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MEDICAL SICENCES

EXPERIMENTAL STUDY OF ULTRASTRUCTURAL CHANGES IN RAT BONE TISSUE FOLLOWING FIREARM AND NON-FIREARM INJURIES TO THE MAXILLOFACIAL REGION

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Abstract

Gunshot wounds remain one of the most severe types of traumatic injuries and are characterized by a complicated clinical course, a high incidence of complications, and a significant level of disability among affected patients. In the context of modern armed conflicts, the increasing availability of firearms, and the growing number of terrorist incidents, the prevalence of such injuries continues to rise, making the problem of their diagnosis and treatment particularly relevant for contemporary clinical medicine.

Despite the considerable number of studies devoted to the problem of gunshot injuries, many aspects of their pathogenesis, tissue morphological alterations, and the processes of restoration of maxillofacial structures remain insufficiently investigated. This necessitates further experimental and clinical research aimed at a deeper understanding of tissue injury mechanisms and the development of effective surgical correction and rehabilitation methods for patients.

To study ultrastructural changes in bone tissue, the following **tasks** were set:

1. To study the ultrastructural changes in bone tissue after firearm-induced jaw injury in experimental rats.
2. To study the ultrastructural changes in bone tissue after non-firearm-induced jaw injury in experimental rats.

Materials and methods

The study was conducted on seven adult Wistar rats, divided into three groups: Group I — control (intact animal); Group II — experimental group with mechanically induced injury; Group III — experimental group with firearm-induced injury.

For electron microscopic examination, fragments of bone and soft tissue from the rat jaws were fixed in a 2.5% glutaraldehyde solution prepared in phosphate buffer at pH 7.4, followed by post-fixation in a 1% osmium tetroxide solution in the same buffer. The samples were then dehydrated in a graded series of ethanol. Impregnation and embedding of the material were performed in a mixture of Epon–Araldite epoxy resins. Subsequently, ultrathin sections were contrasted according to the Reynolds method.

The results of the study revealed that on the 7th day after a gunshot bone injury, which causes significant damage to the bone and periosteal structures, a more intense inflammatory process develops, involving larger areas of tissue compared to that observed after a mechanical fracture. In these regions, edema of the connective tissue framework was detected, accompanied by the destruction of collagen fibrils, the presence of small numbers of leukocytes and erythrocytes, and the accumulation of numerous histiogenic cells. These findings indicate the presence of inflammation accompanied by the ingrowth of single microvessels into the injury zone.

At the same time, active reparative processes were observed in this region, involving connective tissue cells engaged in collagen synthesis and the restoration of connective tissue fibrils, tissue architecture, or the formation of coarse scar tissue. In the fracture zone after mechanical injury, mast cells, macrophages, and mild edema of the connective tissue ground substance were present. In the case of a fracture in an intact bone, isolated cellular elements and macrophages were found, along with activation of individual osteoblasts.

Keywords: gunshot wounds, maxillofacial area, experimental study, electron microscopy.

The problem of treating gunshot wounds and their consequences remains one of the most complex and pressing challenges in military surgery. Since the invention of gunpowder, firearms have continuously evolved, and new types of projectiles have become increasingly destructive [1]. Recent studies in wound ballistics and bone tissue regeneration have revealed the specific effects of high-energy projectiles on bone at the micro-, macro- and ultrastructural levels, as well as their potential impact on the speed and quality of healing [2,3]. However, the ultrastructural changes that occur in bone tissue in gunshot fractures have not yet been fully investigated [4,5,6].

The patterns of firearm injuries, their complications, and the course of wound-related conditions with high mortality indicate the use of projectiles with varying properties in combat. This emphasizes the importance of studying wound ballistics for accurate assessment of injury mechanisms, selection of surgical tactics, and understanding of the mechanisms of firearm damage. The use of modern combat weapons with different projectile types, high velocities, and unstable orientation in flight has led to changes in wound ballistics and an increase in injury severity [7].

Aim of the Study

The aim of the study was to investigate the ultrastructural changes in the skin and oral mucosa at early stages after firearm and non-firearm jaw injuries.

To achieve this goal, the following **tasks** were set:

1. To study the ultrastructural changes in bone tissue after firearm-induced jaw injury in experimental rats.
2. To study the ultrastructural changes in bone tissue after non-firearm-induced jaw injury in experimental rats.

