EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Adrenergic Innervation of the Gingiva in Experimental Periodontitis

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In experiments on guinea pigs, the nerve-tissue relationships in gingival papillae were studied under conditions of experimental inflammation induced by local (turpentine injection) and general (whole-body γ-irradiation). It is found that structural and metabolic changes in the lamina propria and epithelium of the gingival mucosa correlate with disturbances in trophic influences from the sympathetic nervous system.

Key Words: *gingiva; periodontitis; sympathetic innervation; lactate dehydrogenase; succinate dehydrogenase*

High prevalence of periodontitis, difficulties in treating and preventing of this disease, and inconsistent data on its etiology and pathogenesis explain great interest to this pathology. Morphogenesis of periodontitis and morphological and histochemical changes in the gingiva of humans and animals are described in detail [2,3]. However, the mechanisms underlying structural changes of the gingiva and the relationships between local disturbances and innervation of the gingiva are less studied. At present, many Russian and foreign investigators believe that the development of inflammatory process in gingivitis depends on bacterial dissemination of the periodont [5,7,10]. However, published data suggest that pathological process can be also induced by other factors, in particular local traumatic injury [9]. There are practically no data on the mechanisms underlying chronic inflammation in the gingiva and the role of local and general damaging factors in this process.

The aim of the present study was to investigate the nerve-tissue relationships in the gingiva during chronic inflammation induced by local and general factors.

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MATERIALS AND METHODS

Experimental material was obtained from 35 guinea pigs weighing 220-370 g divided into 5 groups. Groups 1 and 2 comprised intact and control animals (injection of physiological saline into the gingivae), respectively. In group 3 animals, experimental periodontitis was modeled during 18 days as described previously [1]. To this end, 0.1 ml 50% turpentine in sunflower oil was injected into the gingivae near the frontal teeth. Inflammatory reaction developed during 24 h: marked hyperemia and edema were observed. Symptoms of acute inflammation increased over 3 days. Necrotic foci appeared in the site of injection. After 10-12 days, inflamed areas cleared from necrotic debris. On day 18, no sings of inflammation were observed: gingival tissue became more dense, no hyperemia and gingival recession were seen. In groups 4 and 5, local injury was combined with whole-body γ-irradiation in a dose of 50 Gy. Turpentine (0.1 ml, 50% solution) was injected on the next day after γ-irradiation. Unlike group 3, in irradiated animals a more protracted inflamma-

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tory response was observed. After 16-18 days, the inflamed area cleared from necrotic debris, but hyperemia, edema, and stomatorrhagia persisted for a longer time. Gingival recession and sometimes sequestration of teeth and dentogingival pouches were observed. Slight hyperemia, gingival recession, and deformation of interdental papillae persisted for about 3 months, some teeth were fractured.

Taking the dynamics of inflammatory processes in the gingivae into account, the material for the study was obtained during acute (day 18) and subacute (day 90) stages. The animals were anesthetized with hexenal (10 mg/100 g, intraperitoneally).

Sympathetic innervation of the gingiva was assessed by measuring catecholamine content by fluorescent histochemical method [8]. Activity of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) in epitheliocytes was assessed as described previously [6]. The intensity of fluorescence in adrenergic fibers and activity of the enzymes of energy metabolism (at 580 nm) were measured using an MPV-2 photometric microscope. In each case, 50 measurements were performed. The data were processed statistically using the Student *t* test.

RESULTS

In intact guinea pigs (group 1) nerve fibers with catecholamine-specific fluorescence are found only in the lamina propria of the gingival mucosa. Sympathetic terminals are primarily observed on blood vessels (Fig. 1, *a*). Solitary thin unequally twisted terminals going from perivascular plexes deep into the tissue are characterized by varicose structure and weaker fluorescence. Solitary adrenergic axons in the lamina propria are arranged along the connective tissue fibers. Free terminals are seen as small point formations and larger helical structures (Fig. 1, *b*). Small varicose terminal plexes adjacent to the basement membrane are observed, nerve fibers do not contact with the basement membrane and no adrenergic terminals are found in the gingival epithelium. The intensity of catecholamine fluorescence is 97.67±6.74 in terminals and 185.02±7.67 in vessels.

Injection of physiological saline (group 2) had no effect on the distribution and intensity of fluorescence in adrenergic structures in comparison with the norm. In the lamina propria, small aggregates of mast cells (orange-yellow luminescence) and macrophages (white-yellow luminescence), and solitary lymphocytes are seen.

