MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructural Analysis of Reorganization of the Periodontium in Simulated Torsion Abnormality and Its Correction with Succinic Acid

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Morphological study confirmed the positive effect of succinic acid on tissue ultrastructure, energy metabolism in cells of fibroblastic differon, reorganization and neogenesis of intercellular substance of the periodontal connective tissue during the retention period after correction of simulated dental torsion abnormality in dogs.

Key Words: *dental torsion abnormality; succinic acid; retention period; ultrastructural analysis of periodontium*

Correction of torsion abnormalities is still a pressing problem in dentistry. According to published reports [1,6,10], this condition is responsible for 9.3-19.3% maxillodental abnormalities. Despite recent progress in this field, the final stage — retention period ensuring stable results and good prognosis – is still associated with considerable difficulties.

Various methods (orthodontic, surgical, orthopedic, and physiotherapeutic) are used for stabilization of the results during the retention period. However positive results are not always fully achieved. We found no published reports about drug therapy used for stabilizing the results of treatment of torsion abnormality at this stage.

Succinic acid and its derivatives (*e. g.* sodium succinate) are involved in the synthesis of organic matrix and depositions of the mineral component, which stimulates reparative regeneration of the bone tissue. Succinic acid preparations inhibit the synthesis of prostaglandins in periodontal tissues, which induce the appearance of osteoclasts and promote resorption of bone structure [4,9].

There are good reasons to use succinic acid as an antiinflammatory agent and for stimulation of epithelial tissue regeneration [5], as well as for preventing osteoporosis resultant from prolonged hypodynamia [8], for improving oxidative and energy processes [3, 5,7]. Antioxidant effects of succinic acid underlie its membrane-stabilizing and antihypoxic action [2,3].

Experimental and clinical studies carried out at the Department of Orthodontics, Kuban' State Medical Academy, and at Laboratory of Krasnodar Research Center (Russian Academy of Medical Sciences) showed that tratment with succinic acid shortened retention period in experimental dogs and patients by 20-25% and considerably reduced the incidence of relapses.

The aim of this study was ultrastructural analysis of succinic acid effect on morphological reorganization of the periodontium during the retention period of repair of torsion abnormality modeled with orthodontic construction.

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MATERIALS AND METHODS

Experiments were carried out on 8-month-old mongrel dogs (*n*=8, 6-7 kg). All animals were divided into 2 groups: main (*n*=6) and control (*n*=2). The main group was subdivided into 3 subgroups, 2 dogs in each. Torsion abnormality (45° torsion of the maxillary canine) was induced in all animals of the main group. In subgroup 1 (experimental control) no succinic acid was used, in subgroups 2 and 3 it was added to food (0.5 g tablets) 3 times a day. The retention period was 70 days in subgroup 1, 30 days in subgroup 2, and 45 days in subgroup 3.

Ultrastructure of the periodontium collected at the level of the neck and apex of the root near the cement was studied. Fragments of the periodontium (~2 mm^3) were plunged for 2 h in 2% glutaraldehyde solution and then for 1 h into 1% osmium tetroxide solution in 0.2 M phosphate buffer (pH 7.4) at 4°C. After washout with cold phosphate buffer the material was dehydrated in ascending alcohols and embedded in araldite. Ultrathin sections (50-90 nm) were prepared using an LKB-8800 ultramicrotome and placed onto nickel grids. After contrasting with 2.5% uranyl acetate (in 50° ethanol) and lead citrate the sections were examined under an EMB-100 B microscope at accelerating voltage of 75 kV.

For photooptic identification of the required section area, serial semithin sections (1-2 μ) were prepared from all blocks embedded in araldite and stained with 1% toluidine blue, methylene blue, and Azur II in succession.