Materials and Methods

The study was conducted on seven adult Wistar rats, divided into three groups: Group I — control (intact animal); Group II — experimental group with mechanically induced injury; Group III — experimental group with firearm-induced injury [8].

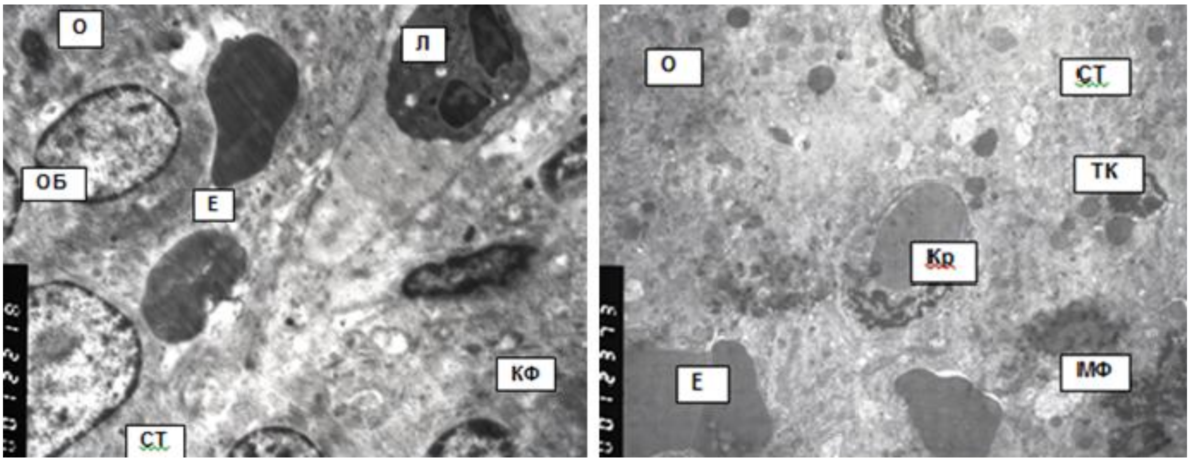
For electron microscopic examination, fragments of bone and soft tissue from the rat jaws were fixed in a 2.5% glutaraldehyde solution prepared in phosphate buffe at pH 7.4, followed by post-fixation in 1% osmium tetroxide solution in the same buffer solution. The samples were then dehydrated in graded ethanol concentrations. Impregnation and embedding of the material were performed in a mixture of Epon–Araldite epoxy resins. Subsequently, ultrathin sections were contrasted according to the Reynolds method [9].

The specimens were examined and photographed using a PEM-100-01 transmission electron microscope (Ukraine). The study was carried out in the Electron Microscopy Group of the Laboratory of Pathological Anatomy and Electron Microscopic Research.

Results and Discussion

1. Ultrastructure of the periosteum in intact rats

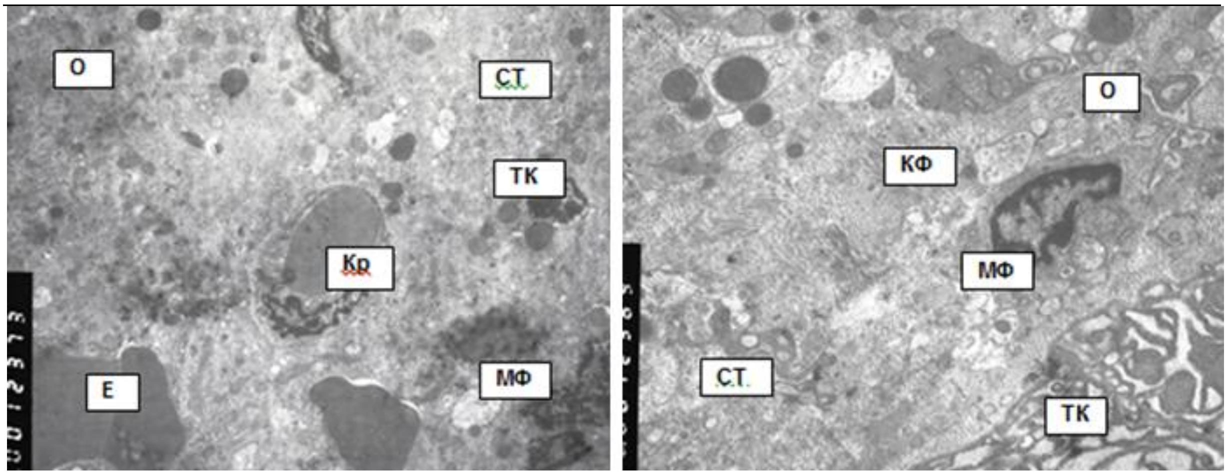
In the periosteal region, minor amorphous areas were observed in the locations of disrupted collagen fibrils, along with noticeable edema of the connective tissue ground substance. Among the connective tissue fibers, groups of osteoblasts, isolated erythrocytes, leukocytes, and macrophages were identified. In the adjacent connective tissue, osteoblasts with hypertrophied granular endoplasmic reticulum (GER) were detected, whose cisternae were filled with granular material — a characteristic sign of active collagen synthesis (Pic. 1, 2).



