Morphological Study of Reparative Processes in the Gingiva during Therapy of Chronic Periodontitis with Energostim and Application of Orthopedic Splinting Constructions

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 9, pp. 336-342, September, 2003
Original article submitted June 24, 2003

We studied morphological characteristics of the regenerative process in gingival tissues during therapy of chronic periodontitis. Energostim stimulated macrophageal reaction and promoted neoangiogenesis in the inflammatory infiltrate. It was not observed after traditional drug therapy. Energostim promoted vascularization in regenerating tissues, normalized the structure at a greater area of the lamina propria of the gingiva, and prevented fibrous and sclerotic changes. The volume of histiolymphocytic infiltrates in regenerating gingival tissues decreased after application of intradental splints.

Key Words: *chronic periodontitis; energostim; regeneration; intradental splint*

Reparative processes in the gingiva during therapy of chronic generalized periodontitis do not necessarily proceed under optimal conditions. The disease is often characterized by a protracted and subacute course and results in the formation of scars consisting of coarse fibrous connective tissue.

Energostim therapy and splinting of shaky teeth with intradental splints improve the clinical situation. Clinical trials showed that this treatment produces the positive effect, which is manifested in rapid disappearance of external signs of gingivitis and remission of the disease.

Here we evaluated structural bases of the regenerative process during the therapy of chronic periodontitis with energostim and application of orthopedic splinting constructions. These data will allow us not only to gain empirical knowledge, but also to perform direct (practical) studies of regenerative morphogenesis [2,6,7].

MATERIALS AND METHODS

Morphological and histochemical assays were performed on samples of gingival mucosa taken from 35 patients with exacerbation of generalized periodontitis. The specimens were taken at admission to hospitals (control) and on days 10 and 30 of therapy.

The patients were divided into groups depending on therapeutic treatment and period of examination. All patients (groups 1-5) received traditional drug drugs. Group 1 patients did not receive any additional preparations. After therapy prosthetic surgeries were performed in group 2 patients by routine methods. Group 3 patients received not only standard pharmaceutics, but also energostim. Prosthetic surgeries were performed routinely in group 4 patients receiving traditional drugs and energostim. Original splinting constructions were applied to group 5 patients after combination therapy with traditional drugs and energostim.

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0007-4888/03/1363-0296$25.00

Energostim was applied locally (douches and applications) and injected daily into the intermediate fold for 10 days (25%, 2 ml).

Shaky teeth were immobilized with an original intradental splint. Pivot crowns were fixed inside dental pulp channels. The splint was covered with a photopolymeric composite material.

Gingival biopsy specimens were fixed in 10% neutral formalin and embedded in paraffin by routine methods. Paraffin sections (7 μ) were stained with hematoxylin and eosin. Staining and treatment of histological sections were performed by various histochemical techniques. The sections were consecutively stained with aldehyde fuchsin, chromotrope, and aniline blue for differential examination of fibrous structures in the connective tissue [3]. The sections were additionally stained with alcian blue and neutral red after preoxidation with an acid solution of potassium permanganate. Nucleic acids were assayed on preparations stained with methyl green (pyronin) [4]. Acid glycosaminoglycans were detected with alcian blue (pH 2.5) and toluidine blue. Neutral glycosaminoglycans were stained by the method of McManus—Hochkiss [5].

Morphometric data were processed statistically. The thickness of the epithelial layer was measured on vertical sections of the gingiva using an ocular micrometer. The diameter of blood capillaries (cross-sections) was estimated in the papillary layer of the dermis. The relative area of inflammatory cell infiltrates and sclerotic regions was determined in the reticular layer using an ocular morphometric grid. Numerical results were analyzed by methods of variational statistics. The differences were significant at $p<0.05$.

RESULTS

Morphological study revealed not only focal sclerotic changes in the lamina propria of the gingiva typical of the chronic disease, but also signs of acute inflammation (Fig. 1, *a*) Alteration in the gingival tissue included exudation and presence of inflammatory cell infiltrates (Fig. 1, *b*). Circulatory disturbances (plethora and stasis, Fig. 1, *c*, *d*), neutrophilia in the inflammatory infiltrate (Fig. 1, *e*), and exocytosis (diapedesis of neutrophilic leukocytes into the epithelial layer of the mucosa, Fig. 1, *b*, *c*) were typical of the acute stage of the disease. Pronounced acanthosis was accompanied by penetration of epithelial processes into adjacent mucosa (Fig. 1, *f*).

