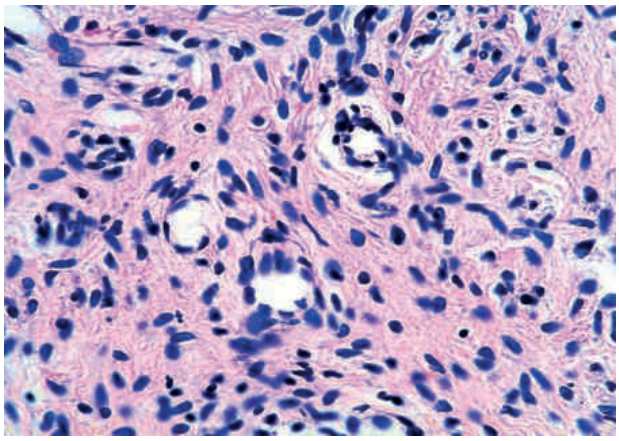

Figure 30. Complete restoration of the structure of the subepithelial connective tissue of the gum. Stained with hematoxylin and eosin. Magnification ×400.

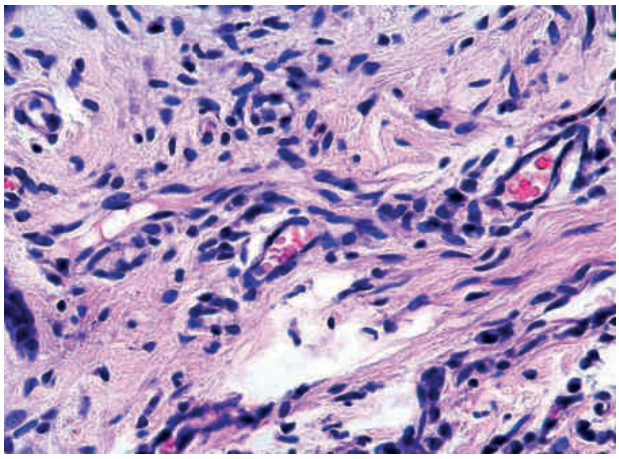

Figure 31. Areas of restoration of the structure of the gingival tissue complex by the type of restitution. Stained with hematoxylin and eosin. Magnification ×400.

The obtained results of morphological studies of the samples of the marginal part of the rats' gingival mucosa with simulated EP in group 5, treated with the complex pharmacotherapy – TDT + Soderm®-Forte gel + NIF of Rexod®, were the best. In this group of animals, morphological sings of regeneration of the tissue complex of the gingival mucosa were revealed, determined by the proliferative inflammatory response to damage with minimal residual signs of damage to the cell structures of the surface epithelium and its lower connective tissue. The absence of destructive necrotic and pronounced exudative processes in rat samples in this group, compared with the other groups, suggests the favorable conditions for faster and more effective restoration of the mucous membrane structure of the animals' gum without previous suppuration and ulceration.

Digital morphometric indicators of the gingival mucosa in animals of the 5th group revealed the most complete, statistically significant ($p<0.05$) normalization (Table 5). The treatment led to a 2-time increase in the thickness of the epithelial layer of the gum with a 4.4-time decrease in the acanthosis depth. The capillary network of the gingival mucosa decreased 2.4 times, and the average diameter of the capillary vessels decreased 1.4 times.

Table 5. Computer morphometry of the gingival mucosa in the 5th group of animals with EP treated with the combination of TDT with Soderm®-Forte gel and the NIF of Rexod®

Group	Me [Q1; Q3]			
	Epithelial thickness, µm	Acanthosis depth, µm	The number of capillaries per 1 mm²	Capillary diameter, µm
2	57.45 [17.75; 82.5]	297.71 [167.08; 361.9]	69.90 [41.16; 82.07]	33.20 [31.30; 42.25]
5	113.3 [66.80; 128.1]*	68.4 [49.7; 81.5]*	29.73 [20.15; 41.70]*	23.11 [19.63; 27.65]*

Note: *– the difference is significant in comparison with group 2 (untreated EP), $p<0.05$.

The values of all indicators were maximally approximated to those in the animals with intact periodontium.

Thus, the morphometric study revealed quantitative signs of damage to the structures of the gingival mucosa in rats with EP and their recovery due to the administration of various pharmacotherapy regimens. Periodontitis with damage to the surface epithelium manifests itself by a marked decrease in its thickness (Fig. 32), as well as a sharp increase in the acanthosis depth (Fig. 33). Exudative inflammatory changes is accompanied by modification of the vascular tree in the proper mucous plate with an increase in the number of capillaries (Fig. 34) and their diameter (Fig. 35).

