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Research Article

Changes of ultrastructural organization in periodontal complex components in experimental periodontitis and its correction with quercetin

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Abstract

The article presents the results of study the changes of ultrastructural organization in the periodontal complex under conditions of experimental bacterial-immune periodontitis development and treatment effect of flavonol quercetin. Experimental bacterial-immune periodontitis in experimental animals was caused by injection of complex mixture of microorganisms diluted with egg protein into periodontal tissue. The evaluation of the ultrastructural character changes in the gingival area of the periodontal complex was carried out on the basis of electron microscopic examination. In a result study on the 7th day of the experiment were observed changes that manifested disorders of the connective tissue and vessels structure. It were also marked increased diffusive leukocytic infiltration of the connective tissue of the mucosal gingival lamina propria. There was some improvement in respect of the electron microscopic structure of the microcirculatory vessels on the 14th and 30th days of the experiment. The performed studies of hemocapillaries submicroscopic organization of the animal's gums mucous membrane in conditions of application quercetin for experimental periodontitis has shown that flavonol improved structural organization considerably. The use of quercetin improves the organization of all ultrastructured components of the periodontal complex in experimental periodontitis.

Keywords

Periodontitis, inflammation, microcirculation, fibroblast, lymphocyte, quercetin

Introduction

Periodontal diseases by the nature of the clinical course are mainly chronic, ending with inflammatory and destructive changes in the tissues that hold the teeth in the socket, and lead to progressive growth of connective tissue (Demkovych et al. 2019; Bandrivsky et al. 2022). Epidemiological data shows the growth and prevalence of periodontal disease worldwide (Slots 2017). Generalized inflammatory periodontal diseases attract the increased interest of researchers and clinicians, because the consequence of this pathology is tooth extraction, increasing the risk of associated systemic pathology (Dankevych-Kharchyshyn et al. 2019). It is known that inflammatory processes that develop in the periodontal complex are often the main cause of tooth loss (Di Benedetto et al. 2013).

A feature of the ultrastructural organization of periodontal tissues is their vulnerability to damage by mechanical, chemical, bacterial and immune factors. Integrity of the structure depends on maintaining the appropriate level of metabolic, microcirculatory processes, neuroendocrine regulation (Demkovych et al. 2017, 2022).

At present, scientific research has provided sufficient material about ultrastructural changes in periodontal tissues during inflammation, but the sequence and regularity of its formation is far from being studied (Hasiuk et al. 2016, 2017, 2021). In this regard, it is expedient to experimentally study the sub microscopic changes in the soft and bone tissues of the periodontium, which are formed under the influence of pathogenetic processes that underlie inflammatory-dystrophic disorders in the periodontal complex (Hyde et al. 2017).

In the diagnostic of periodontal disease with electron microscopic examination the tasks are determining the depth, activity, and duration of the destructive process. Based on which an objective assessment of the severity of the lesion and develops adequate treatment tactics that require extraordinary approaches to their solving. (Gao et al. 2015). In this case, the attention is drawn to quercetin, which is a flavanol with antioxidant, anti-ischemic, membrane-stabilizing and immunomodulatory properties (Miles et al. 2014; D'Andrea 2015). It has a great restorative potential and exhibits anti-inflammatory, anabolic, anti-apoptotic properties (Li et al. 2016). An antioxidant property of the drug is associated with its ability to inhibit lipid peroxidation, reduce the concentration of free radicals and toxic peroxidation products, stimulate catalase and superoxide dismutase activity (Kumar and Pandey 2015; Demkovych et al. 2021a, b, c). Anti-inflammatory and anti-allergic effects are also associated with the ability of quercetin to suppress calcium ATPase and leukotriene synthesis (Zabolotny et al. 2016). This flavanol is able to inhibit the activity of hyaluronidase, increase the content of cells of the immune system (phagocytes, T- and B-lymphocytes) in the blood, resulting in reduced manifestations of secondary immunosuppression (Terao 2017). Finding out the pathomorphological characteristic of the ultrastructural changes will determine the mechanisms of damage to the structures of the periodontal complex, the patterns of formation of the inflammatory process and the possibility of their correction (Chapple et al. 2015; Livingstone et al. 2015).