RESULTS

The periodontium of intact controls was formed mainly by elements of solid connective tissue intercellular substance and by fibroblastic differon cells, which participated in its renewal. The maximum number of these latter cells was detected in the interstitial matter. Fibrocytes constituted the greater part of differentiated cells of connective tissue of the periapical and pericervical periodontium. These cells were characterized by poorly expressed contours usually bordering with relatively amorphous main substance of the connective tissue (Fig. 1, *a*). The cytoplasm of these cells rarely contained mitochondria, small osmiophilic lysosome-like bodies, free ribosomes, short dilated cisterns of the granular endoplasmic reticulum, numerous vacuolar vesicles, elements of the cytoskeleton, and particularly microfilaments, which indicated the capacity of fibrocytes to modify their shape and migrate in the intercellular substance. Typical morphofunctionally active fibroblasts were often present among the fibroblastic differon cells; the cytoplasm of these fibroblasts contained fragments of collagen fibrils near elements of granular endoplasmic reticulum, which indicated the possibility of their intracellular assembly and subsequent release. Fibroclasts (cells containing not only biosynthesis organelles in the cytoplasm, but also numerous lysosomes and autophagosomes, destroying the intercellular substance of periodontal connective tissue) were also characteristic of the periodontal fibroblastic differon.

Sometimes small spindle cells with short processes with euchromatic nuclei, numerous free ribosomes, and solitary elements of the granular endoplasmic reticulum in the cytoplasm were seen in various compartments of the periodontium between bundles of collagen fibrils. These cells, which we identified as poorly differentiated, were also seen near periodontal blood vessels. The presence of mitotic figures suggests that these cells served as the possible source of regeneration of periodontal cellular composition (Fig. 1, *b*). New formation and differentiation of fibroblastic differon cells were equilibrated by apoptosis (Fig. 1, *c*).

Along with the fibroblastic differon cells (usually in the interstitial component of the periodontium) we observed labrocytes and leukocytes. Blood capillaries, most numerous round the root part of the tooth, had abundant markedly thinned sites of endotheliocyte cytoplasm. The endotheliocyte luminal membrane could participate in the formation of microvilli, numerous pinocytous vesicles even in clasmatosis phenomena. The main type of interendothelial contacts were short simple adhesive connections. The ultrastructure of perinuclear and peripheral (exchange) zones of the cytoplasm were characterized by manifestation of multiple functions of endotheliocytes.

In the experimental control subgroup torsion abnormality still had a traumatic effect on the dog periodontium even after 70 days of the retention period. Analysis of panoramic electronoptic images confirmed the presence of extensive areas of the periodontium with markedly rarefied collagen fibrils or very few fibrils per units of area because of persistent edema of the amorphous component of the connective tissue intercellular substance. Moreover, not only periodontal tissues and vessels were deformed, but cement and bone tissue were involved too. This was paralleled by accelerated biosynthesis or resorption of collagen fibrils and other components of the connective tissue intercellular substance in some areas of the periodontium. Our observations showed that orthodontic loading simulated in animals of this subgroup involved more diverse and essential changes in the periodontium. Fibroblastic differon cells capable of proliferation at different levels of differentiation were seen here more often. Mast cells were detected near blood vessels along with fibroblasts, their cytolemma con-

