Morphological Analysis of the Effect of Richlocaine on Gingival Mucosa during Exacerbation of Chronic Periodontitis

V. L. Popkov*, L. A. Faustov, N. L. Sycheva, A. V. Zadorozhnii, and V. P. Galenko-Yaroshevskii

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We studied morphology of the regeneratory process in gingival tissues during treatment of exacerbation of chronic generalized periodontitis. Richlocaine treatment led to more pronounced activation of neoangiogenesis in inflammatory cellular infiltrate in comparison with traditional drug treatment. Richlocaine stimulated vascularization of regenerating tissues, promoted more extensive normalization of the structure of the gingival mucosa, and prevented the development of fibrosclerotic changes.

Key Words: *chronic periodontitis; richlocaine; regeneration; neoangiogenesis*

Drug therapy of chronic generalized periodontitis (CGP) is not always effective [3], which can be due to our insufficient knowledge of the etiology and pathogenesis of this disease and low efficiency of drugs.

Richlocaine (local anesthetic) used in combined therapy of patients with CGP, along with its high analgesic effect, exhibited a pronounced therapeutic action characterized by appreciable reduction or arrest of periodontal tissue damage and earlier remission.

Morphological criteria are important for evaluation of the course of many diseases, including CGP [3,6,8,9]. We studied the effect of richlocaine on structural, including histochemical, changes in the gingival mucosa of patients with exacerbation of CGP.

MATERIALS AND METHODS

Morphological and histochemical studies were carried out on mucosa specimens collected from the gingival edge of 64 patients with CGP exacerbation. Biopsy specimens were collected during the first consultation and on day 15 of treatment.

Patients with CGP were divided into 2 groups: 1) 29 patients received traditional drug therapy, including antibacterial, antiinflammatory, stimulatory, common fortifying, and desensitizing drugs and 2) 35 patients received traditional therapy supplemented with richlocaine (local irrigations and applications of 0.5% solution, injections of 2 ml 0.25% solution in the transitional fold daily for 14 days; 5% ointment on turundae was introduced into periodontal pouches).

Gingival biopsy specimens were fixed in 10% neutral formalin and embedded into paraffin [4]. Paraffin sections (7 μ) were stained with hematoxylin and eosin. A complex of histochemical methods for staining and processing of histological sections was used. The sections were successively stained with aldehyde fuchsin, chromotrope, and aniline blue for differentiated detection of the connective tissue fibrous structures [10] and post-stained with alcyan blue and neutral red after their preoxidation with acid potassium permanganate. Mast cells were studied on preparations stained with

Krasnodar Affiliated Branch of Southern Bureau of Russian Academy of Medical Sciences; *Kuban State Medical Academy

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toluidine blue [4]. Acid glycosaminoglycans were detected by alcyan blue staining at pH 2.5. Neutral glycosaminoglycans were stained by the method of McManus—Hochkiss [7].

In order to obtain more objective results, morphometry was used. The thickness of the epithelium was measured by ocular micrometery on vertical sections of the gingiva. The diameters of blood capillaries in the papillary layer of the derma were measured on their cross-sections. Areas occupied by inflammatory cell infiltration and sclerosis zones were measured in the reticular layer using an ocular measuring grid [1].

Numerical data were processed by the differential method of variation statistics [5]. The differences were considered significant at $p<0.05$.

RESULTS

Acute inflammatory processes against the background of previous sclerotic changes were observed in the gingiva of patients with CGP. Circulatory disorders (plethora, stasis, hemorrhages) and the exudative component (polymorphocellular infiltration with obligatory macrophages and numerous neutrophilic leukocytes; Fig. 1, *a*) indicated acute form of the process. Intensive leukodiapedesis in the epithelium supplemented the morphological picture of exacerbation. Pronounced acanthosis with submerged growth of epithelial processes into the underlying tissues of gingival mucosa was observed (Fig. 1, *b*).

Inflammatory cell infiltration was situated in the reticular layer of the gingiva and rarely involved the papillary layer, where pathological changes were confined to edema. More severe involvement of the papillae manifested in destruction of their tissue, replaced by empty vacuoles of different size. These serious injuries to the papillae with destruction of the connective tissues were observed in just few cases.

Traditional drug therapy alone and in combination with richlocaine effectively suppressed the exudative component and promoted the development of proliferative processes. This effect was due to the drugs used as obligatory components of therapy for CGP exacerbation.