The objective of this study was to investigate the changes in the ultrastructural organization of the periodontal complex in experimental bacterial-immune periodontitis development and treatment effect of flavanol quercetin.

Materials and methods

The study was conducted with use of non-breeding clinically healthy male rats (25 animals) weighing 150–200 g in vivarium conditions in accordance with sanitary-hygienic norms and GLP requirements. The animals were in a standard diet balanced by the main elements of nutrition. Experiments were carried out in compliance with the general rules and provisions of the "European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments" (Kyiv, 2001). The rats were divided into groups: I – intact animals, control (n = 5); II – animals with experimental periodontitis on the 7th day of the research (n = 5), III – animals with experimental periodontitis on the 14th day of the research (n = 5), IV – animals with experimental periodontitis on the 30th day of the research (n = 5), V – animals with experimental periodontitis on the 14th day of the research, which was introduced to quercetin (n = 5). Experimental bacterial-immune periodontitis in experimental animals was caused by introducing complex mixture of microorganisms diluted with egg protein into periodontal tissue (Demkovych 2017). Simultaneously with the injections of the microbial pathogen, a complete Freund's adjuvant was injected in the rat's paw to enhance the immune response. Systematically healthy rats of the same age were used as controls. The pathogen and adjuvant were injected repeatedly on the 14th day of the experiment. The experimental animals of the fifth group were treated by intramuscular injections with water-soluble quercetin (Corvitin, PJSC SIC "Borshchahivskiy Chemical Pharmaceutical Plant", Kyiv) in a dose 100 mg / kg of animal weight for 7 days (from the 7th to the 14th day).

Sampling for electron microscopy was performed immediately after the animals were removed from the experiment according to generally accepted rules (Bilash et al. 2019). Small pieces of periodontal tissue were taken for electron microscopic studies. The material was fixed in 2.5% glutaraldehyde solution with an active medium pH reaction of 7.3–7.4 prepared on Millonig phosphate buffer. The fixed material was transferred to the buffer solution in 50–60 min and washed for 20–30 min. Post fixation was performed on a 1% solution of osmium tetroxide on Millonig buffer for 60 min, followed by dehydration in alcohols and acetone and poured into a mixture of epoxy resins. Ultra-thin sections made on ultramicrotome LKB-3 were stained with 1% aqueous uranyl acetate solution, contaminated with lead citrate according to the Reynolds method and studied in an electron microscope of PEM-125K. Micro photographing of images was done using a digital video-camera Delta Optical DLT – Cam Basic 2 MP. Photos were processed on computer Intel (R) Celeron (R) 2.7 GHz with the help of Toup View program.

Results and discussion

As a result of electron microscopic studies of the rat's gingival mucosa lamina propria on the 7th, 14th and 30th day with the experimental bacterial-immune periodontitis, pronounced changes were found in the all structural components. Thus, on the 7th day, in the lamina propria of the mucous membrane of the gums, changes were observed. That was characteristic of the violation of the structure of connective tissue and vascular disorders.

Damage to the intercellular substance, edema of the amorphous component, destructive changes in the fibres, thinning and fragmentation of collagen fibres were noted. An electron-light amorphous component was observed in the reticular layer of the gingival mucosa, and there were thick compacted bundles of collagen fibres, between which there were light, irregularly shaped areas of the amorphous component. This sub microscopic condition of the connective tissue of the gums indicates its edema, which appears in the early period of experimental periodontitis.

At the same time, fibroblasts and fibrocytes were destructively changed in these conditions. The nuclei of fibroblasts had an irregular shape due to karyolemma invagination. Its structure was fuzzy in places; there were heterochromatin granules in the karyoplasm. There was destruction of organelles, irregular extension of the endoplasmic reticulum tubules, illumination of the mitochondrial matrix, and the destruction of cristas were noted. In the cellular cytoplasm occurred homogeneous sites and a small number of organelles. The nuclei and significant intussusception of the karyolemma were also altered in the fibrocytes, and heterochromatin predominated in the karyoplasm.

The fragmented collagen fibrils and amorphous intercellular substance of the connective tissue located around the fibrocytes in the intercellular substance of the connective tissue. The swelling of the intercellular amorphous component of the connective tissue was increased (Fig. 1).