A 10-day course of treatment with standard preparations alone or in combination with energostim relieved symptoms of acute inflammation. It was related to the action of antibiotics, sulfanilamides, biostimulators, and vitamins used as traditional pharmaceutics for the therapy of chronic periodontitis.

TABLE 1. Morphometric Characteristics of Gingiva Mucosa during Therapy of Chronic Generalized Periodontitis ($M \pm m$)

Group	Duration of therapy, days	Thickness of epithelial layer, μ	Diameter of capillaries in the papillary layer, μ	Total area of cell infiltrates in the reticular layer, %	Total area of sclerotic focuses in the reticular layer, %
Control (before therapy, n=35)	—	568±25	9.9±0.2	81.2±7.3	10.4±0.9
1 (n=10)	10	422±29*	9.1±0.3**	52.3±5.4*	25.3±2.2**
2 (n=10)	30	182±21* $p_1<0.001$	4.2±0.1* $p_1<0.001$	24.1±6.0* $p_1<0.001$	45.4±5.1* $p_1<0.001$
3 (n=25)	10	313±38* $p_1<0.05$ $p_2<0.001$	7.3±0.2* $p_1<0.001$ $p_2<0.001$	31.3±7.2* $p_1<0.001$	16.1±1.7* $p_1<0.02$ $p_2<0.001$
4 (n=11)	30	274±32* $p_1<0.001$ $p_2<0.05$	7.7±0.4* $p_1<0.001$	10.1±1.8* $p_1<0.001$ $p_2<0.05$ $p_3<0.01$	18.6±3.0* $p_2<0.05$
5 (n=14)	30	258±30* $p_1<0.001$ $p_2<0.05$	8.0±0.4* $p_1<0.05$ $p_2<0.01$	4.3±1.1* $p_1<0.001$ $p_2<0.01$ $p_3<0.001$ $p_4<0.02$	18.5±3.6** $p_2<0.05$

Note. Index mark near the degree of confidence: reference group. *$p<0.001$ and **$p<0.05$ compared to the control.