Hence, by day 15 the development of regeneratory processes was observed in the gingival tissues. However, morphological study showed that in patients receiving richlocaine cell infiltrations were better vascularized (due to neoangiogenesis), than in patients receiving traditional drug therapy. Inflammatory cellular infiltration in patients receiving richlocaine presented as well vascularized granulation tissue with well discernible numerous capillaries (Fig. 1, *c*) and with proliferating endothelium in fine-wall capillaries (Fig. 1, *d*).

Neoangiogenesis was always accompanied by fibroblastic cells and was associated with an increase in the count of these cells, because the same factors, produced by macrophages, platelets, and other cells, stimulated endothelial and fibroblast growth [6,11]. Fibroblast activity determines the composition and structure of the intercellular matrix. The volume of created full-value regenerate depends on the participation of tissue specific fibroblasts in regeneration of this tissue, while cells of the bone marrow origin (fibroblasts) can form only nonspecialized cicatricial tissue [6]. It seems that fibroblasts of this tissue are differentiated from pericytes (adventitial vascular cells), while fibroblasts forming the cicatrices are transformed from precursor cells of the bone marrow origin [6].

We consider that synthetic activation of specialized fibroblasts is essential for the type of forming regenerate in patients receiving richlocaine. This manifested in the production of the main substance of the connective tissue and fibrous structures by fibroblasts, these structures being characteristic of the supraepithelial layer of the gingiva before injury, that is, the subepithelial connective tissue was restored by restitution (Fig. 1, *e*).

Traditional drug therapy without richlocaine led to the formation of mainly cords of multicellular and coarse fibrous connective tissue in the cell infiltration (Fig. 1, *f*) with far lesser amounts of the main substance of connective tissue between fibrous structures (mainly nonspecific connective tissue was formed). It seems that this is intrinsic of unspecialized fibroblasts which can form only cicatricial tissue under hypoxic conditions. This was reflected by morphometric values characterizing dissemination of cell infiltration and sclerotic foci in the gingival tissues (Table 1). In patients treated with richlocaine the area of inflammatory cell infiltration decreased by 2.33 times, while in those receiving no richlocaine only by 1.56 times. The growth in sclerotically changed tissues was 21.7 in patients receiving no richlocaine and 11.5% after richlocaine treatment. Traditional drug therapy led to recovery of intercellular matrix of the gingival mucosal layer on 7.1% of area, while richlocaine increased this value to 34.3%. The intensity of acanthosis in the epithelial layer decreased to a greater extent in patients treated with richlocaine. The mean thickness of the epithelial layer after richlocaine treatment approximated the normal value (255 μ [2]), surpassing it by 95 μ, while without richlocaine it was 1.7-fold thicker (by 179 μ).