On day 18 after turpentine injection, perivascular adrenergic plexes are seen in the lamina propria of the gingival mucosa; solitary nerve terminals arising from these plexes go to the adjacent tissues. Perivascular plexes are formed by thin and sparse fibers. The intensity of fluorescence in these fibers is decreased in comparison with the control (Fig. 1, *c*). Similar changes are noted in free nerve fibers. The intensity of luminescence (LC) decreases (Fig. 1, *d*). Numerous cell structures with bright fluorescence are seen in the lamina propria, they sometimes occupy separate fields, but more often densely infiltrate the stroma of the gingiva. Some cell structures, in particular labrocytes, macrophages, and cells of leukocytic infiltrates (neutrophil granulocytes, lymphocytes, and monocytes) selectively accumulate biogenic amines possessing intrinsic LC. Aggregates of LC cells are also arranged outside the leukocytic infiltrates, along blood vessels and free nerve fibers and terminals (Fig. 1, *c, d*). Apart from inflammatory cells, granular mast cells with signs of degranulation are seen nearby blood vessels and leukocytic infiltrates.

On day 18 after turpentine injection against the background of preliminary irradiation (group 4), adrenergic fibers and terminals are extremely depleted of the transmitter; perivascular plexes and free terminals in the lamina propria become practically invisible. Cross-sections with developed muscle layer and adventitia without LC structures are seen on preparations (Fig. 1, *e*).

Against the background of the absence of LC elements of sympathetic innervation, abundant LC structures characteristic of inflammatory processes are present in the lamina propria (leukocytes, monocytes, lymphocytes, Fig. 1, *e*). Abundant bright yellow droplet-like structures with intense fluorescence fill gingival papillae protruding the epithelial layer.

In group 5 animals on day 90 after turpentine injection against the background of irradiation, the content of the transmitter in gingival adrenergic structures gradually returns to normal. Innervation of the lamina propria is extremely reduced, only rare adrenergic structures with very weak fluorescence are noted. Nervous plexes in the perivascular adventitia are characterized by the presence of solitary point varicoses and short fragments of adrenergic fibers with weak LC. At the same time, aggregates of LC cells (lymphocytes, monocytes, and mast cells with signs of degranulation) lie nearby the blood vessels (Fig. 1, *f*).

Thus, combined action of local and general factors sharply reduced functional activity of the sympathetic apparatus of the gingiva to the level when adrenergic structures cannot be identified. Local damage alone considerably reduced the content of neuroamines in the sympathetic nervous structures, but histoarchitectonics of the sympathetic apparatus was preserved. A high content of LC cells (neutrophil granulocytes, monocytes, and lymphocytes) typical of inflammatory processes was observed in both cases.