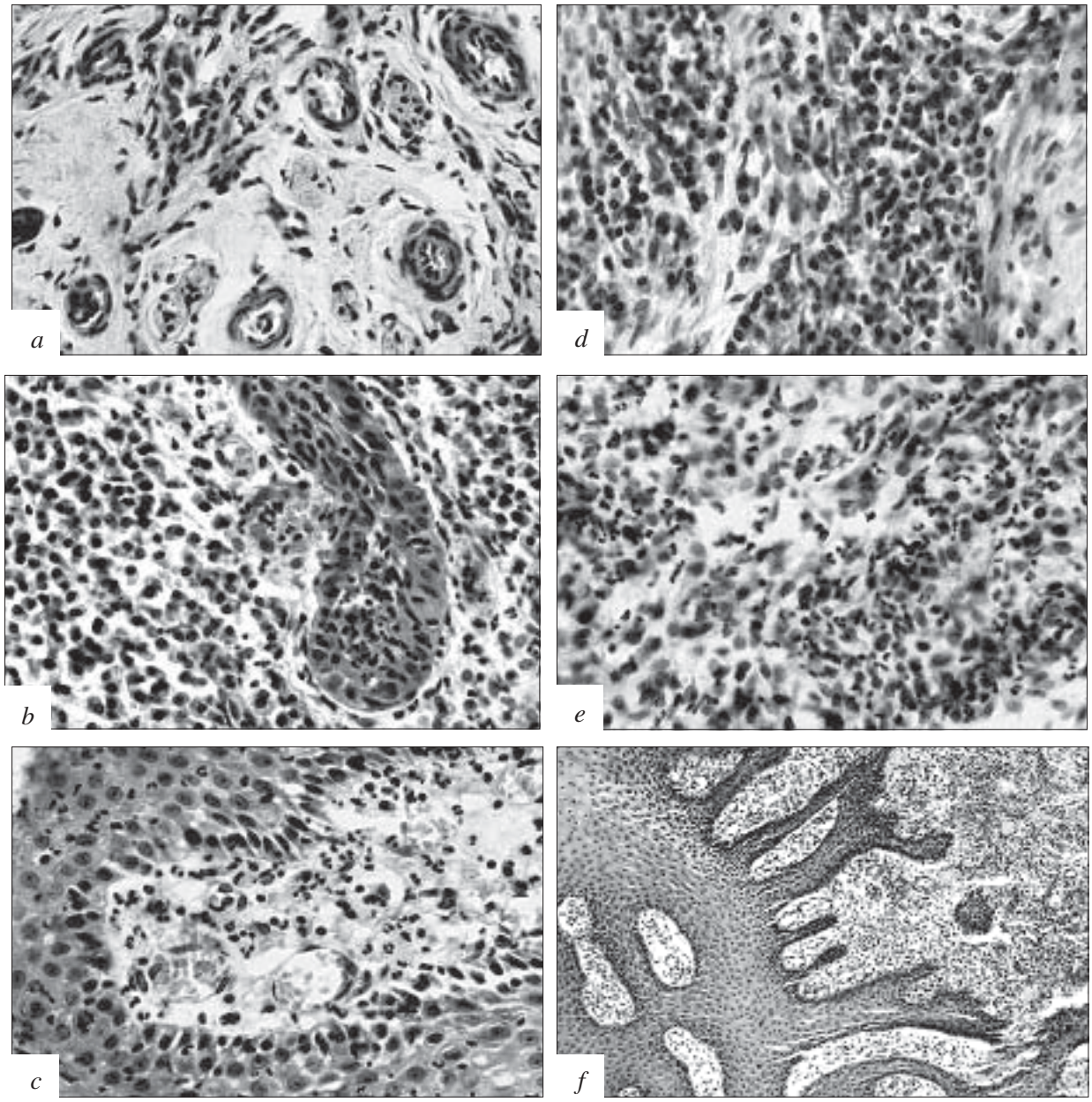

Fig. 1. Morphological changes in the gingival tissue during exacerbation of chronic periodontitis. Region of sclerosis, histiolymphocytic infiltration, and sclerosis of small arteries (*a*); dense inflammatory infiltrate in the reticular layer, acanthotic process, and exocytosis (*b*); plethora, edema, widened capillaries in the papilla, and leukodiapedesis in the epithelial layer (*c*); stases and hemorrhages in the inflammatory infiltrate (*d*); neutrophilia of the inflammatory infiltrate (*e*); branching acanthotic processes and polymorphous inflammatory infiltrate in the subadjacent tissue (*f*). Staining with hematoxylin and eosin. *a-e*, ×400; *f*, ×100.

On day 10 exudative changes in the gingival tissue developed into the regenerative process. Study of morphological characteristics in inflammatory cell infiltrates showed that energostim markedly activated macrophages. It was manifested in an increase in the number of macrophages and amount of neutral glycosaminoglycans in the cytoplasm (Fig. 2, *a*). As differentiated from the effect of treatment with traditional drugs, combination therapy promoted the formation of new capillaries in infiltrates (neoangiogenesis, Fig. 2, *b*, *c*). These changes improved blood supply to infiltrates that looked like a well vascularized granulation tissue (Fig. 2, *d*). Taking into account that macrophages secrete vascular growth factor [8], it is clear that the intensity of neoangiogenesis correlates with high macrophageal activity.

The number of fibroblasts differentiating from vascular adventitial cells increased due to angiogenesis. The connective tissue matrix was formed more intensively than fibrous structures, which affected the type of regenerates (Fig. 2, *e*). It should be emphasized