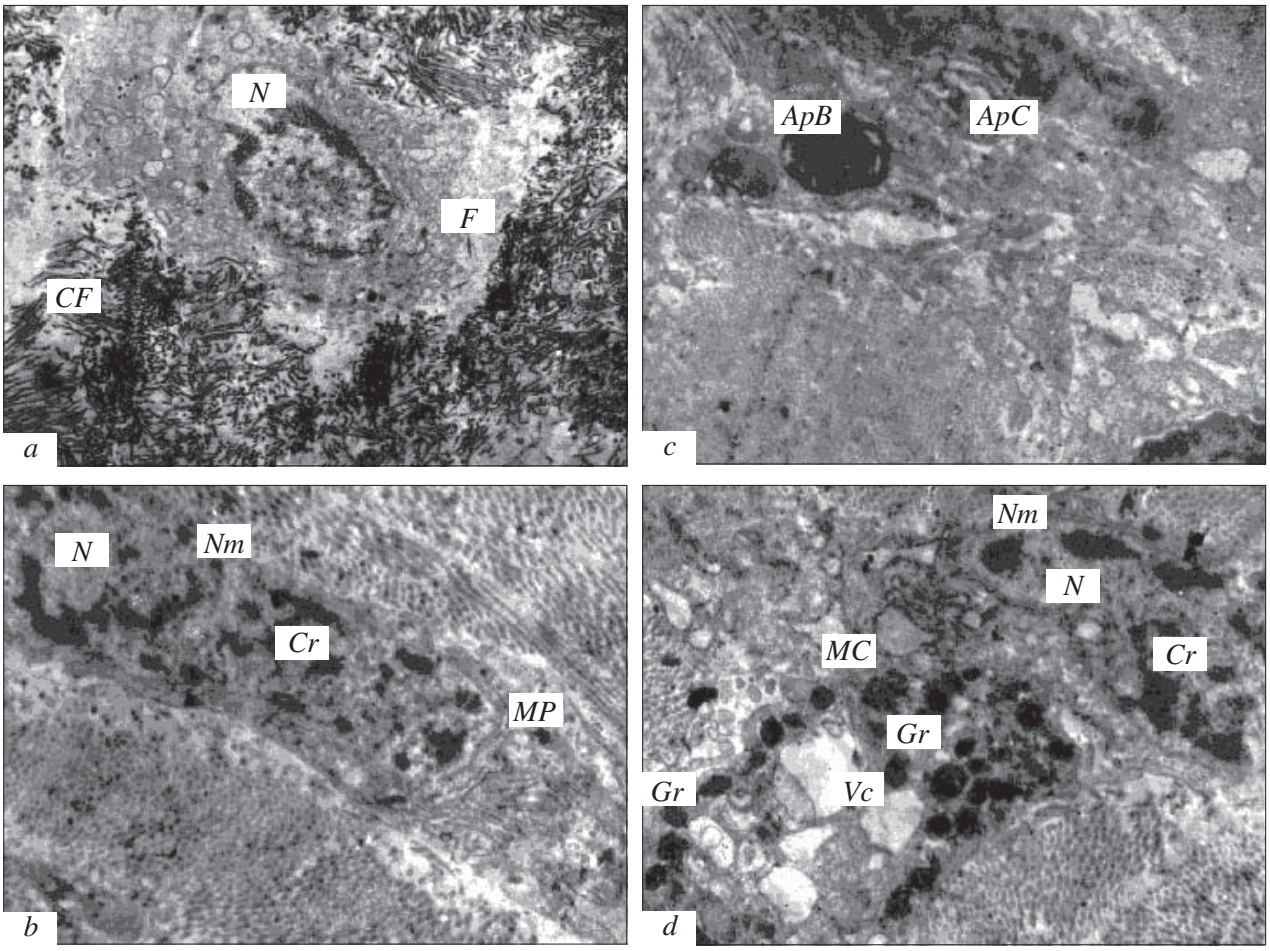

Fig. 1. Ultrastructure of periodontium in intact (*a-c*) and experimental (*d*) animals on day 70 of retention period after rotation of the tooth by 45°. *a*) fibrocyte in periapical periodontium; *CF*: collagen fibrils; *F*: fibroblast; *N*: nucleus; ×5200. *b*) early prophase of mitotic division of poorly differentiated fibroblast in the periodontium at the level of the tooth neck; *MP*: mitosis prophase; *Cr*: chromatin; *Nm*: nuclear membrane; ×7475. *c*) fibroblastic differon cell apoptosis in periodontium at the level of tooth neck; *ApC*: apoptotic cell; *ApB*: apoptotic body; ×5600. *d*) mast cells of experimentally modified periodontium with manifestation of destruction of their specific granules in the cytoplasm on day 70 of retention period after tooth rotation by 45°; *Vc*: vacuole; *Gr*: granules; *MC*: mast cells; ×8050.

taining foci of vesiculation, while large vacuoles and manifestations of intracellular degradation of the specific contents of the granules in the cytoplasm indicated liberation of histamine (Fig. 1, *d*). This latter phenomenon could be the cause of edema of perivascular spaces containing amorphous component of connective tissue in abundance. Obvious morphological changes were observed in endothelial cells of capillaries. Many of them possessed gel-like cytoplasmic matrix and were identified as "dark". Their cytoplasm contained few intracellular structures, except cytofilament bundles attesting to contractile activity of endothelial cells and their capacity to modify their shape. "Light" endotheliocytes were characterized by less compact cytoplasmic matrix and more pronounced margination of chromatin under the nuclear membrane, often forming local dilatations of the perinuclear space. Biosynthetic organelles were better developed in the cytoplasm of "light" endotheliocytes; lysosomes were seen more often. More clear contours of the basal membrane were seen under such cells.