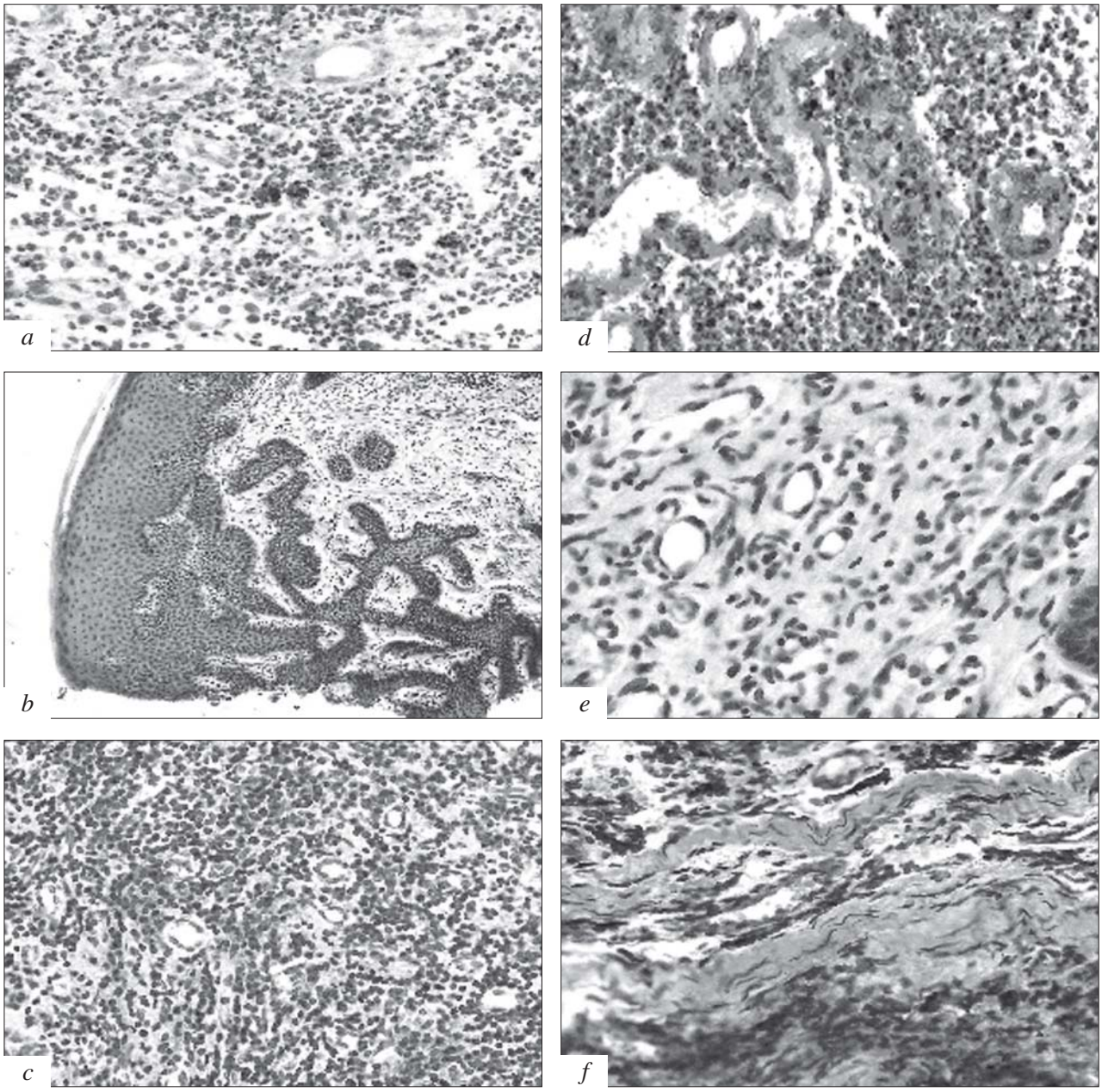

Fig. 1. Morphological manifestations of exacerbation of chronic generalized periodontitis (*a*, *b*), regeneration processes in the gingiva under the effect of drug therapy including richlocaine (*c-e*) and traditional treatment (*f*). *a*) macrophages and numerous neutrophils in inflammatory infiltration; *b*) pronounced acanthosis with submerged growth of epithelial processes into underlying tissues of gingival mucosa; *c*) numerous new capillaries in inflammatory cellular infiltration; *d*) proliferating endothelium of blood vessels in granulation tissue; *e*) area of gingival mucosa reticular zone restored by restitution; *f*) cords of fibrous connective tissue in inflammatory infiltration of gingival reticular layer. Staining: *a*, *c*: alcyan blue and neutral red; *b*, *d-f*: hematoxylin and eosin; ×400 (*a*, *c-f*), ×100 (*b*).

Hence, qualitative and quantitative morphological characteristics of regenerating tissues in the gingival mucosa on day 15 of treatment were better in patients receiving richlocaine compared to traditional therapy alone. It seems that recovery of the typical tissue structure in the subepithelial layer is of priority importance, because it provides normalization of the metabolic processes in the gingiva.

Richlocaine had a favorable effect on the inflammatory regeneratory process in the gingival tissues by activating angiogenesis in the regenerating structures. It promotes recovery of damaged connective tissue on a large surface of the mucosa by restitution. This process is paralleled by less intensive development of fibrosclerotic processes in the gingival tissues. The type of regeneration depends on the development of vascular network in

TABLE 1. Results of Morphometrical Studies of Gingival Mucosa during Treatment of CGP with Richlocaine ($M \pm m$)

Group of patients	Thickness of epithelium, μ	Diameter of capillaries in papillary layer, μ	Summary area of cellular infiltration in reticular layer, %	Summary area of sclerotic foci in reticular layer, %
Before therapy (n=64)	576±21	11.8±0.2	80.2±8.1	8.3±0.8
Traditional drug therapy (n=29)	434±32*	8.6±0.2*	51.4±6.0*	30.0±3.3*
Traditional drug therapy (15 days)+ richlocaine (n=35)	350±31*++	9.6±0.2*+	34.4±6.4*+	19.8±1.1*+

Note. n: number of patients. *p<0.001 compared to the corresponding parameter before treatment; + p<0.001, ++ p<0.05 compared to traditional drug therapy.

regenerating tissues. Our findings confirm that blood vessels play the key role in regeneratory processes [10], which is determined not only by delivery of required substances to regenerating tissues, but also by the fact that vascular cells serve as the main source of the formation of the cell ensemble in the regenerate participating in recovery of tissue structures.

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