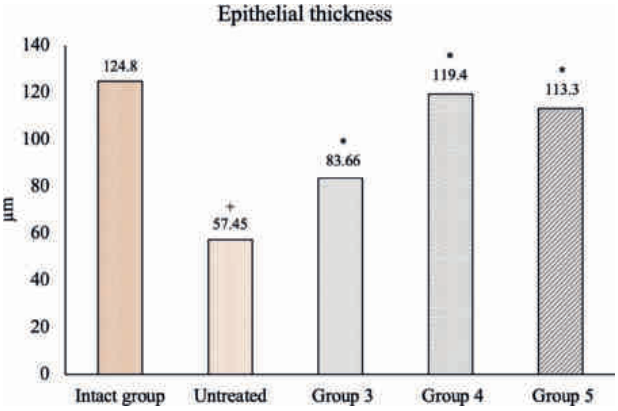

Figure 32. Epithelial thickness in the mucous membrane of the rats' gum (µm). **Note:** +– significant difference from group 1 (IP), $p<0.05$; *– significant difference from group 2 (untreated EP), $p<0.05$.

TDT of periodontitis creates a clear tendency to normalize three quantitative parameters: epithelial thickness, acanthosis depth, and the number of capillaries. Nevertheless, the average capillary diameter continued to significantly increase, indicating the presence of inflammatory changes in the gingival mucosa. The combination of TDT with Soderm®-Forte gel caused a more pronounced normalization of indicators. At the same time, the capillary diameter in the vascular tree decreased, though, without a statistically significant difference from the untreated animals. When TDT was combined with Soderm®-Forte gel and the NIF of Rexod®, the most complete normalization of all morphometric indicators was noted

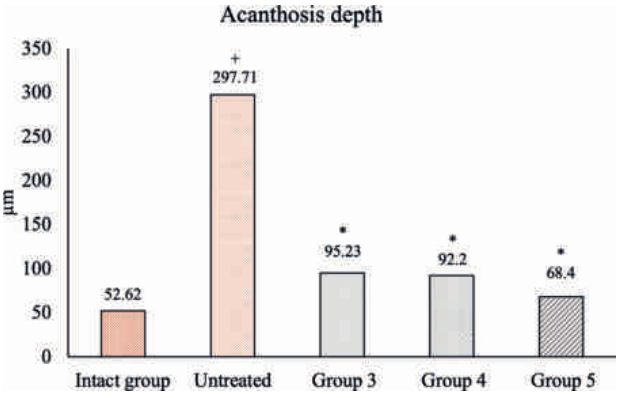

Figure 33. Acanthosis depth in the mucous membrane of the rats' gum (µm). **Note:** +– significant difference from group 1 (IP), $p<0.05$; *– significant difference from group 2 (untreated EP), $p<0.05$.

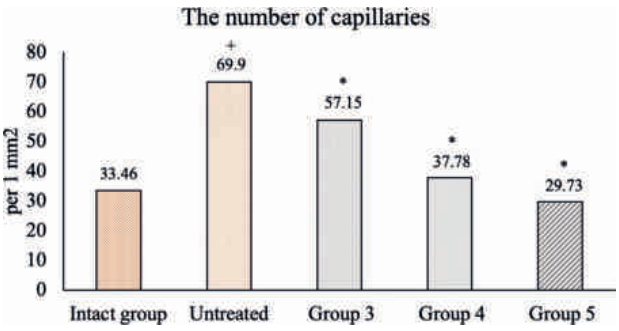

Figure 34. The number of capillaries in the mucous membrane of the rats' gum (per 1 mm²). **Note:** +– significant difference from group 1 (IP), $p<0.05$; *– significant difference from group 2 (untreated EP), $p<0.05$.

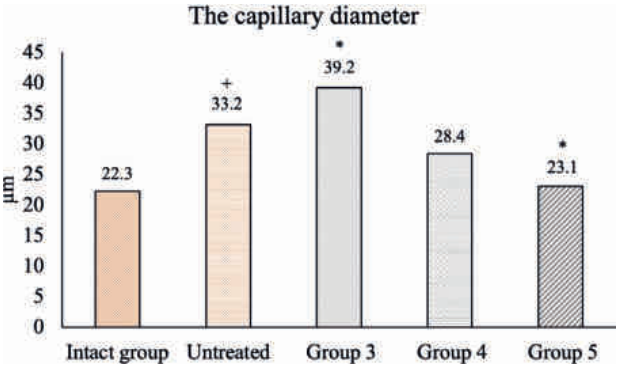

Figure 35. The capillary diameter in the mucous membrane of the rats' gum (µm). **Note:** +– significant difference from group 1 (IP), $p<0.05$; *– significant difference from the group 2 (untreated EP), $p<0.05$.

with the greatest degree of approximation to those in animals of the control group without EP.

Conclusion

The complex pharmacotherapy of EP in rats using the combination of TDM with Soderm®-Forte gel and the NIF of Rexod® for 12 days has the most beneficial effect on the pathological processes in the gum. The resulting

restorative effect (by the type of restitution), induced by the studiedcombination of drugs, promotes the earliest regeneration of the gum tissues and reduces the risk of persistent pathomorphological changes.

Conflict of interest

The authors have no conflicts of interest to declare.

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