An obvious manifestation of specific reactive changes in the periapical periodontium was more frequent presence of epithelial Malassez residues (islets) (Fig. 2, *a*). These islets presented as compact accumulations of small "dark" and "light" epitheliocyte-like cells in a different structural and functional state and in different periods of cell cycle. Increasing concentration of the main elements forming the connective tissue (collagen fibrils, fibroblast cells, particularly fibrocytes, and even myofibroblasts) was most obvious in this area, particularly near cement (Fig. 2, *b*).

Hence, functional activity of the greater part of periodontal cells, including capillary endotheliocytes, decreased (normalized) by the end of retention period.

Electron-microscopic examination of the periodontium from animals of subgroup 2 of experimental group showed ultrastructural manifestations of pro-

gressing restructuring of the periodontium in the pericervical and periapical zones. Obvious structural and functional activity of fibroblasts and accumulation of collagen fibrils in the intercellular substance were detected at the level of the tooth neck (Fig. 2, *c*, *d*).

Along with typical fibroblasts, fibroclast type cells were often seen among collagen fibril bundles; these cells were characterized by similar ultrastructure, but possessed better developed lysosomal system, myelin bodies, mitochondria, and showed manifestations of intracellular degradation of osmiophilic material, a possible object of phagocytosis (Fig. 3, *a*).

Mitotically dividing cells were seen among poorly differentiated or differentiating cells of the fibroclastic differon, but we detected no appreciable changes in their proliferative activity in comparison with other control and experimental material. The main substance was present in greater amounts in the periapical periodontium and signs of edema were more pronounced in it, which was confirmed by the presence of labrocytes with signs of degranulation and intracellular destruction of their specific granules (Fig. 3, *b*). Rarely seen apoptotic bodies were regarded as a result of "delayed" physiological cell death of some mature connective tissue cells which "survived" the trauma.

Cells with numerous processes and few organelles were seen in tissue elements of the periodontium, they often formed small clusters. Chromatin marginates were numerous in the nuclei of these cells, while the nuclear membrane was scalloped. Their peripheral cytoplasm was characterized by accumulation of filamentous material. The cytoplasm contained numerous large vacuoles formed by granular endoplasmic reticulum and lysosomes. Homogeneous material seen in some vacuoles corresponded by electron density to free lipids, which attested to possible involvement of