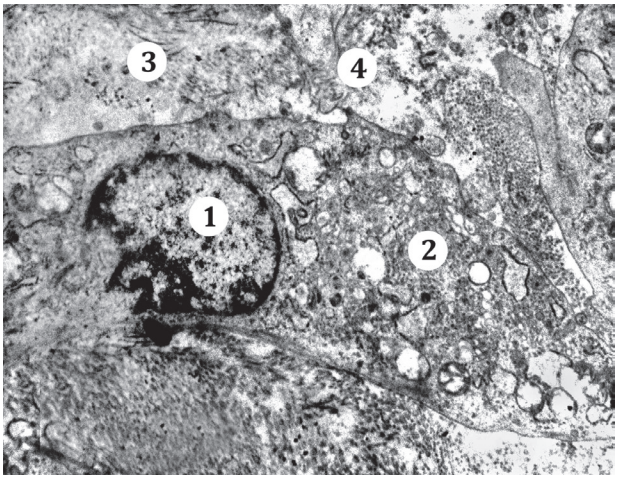

Figure 1. Electron microscopic changes in the rat's gingival mucosa lamina propria with experimental periodontitis on the 7th day of research. × 7000. **Notes:** 1. the fibroblastic nucleus; 2. the fibroblastic cytoplasm; 3. the intercellular substance; 4. the collagen-damaged fibrils.

At the same time, it is necessary to note the increased leukocyte infiltration of the connective tissue of own plate of a gums mucous membrane. Small focal infiltrates were found in the perivascular spaces, containing lymphocytes, neutrophil granulocytes, plasmacytes and macrophages (Fig. 2).

Macrophages with secondary lysosomes of phagocyted material in the cytoplasm were observed in the visual field.

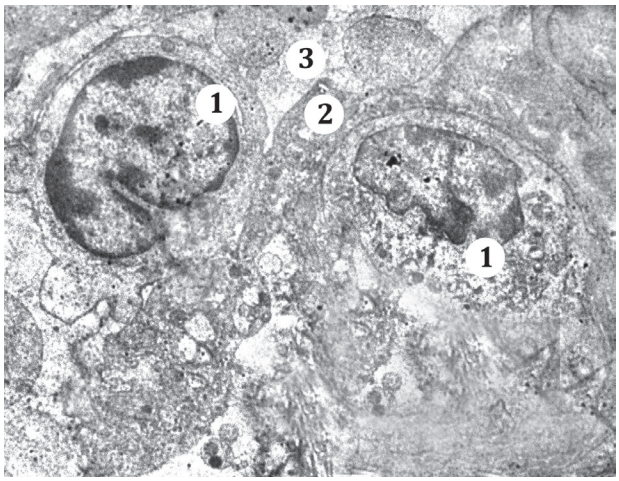

Figure 2. Ultrastructural image of the rat's gingival mucosa lamina propria with experimental periodontitis on the 7th day of research. × 7000. **Notes:** 1. the lymphocyte; 2. the fibroblastic processes; 3. the intercellular substance.

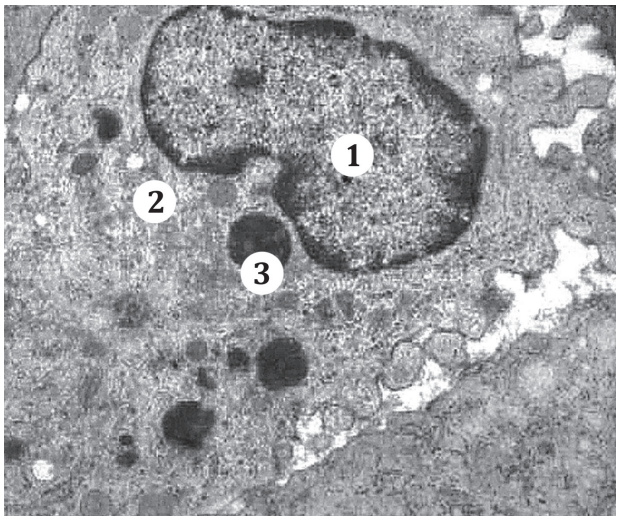

Figure 3. Ultrastructural changes in the of the rat's gingival mucosa lamina propria with experimental periodontitis on the 7th day of research. × 7000. **Notes:** 1. the macrophage nucleus; 2. the macrophage cytoplasm; 3. the secondary lysosome.