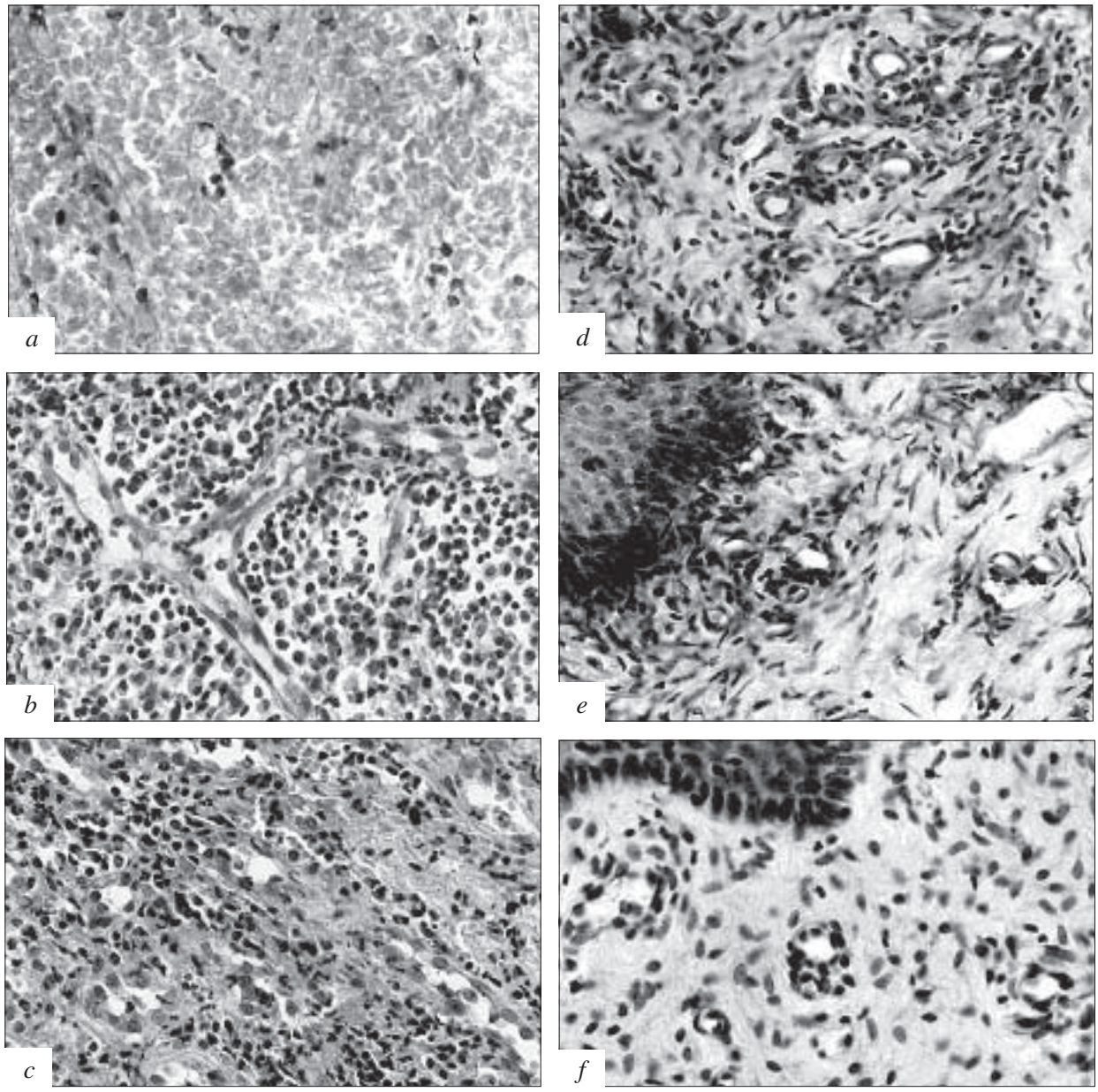

Fig. 2. Morphological characteristics of the regenerative process in the gingiva during therapy with energostim (×400). Numerous macrophages in the cell infiltrate with high content of neutral glycosaminoglycans (*a*); newly formed capillaries (*b*); proliferation of endothelial cells with the formation of capillaries (*c*); numerous newly formed capillaries in the infiltrate (*d*); fibroblasts and considerable amount of the connective tissue matrix in recovered tissues of the reticular layer (*e*); region of complete regeneration of the lamina propria of the gingiva (*f*). Staining by the method of McManus-Hochkiss and methyl green (*a*); staining with hematoxylin and eosin (*b*, *c*, *f*); staining with alcian blue and neutral red (*d*, *e*).