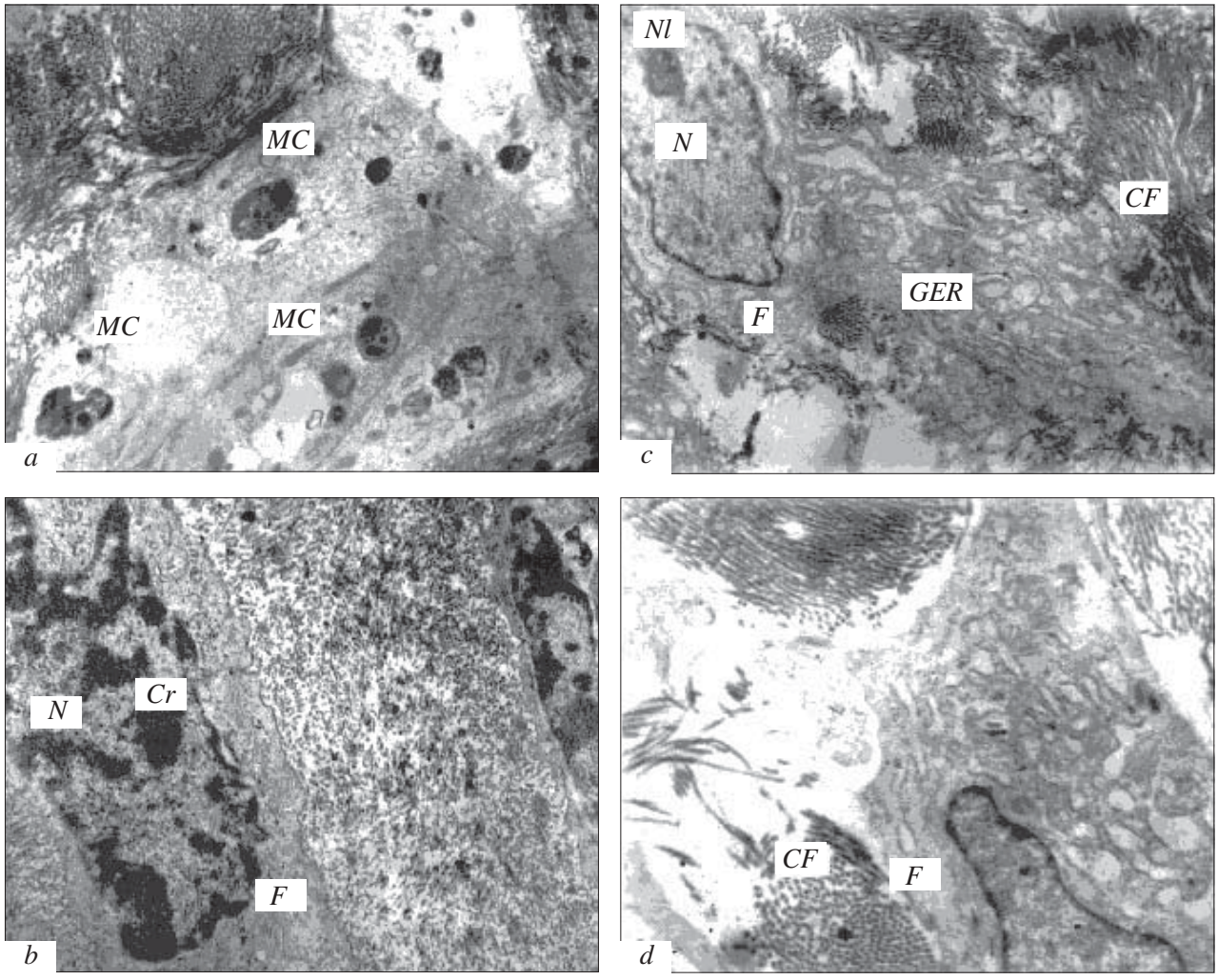

Fig. 2. Ultrastructure of periodontium on day 70 (*a*, *b*) and 30 (*c*, *d*) of retention period after experimental 45° rotation of the tooth after treatment with succinic acid. *a*) Mallasez islet cells (*MC*) in experimentally modified periodontium at the level of the tooth apex; ×4200. *b*) myofibroblasts of periapical periodontium; ×6300. *c*) pericervical periodontium fibroblasts; *GER*: granular endoplasmic reticulum; *Nl*: nucleolus; ×440; *d*) periapical periodontium fibroblast, ×6400.

differentiating fibroblastic cells in energy metabolism. This also can be a manifestation of the early stages of lipocyte differentiation from poorly differentiated cells of fibroblastic differon. Other findings included perivascular extravasates (mainly erythrocytes or their fragments), characteristic changes in capillary wall ultrastructure and bimorphism of endotheliocytes, signs of structural and functional activation of tissue elements of experimentally damaged periodontium, cells dying by "delayed" apoptosis, and apoptotic bodies.