The plasma of those cells had cytoplasmic pseudopodia and invaginations (Fig. 3).

Significant electron microscopic changes in the components of the microcirculatory bloodstream of the gingival lamina propria with experimental periodontitis on the 7th day of research were found. At the same time, the lumens of venules and capillaries were significantly increased, they were blood overfilled, the walls were thinned, and perivascular edema has occurred. The red blood cells were formed in the hemocapillary lumens (Fig. 4).

On the 14th and 30th days of the experiment, the ultrastructural organization of the gingival plate became more simplified by changes in vascular and tissue components, but cellular disorganization persisted. In particular, the fibroblastic nuclei had profound invagination of karyolemma, in which a significant area was occupied

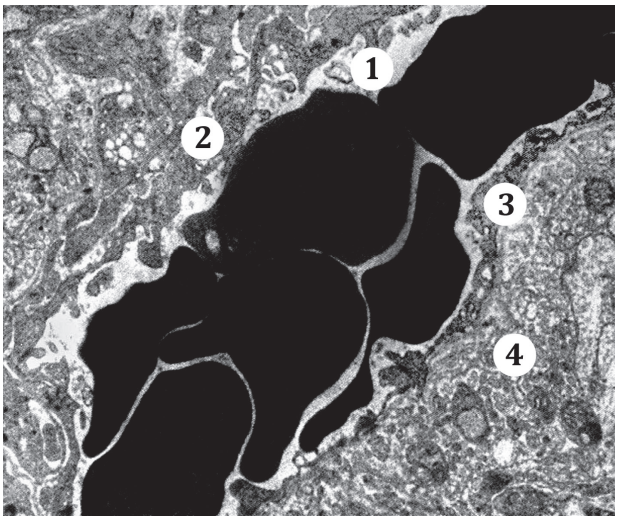

Figure 4. Ultrastructural organization of the hemocapillaries of the rat's gingival mucosa lamina propria with experimental periodontitis on the 7th day of research. × 7000. **Notes:** 1. the lumen of the capillary with red blood cells; 2. the endothelial cell cytoplasm; 3. the basement membrane; 4. the perivascular space.

by euchromatin. The organelles of the cytoplasm were less damaged than before, the Endo plasmatic reticulum canals were dilated, and ribosomes were located on their membranes. Mitochondrial hyperplasia was observed, they had better preserved cristae. There was also less swelling of the amorphous component of the intercellular substance of the connective tissue, collagen fibrils were partially damaged (Fig. 5).

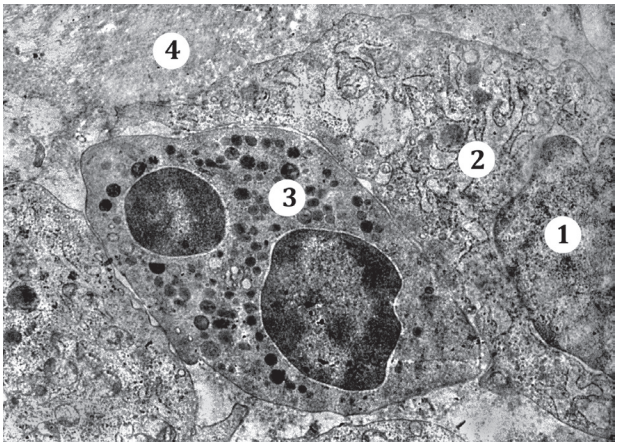

Figure 5. The electron microscopic image of the rat's gingival mucosa lamina propria with experimental periodontitis on the 30th day of research. × 9000. **Notes:** 1. the fibroblastic nucleus; 2. the fibroblastic cytoplasm; 3. the neutrophil; 4. the intercellular substance.

However, markedly damaged fibroblasts with kariopyknotic nuclei, osmiphilic cytoplasm, profound invaginations karyolemma and destructively altered organelles were observed sub microscopically, on the 14th day of the experiment (Fig. 6).