Pic.1,2. Ultrastructure of the periosteum and connective tissue of a rat on the 7th day after a mechanical bone fracture. ×3,000.
О — periosteum; СТ — connective tissue; ОБ — osteoblast; КФ — collagen fibrils; Е — erythrocyte; Л — leukocyte.

2. Ultrastructure of the periosteum in rats on the 7th day after mechanical bone fracture

On the 7th day after the mechanical bone fracture in rats, the periosteal connective tissue, similar to that of the intact animals, contained isolated leukocytes, erythrocytes, macrophages, and capillaries. However, in this group of animals, a significant number of mast cells, lipid inclusions, and an increased number of macrophages compared to normal were observed (Pic. 3, 4).

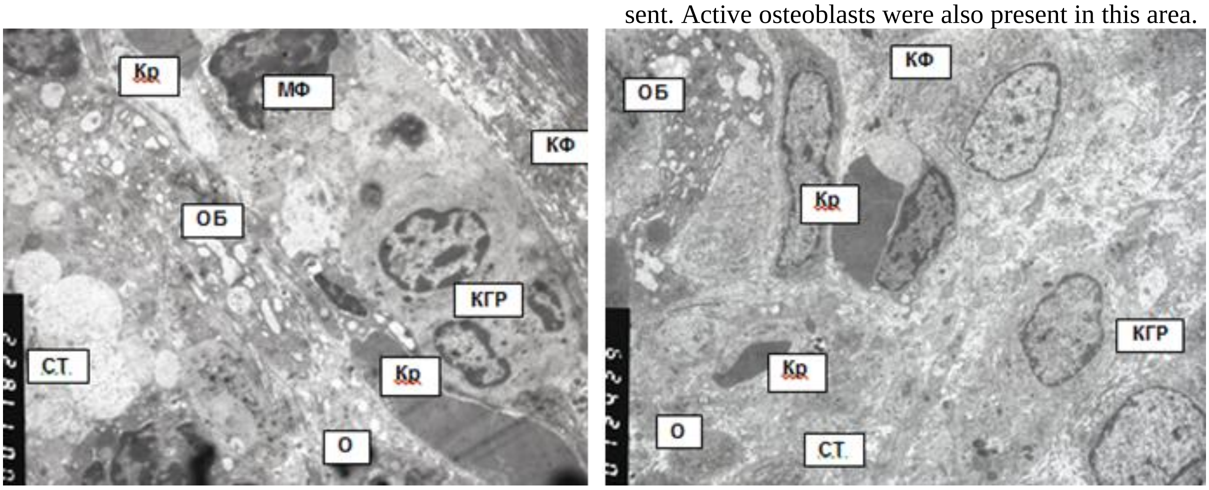


Pic.3,4. Ultrastructure of the tissue adjacent to the periosteum of a rat on the 7th day after a mechanical bone fracture. ×3,000.
О — periosteum; СТ — connective tissue; КФ — collagen fibrils; МФ — macrophage; ТК — mast cell; Е — erythrocyte; Кр — capillary.

3. Ultrastructure of the periosteum in rats on the 7th day after a gunshot bone injury

On the 7th day after the gunshot injury to the rat’s bone, the periosteal wound area exhibited regions with signs of active productive inflammation. In the markedly edematous ground substance of the connective tissue, accumulations of histiogenic cells were observed, including macrophages, mast cells, epithelioid cells, and giant multinucleated cells (Pic. 5, 6).