The study of the ultrastructure of experimentally deformed periodontium in experimental dogs of subgroup 3 also demonstrated certain specific morphofunctional changes in tissue elements of the periodontium. The greater part of these elements in the pericervical zone had ultrastructure and ratio of the intercellular substance components similar to the control. Orientation of collagen fibrils in bundles tightly adhering to each other was approaching the mutually perpendicular. By morphological characteristics we identified the greater part of cells as fibrocytes (Fig. 3, *c*). Fibroclasts with characteristic ultrastructural organization were sometimes seen. The concentration of collagen fibrils per unit of the periapical periodontium area notably decreased in it, and they were oriented in opposite directions; residues of erythrocytes, autophagosomes, residual and apoptotic bodies, and lipid droplets were sometimes seen in amorphous substance (Fig. 3, *d*). The ultrastructure of fibroblasts surrounded by typical collagen fibrils is worthy of note. The karyoplasm contained solitary small condensations of chromatin, the nucleoli were activated, nuclear membrane had abundant pores. Extracellular biosynthesis organelles were distributed over the entire cytoplasm. Exocytosis signs were obvious. Fibroblast activity was the most pronounced near blood capillaries, in which endotheliocytes were flattened, contained few organelles, and formed numerous thinned zones (fenestrae) in the peripheral exchange zones, as well as microvilli protruding in the capillary lumen. Intensive transport of pinocytous vesicles, thinning and loosening of the basal membrane indicate high permeability of capillary walls.