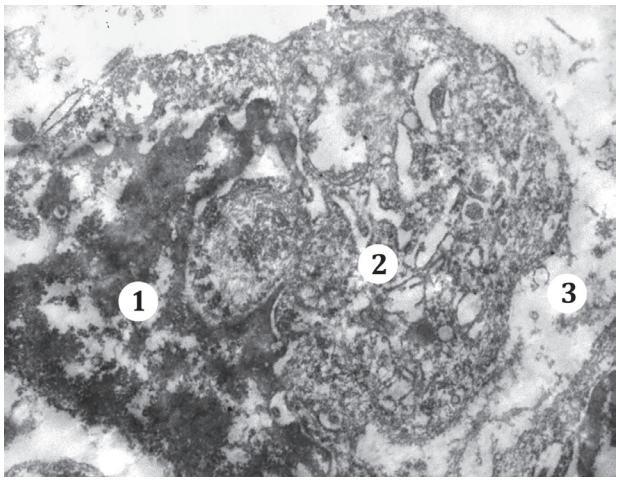

Figure 6. The electron microscopic image of the rat's gingival mucosa lamina propria with experimental periodontitis on the 14th day of research. × 12000. **Notes:** 1. osmiophilic nucleus of the fibroblasts; 2. cytoplasm with damaged organelles; 3. swollen amorphous component of the intercellular substance.

Leukocytic infiltration was observed in the damaged areas of the gum lamina propria and macrophages were present in the swollen connective tissue. There were various electron density inclusions in the leukocytic cytoplasm of the phagocytic material (Fig. 7).

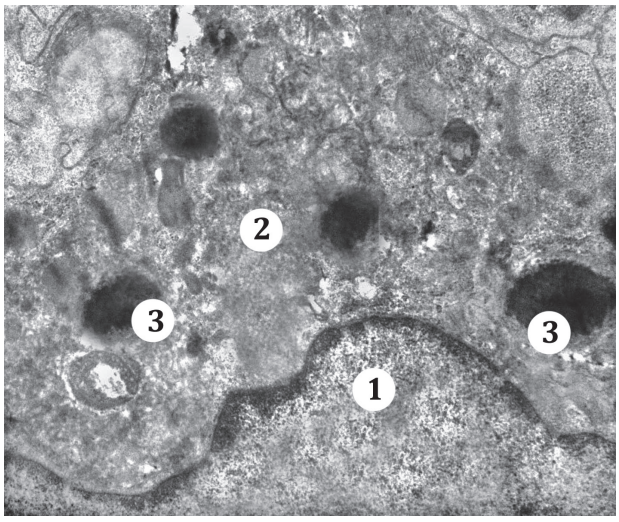

Figure 7. Macrophage fragment of the rat's gingival mucosa lamina propria with experimental periodontitis on the 14th day of research. × 17000. **Notes:** 1. the macrophage nucleus; 2. the macrophage cytoplasm; 3. the secondary lysosomes.

The electron microscopic study on the 14th and 30th days of the experiment showed some structural improvement of the microcirculatory vessels. At the same time erythrocytic sludges with dense packings were absented in hemocapillares, endothelial cells and basal membrane were more clearly contoured. However, in the connective tissue of the gingival mucosa lamina propria, the capillaries and venules were with dilated lumen, which contained

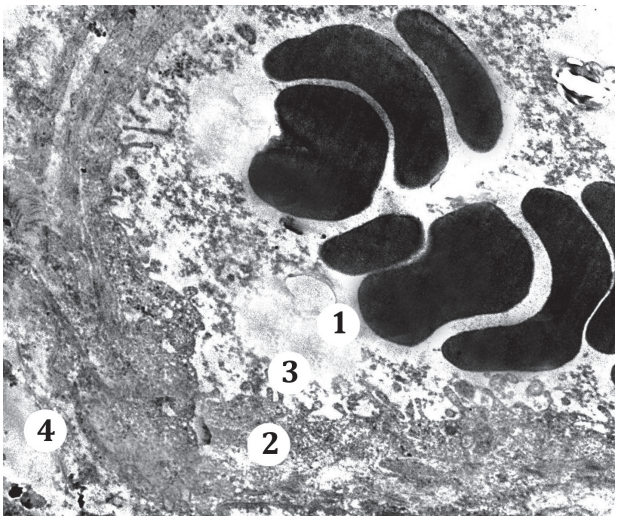

Figure 8. Sub microscopic image of the blood capillary of the rat's gingival mucosa lamina propria with experimental periodontitis on the 14th day of research. × 7000. **Notes:** 1. the capillary lumen with red blood cells; 2. the endothelial cytoplasm; 3. the microvilli; 4. the perivascular space.