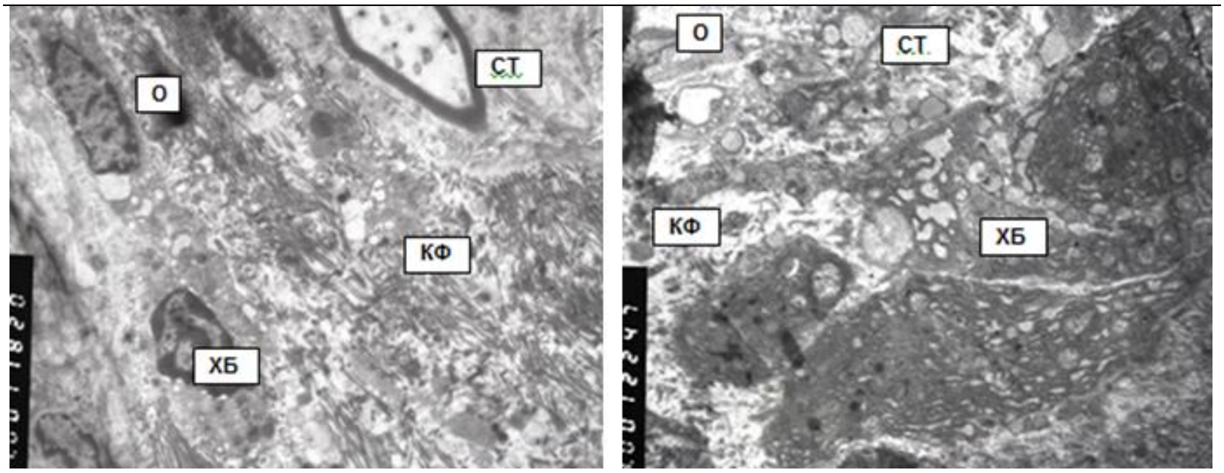
Among them, capillaries containing erythrocytes in their lumen were identified, and individual erythrocytes were also found within the surrounding tissue. However, the endothelial cells of these capillaries were in a destructive state, and the basal membrane was absent. Active osteoblasts were also present in this area.



Pic.5,6. Ultrastructure of the periosteum of a rat on the 7th day after a gunshot bone injury. ×3,000.
О — periosteum; СТ — connective tissue; ОБ — osteoblast; МФ — macrophage; КФ — collagen fibrils; КГР — histiogenic cells; Кр — capillary.

In another animal, a pronounced edema of the ground substance of the connective tissue was observed in the periosteum, along with large electron-lucent amorphous areas containing clusters of hypertrophied chondroblasts and isolated bundles of collagen fibrils between them (Fig. 7, 8).

In parallel, nearby, hypertrophied active connective tissue cells exhibiting signs of intensive collagen synthesis were also detected.



Pic.7,8. Ultrastructure of the periosteum of a rat on the 7th day after a gunshot injury. ×3,000.
О — periosteum; СТ — connective tissue; ХБ — chondroblast; КФ — collagen fibrils.

Thus, analysis of the material showed that on the 7th day after a gunshot bone injury, which causes extensive damage to the bone and periosteal structures, a more intense inflammatory response develops, affecting larger areas of tissue compared to that observed after a mechanical fracture. In these regions, edema of the connective tissue framework was observed, accompanied by the destruction of collagen fibrils, the presence of small numbers of leukocytes and erythrocytes, and the accumulation of numerous cells of histiogenic origin. These findings indicate inflammation with the sprouting of isolated microvessels within the damaged area.

At the same time, active reparative processes occur in this region, involving connective tissue cells engaged in collagen synthesis and the restoration of connective tissue fibrils, tissue architecture, or the formation of coarse scar tissue. In the fracture zone following mechanical injury, mast cells, macrophages, and mild edema of the connective tissue ground substance were observed. After the fracture of an intact bone, isolated cellular elements and macrophages were found, along with activation of individual osteoblasts.

Conclusions

1. Seven days after the gunshot injury, signs of productive inflammation were observed in the periosteum, including connective tissue edema, accumulation of numerous histiogenic cells, capillary localization among them, and activation of osteoblasts. Active bone regeneration was evident in the periosteal region.

2. Gunshot bone injury causes deeper pathological changes involving a larger area of the periosteum and adjacent soft tissues, with manifestations of productive inflammation, compared with mechanical fracture.

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