that after traditional drug treatment cell infiltrates included layers of multicellular fibrous connective tissue (Fig. 3, *a*). The amount of the matrix was low between fibrous structures. These specific features of fibroblasts involved in regenerative process under hypoxic conditions can be explained by insufficient vascularization of formed regenerates. Our suggestion was confirmed by morphometric study of cell infiltrates and sclerotic areas in gingival tissue (Table 1). Energostim and standard drugs reduced the area of cell infiltrates by 2.6 and 1.6 times, respectively. The area of sclerotic tissues increased by 14.9 and 5.7% in untreated patients and patients receiving energostim, respectively. The degree of acanthosis in the epithelial layer decreased more significantly in patients treated with energostim. The mean thickness of the epithelial layer in patients receiving energostim group surpassed the normal (255 μ) only by 58 μ [1]. In patients not receiving energostim this parameter surpassed the normal by 167 μ (1.6 times).

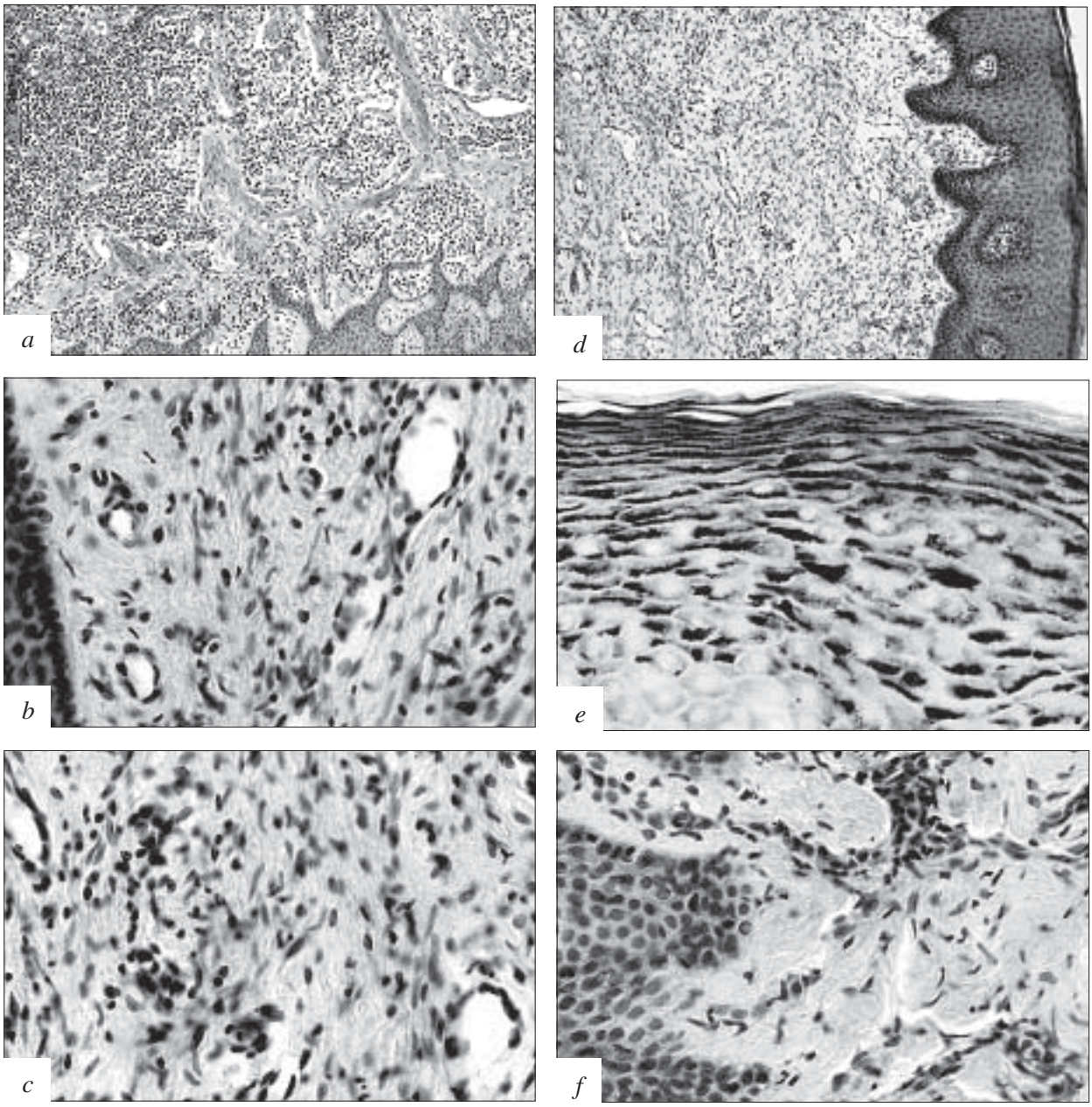

Fig. 3. Morphological characteristics of the regenerative process in the gingiva during standard drug treatment. Layers of multicellular proliferating connective tissue in the inflammatory infiltrate (*a*); endothelial proliferation in capillaries and narrowing of their lumen (*b*); sclerosis and obliteration of capillaries in the reticular layer (*c*); atrophy of gingival epithelium (*d*); site of intensive keratinogenesis in the epithelium (*e*); sclerotic changes in the reticular layer with histiolymphocytic infiltrates (*f*). Staining with hematoxylin and eosin (*a*-*d*, *f*); staining by the method of McManus—Hochkiss and methyl green (*e*). *a*, *d*, ×100; *b*, *c*, *e*, *f*, ×400.

Our results show that 10-day therapy with energostim improved the state of gingival mucosa compared to that observed after traditional drug treatment.

Significant differences in the characteristics of the regenerative process were observed on day 30 of therapy. In group 5 patients regeneration of the gingiva on a considerable area (77.2% area on histological preparations) was followed by the recovery of its connective tissue structures (Fig. 2, *f*). In group 2 patients the structure of the gingiva was normalized only on 30.5% area after 30-day therapy (Table 1). We revealed differences in qualitative characteristics of recovered tissue (*e.g.*, blood supply). After energostim therapy the gingival tissue had well-developed capillary network (Fig. 3, *b*). In patients not receiving energostim blood vessels were narrowed or obliterated due to productive processes (Fig. 3, *b*, *c*). Differences in blood supply to recovered tissues affected the state of the epithelial layer. The structure of the epithelial layer and degree of keratinization returned to normal