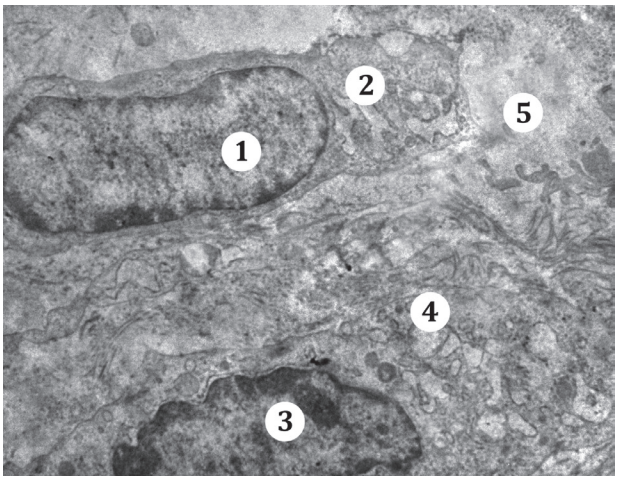

Figure 9. Electron microscopic image of the rat's gingival mucosa lamina propria with experimental periodontitis on the 14th day of research after quercetin treatment. × 9000. **Notes:** 1. the nucleus of young fibroblasts; 2. the cytoplasm of the young fibroblasts, 3. the nucleus of the mature fibroblast; 4. the cytoplasm of the mature fibroblast; 5.the intercellular substance.

a lot of the red blood cells. The vascular wall was thinned, and endothelial cells had a lot of microvillus on the surface and pinocytic vesicles in the cytoplasm, indicating a very active transport of fluid from the microcirculatory bloodstream (Fig. 8).

Electron microscopic studies of the rat's own gingival mucosal plate in experimental bacterial-immune periodontitis under quercetin treatment showed that the ultrastructure of all connective tissue components was significantly better compared to animals without treatment. At the same time the sample had fibroblasts of varying degrees of differentiation. Young cells had

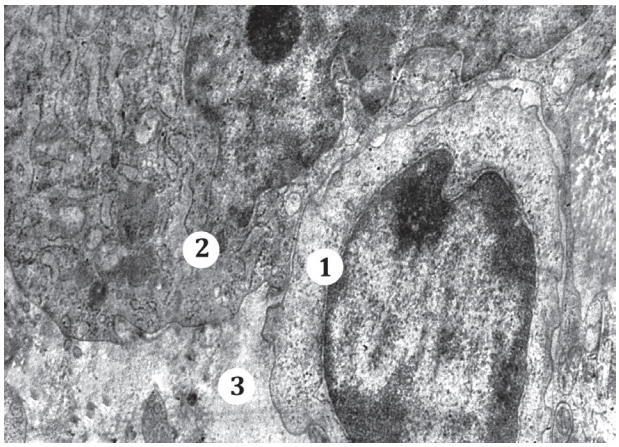

Figure 10. Ultrastructure image of the rat's gingival mucosa lamina propria with experimental periodontitis on the 14th day of research after quercetin treatment. × 12000. **Notes:** 1. the lymphocyte; 2. the fibroblast; 3. the intercellular substance.

small size, round oval nuclei, around of which there was little cytoplasm. In its structure there were a small number of organelles, moderately dilated short tubules of the endoplasmic reticulum, small mitochondria with single crystals. There were also mature fibroblasts that had considerably larger sizes of the perikaryon and processes. The area of the cytoplasm of those cells was greater, it contained developed organelles, many ribosomes, cisterns and vacuoles of the Golgi complex, rough endoplasmic reticulum, longitudinal and rounded mitochondria (Fig. 9).

The collagen fibrils and amorphous substance of the intercellular substance of the gingival connective tissue were observed around fibroblasts, in which there were no signs of edema or they were moderate. Migrated lymphocytes with well structured nucleus and nucleolus, clear nuclear membranes were detected; a considerable amount of ribosomes in the cytoplasm of these cells was found (Fig. 10).