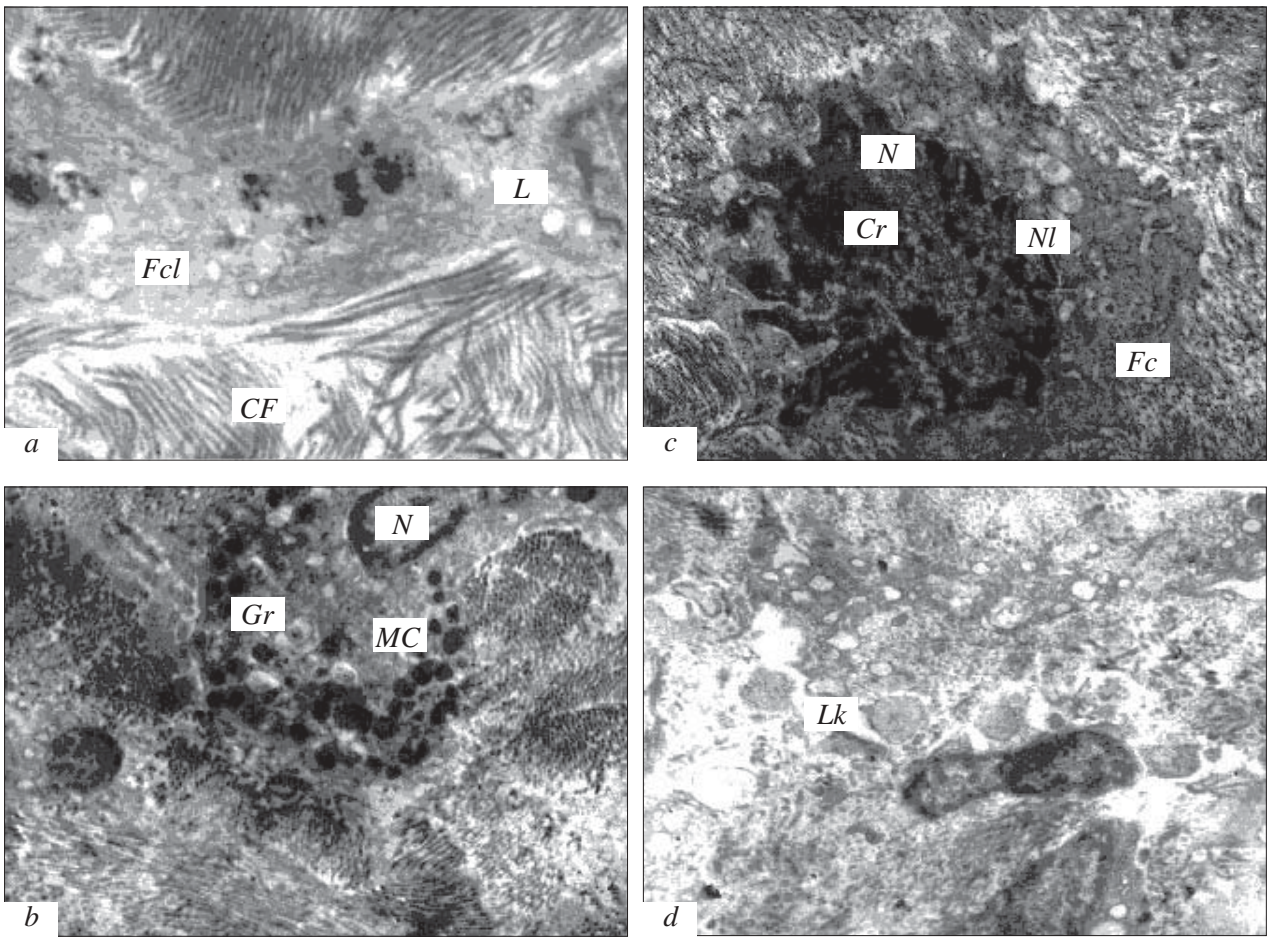

Fig. 3. Ultrastructure of periapical periodontium on day 30 (*a*, *b*), 70 (*c*), and 45 (*d*) after experimental 45° rotation of tooth followed by treatment with succinic acid. *a*) fibroclast (*Fcl*); *L*: lysosomes; ×6300. *b*) mast cells; *Gr*: granules; ×5600. *c*) fibrocytes (*Fc*), ×11,200. *d*) free lipids and loci of their utilization in connective tissue intercellular substance. *Ld*: lipid droplets; ×6650.

Hence, ultrastructural analysis of the periodontium in control (not included in experiment) animals indicated constant regeneration processes in its tissue elements. This is true about the fibroblast cells, capable of mitotic division, and about the components of intercellular substance produced by differentiating and differentiated fibroblasts. Ultrastructural manifestations of the lysosomal system activation in fibroblast cells objectively corresponded to destruction and elimination of collagen fibrils. It is therefore logical to assert that the structure and functions of the periodontium in its different parts was determined by a balanced fibroblast-fibroclast system. Hence, the formation of collagen by fibroblasts and disintegration of its macromolecules are constant processes adapting the periodontium to the conditions of the maxillodental system functioning, which was confirmed during experimental deformation (tooth rotation round its longitudinal axis). Structural reorganization of the periodontium associated with it was based on accelerated synthesis or resorption of collagen fibrils and its other components. This conclusion is also true for other experiments of our study. Signs of pronounced biosynthetic activity of the fibroblastic differon cells appeared by day 30 of the retention period in animals receiving succinic acid with food. These signs were particularly obvious in the periapical periodontium. The data on the effect of succinic acid on energy and plastic metabolism in tissues, on their reparation under conditions of exposure to damaging factors also conform to the modern concepts. Presumably, succinic acid is used as energy substrate in the biosynthesis of lipids accumulating in differentiating fibroblastic elements, which are liable to transformation into lipocytes. Experiments with a longer retention period (45 days) during which the animals received succinic acid showed rather specific and effective reorganization and reparation of damaged periodontium, obvious in all its compartments. Despite such a permanent factor as masticatory movements, the ultrastructural characteristics of tissue elements and the ratio of relative volumes of collagen fibrils and main substance in the periodontium were close to the control values. Signs of inflammation were no longer characteristic, but a certain disorientation of some fibrils and even their bundles were still present, which was more typical of the periapical periodontium. Ultrastructure of the fibroblastic differon cells objectively attested to a decrease in the biosynthesis of intercellular substance: intracellular volumes of granular endoplasmic reticulum and other organelles concentrating around the nucleus were notably decreased. Fibroclasts were much more rarely seen; normalization of the capillary wall ultrastructure and histohematic barrier were observed. A possible results of treatment with succinic acid was pronounced fenestration and vesiculation of blood capillary endothelium. We consider that intense trophics of the damages areas of the periodontium and effect of succinic acid are responsible for long presence of erythrocytic extravasates and even some intracellular structures (mitochondria, autophagosomes, flattened lamellae, lysosomes, *etc.*) in these areas. The detected intra- and extracellular free lipids indicate energy capacity of tissue composition of the periodontium.

Hence, the positive effect of succinic acid on tissue ultrastructure, energy metabolism of the fibroblastic differon cells, reorganization and new formation of the connective tissue intercellular substance during the retention period after repair of torsion abnormality can be considered morphologically confirmed.

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