in group 3 patients. However, group 1 patients receiving standard drugs were characterized by atrophic changes in the epithelial layer (Fig. 3, *d*) and impairment of keratinogenesis (Fig. 3, *e*).

In group 1 patients incomplete regeneration was observed on a greater area of the gingiva than in patients treated with the proposed method. We assayed a part of the regenerate that included abnormal tissues with fibrous and sclerotic changes and cell infiltrates (Fig. 3, *f*). In patients receiving only standard therapy the area of these tissues was 69.5%. After energostim and application of intradental splints the area of abnormal tissues decreased by 3 times (22.8%).

It should be emphasized that energostim produced a positive effect also in patients subjected to routine teeth splinting with standard constructions. In these patients the tissues recovered, and the state of the epithelium returned to normal at a considerable area of gingival reticular zone on day 30. However, standard splinting constructions could traumatize the gingiva. The volume of abnormal tissues increased in the regenerate, which was related to the appearance of histiolymphocytic infiltrates. These changes reflect intensification of autoimmune processes that produce the adverse consequences.

The proposed method for therapy of patients with exacerbation of chronic periodontitis is more effective compared to traditional drug treatment and can be used in stomatological practice. However, a morphological study showed that this therapy cannot completely prevent progressive chronic disorders in periodontal tissues. The slight increase in the area of sclerotic tissues with focal histiolymphocytic infiltrates indicates that the proposed therapeutic treatment reduces the severity of acute inflammation, but does not relive the symptoms of chronic disease. Recurrences of the chronic disease cannot be excluded and, therefore, preventive treatments are required.

The morphological study indicate that energostim activating the macrophageal reaction, stimulating angiogenesis, improving microcirculation, and replenishing the supply of metabolites necessary for bioenergetics of the cell affects the course and outcome of regenerative processes. It is very important to exclude local mechanical factors producing the adverse effect on regenerative processes. Application of splinting constructions for immobilization of shaky teeth that would not traumatize periodontal tissues is an urgent problem.

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