Studies of submicroscopic organization of hemocapillaries of the mucous membrane of the gums of animals after treatment by flavanol in this period of experimental periodontitis showed that structural organization has improved significantly. In particular, the blood elements were found in moderately enlarged vascular lumens, mainly erythrocytes. The wall of the blood capillary was formed by endothelial cells and the basement membrane. Endothelial cells had an elongated shape, a thicker nuclear and a thin cytoplasmic area that had microvillus. A small number of organelles were located predominantly in the paranuclear sites of the cytoplasm. The basement membrane is clearly contoured (Fig. 11).

Significantly fewer migrating lymphocytes and neutrophils were observed in the lamina propria of the gingival mucosa, but some separate macrophages were present. Their cytoplasm had a typical structure, mostly primary lysosomes were observed, plasmalemma formed prominences, microvillus (Fig. 12).

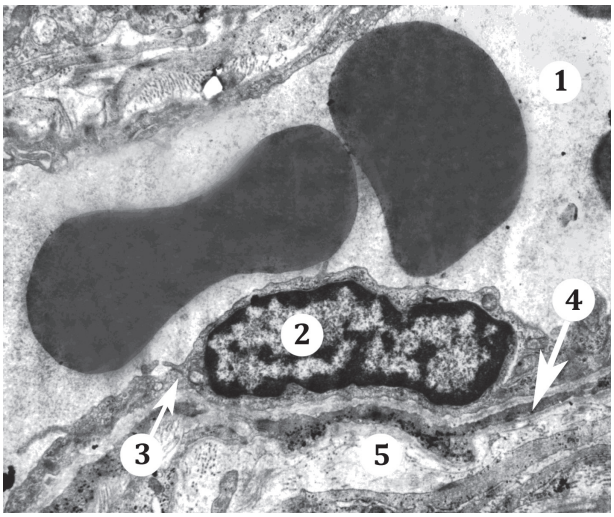

Figure 11. Ultrastructure organization of the blood capillary of the rat's gingival mucosa lamina propria with experimental periodontitis on the 14th day of research after quercetin treatment. × 7000. **Notes:** 1. the capillary lumen with red blood cells; 2. the endothelial cell nucleus; 3. the endothelial cell cytoplasm; 4. the basement membrane; 5. the perivascular space.

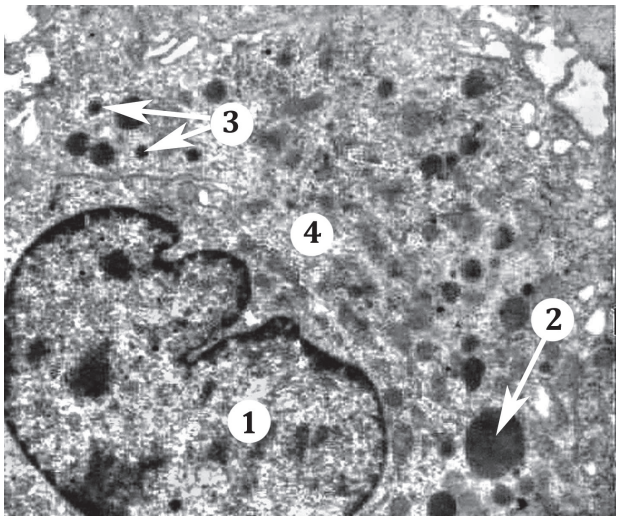

Figure 12. A macrophage fragment of the rat's gingival mucosa lamina propria with experimental periodontitis on the 14th day of research after quercetin treatment. × 14000. **Notes:** 1. the macrophage nucleus; 2. the secondary lysosome; 3. the primary lysosomes; 4. the macrophage cytoplasm.

Conclusion

Development of the inflammatory process in the periodontal complex of bacterial-immune genesis is accompanied by reorganization of all structural components of the gingival mucosa lamina propria with the most pronounced damages of vascular-tissue components, fibrous structures of the connective tissue with leukocyte-macrophage reaction in the early period (on the 7th day). The ultrastructural changes in the nucleus and cytoplasm of fibroblasts, macrophages and endothelial cells predominate in the late period (on the 14th and 30th days).

Flavanol significantly improves the ultrastructure of the hemocapillary bloodstream, the connective tissue of the gingival mucous membrane, intracellular structuration of the organelles, reduces the swelling of the amorphous component of the intercellular substance, destruction of collagen fibres, and leukocytic infiltration for the experimental bacterial-immune periodontitis.

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