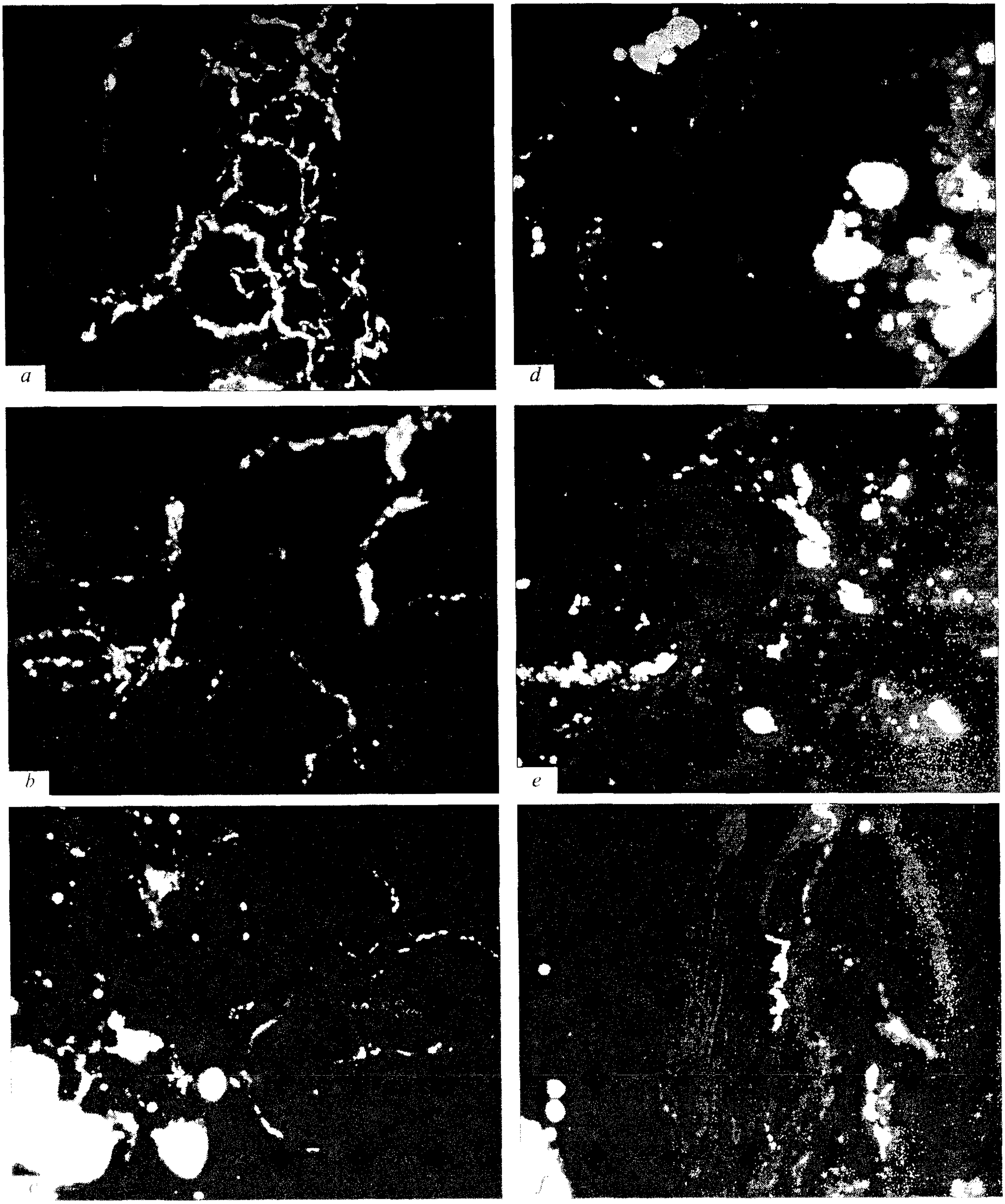

Fig. 1 Adrenergic structures in the lamina propria of guinea pig: perivascular nerve plexes in the norm (*a*), 18 h after turpentine injection (*c*), 18 and 90 days after combined action of turpentine and γ-irradiation (*e* and *f*), respectively; free nerve terminals in the norm (*b*) and 18 days after turpentine injection (*d*). Luminescent technique [8]. ×120

TABLE 1. Activity of Enzymes of Energy Metabolism in Gingival Epithelium of Guinea Pigs with Experimental Periodontitis ($x \pm S_x$, n=7)

Group	SDH		LDH	
	basal cells	spinous cells	basal cells	spinous cells
1 (norm)	0.97±0.03	0.54±0.01	1.49±0.04	0.97±0.02
2 (control)	0.81±0.04*	0.47±0.01*	1.51±0.04	1.11±0.08
3 (turpentine, day 18)	0.89±0.07	0.59±0.04+	1.54±0.05	1.33±0.05*+
4 (turpentine+γ-irradiation, day 18)	1.12±0.07*+	0.72±0.04*+	1.33±0.02*+	0.89±0.02*+
5 (turpentine+γ-irradiation, day 90)	1.18±0.02*+	0.81±0.07*+	1.39±0.06+	0.97±0.04

Note. *: significant differences from the norm; +: significant differences from the control.

During chronic inflammation induced by combination of local and general damaging factors, the level of sympathetic innervation decreases in comparison with the control; however in comparison with the early stage a positive dynamics is noted. Inflammatory cells are characterized by intense LC and form multiple aggregates in the lamina propria of the gingival mucosa.

In parallel, the state of energy metabolism in epithelial cells of the gingival mucosa and activity of SDH and LDH in the basal and spinous layers are evaluated (Table 1). In the gingiva involved into inflammation process caused by turpentine injection, the decrease in sympathetic innervation is accompanied by changes in the energy metabolism in cells of the basal and spinous layers. In the spinous cells glycolytic processes are activated without changes in aerobic oxidation. In the basal layer no such changes are observed (Table 1).

Chronic inflammation of the gingiva in irradiated animals caused by turpentine injection (combination of local and general factors) induces most pronounced changes in glucose metabolism in epitheliocytes. On day 18, energy metabolism in the basal and spinous layers is enhanced due to activation of aerobic glucose oxidation. These changes are also observed in chronic inflammation (Table 1).

Hence, energy metabolism in gingival epithelium is enhanced in chronic periodontitis due to activation of aerobic glucose oxidation in the Krebs cycle. These metabolic changes develop against the background of depletion of transmitter stores in adrenergic fibers and terminals of the gingival papillae confirmed by the absence of characteristic catecholamine fluorescence. These changes can be attributed to structural (sometimes degenerative) alterations in nerve fibers [4]. A relatively high level of aerobic glucose oxidation observed 3 months after induction of chronic periodontitis reflects the reserves of gingival epithelium.

Thus, changes in the gingiva observed after injection of turpentine alone or in combination with γ-irradiation and the data of neurohistochemical analysis suggest that combined action of local and general damaging factors plays an important role in the development of chronic inflammation of the periodont. Structural changes in the lamina propria and epithelium of the gingival mucosa result from disturbed nerve-tissue relationships and reduced trophic influenced from the sympathetic nervous system.

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