MEDICAL SCIENCES

EXPERIMENTAL STUDY OF ULTRASTRUCTURAL CHANGES IN THE ORAL MUCOSA OF RATS AFTER FIREARM AND NON-FIREARM INJURIES TO THE MAXILLOFACIAL REGION

Logai V.
Candidate of Medical Sciences
Associate Professor
Department of Traumatology, Orthopedics and Military-Field Surgery
Odessa National Medical University
Odesa, Ukraine
ORCID 0009-0007-9977-5162

ABSTRACT

Gunshot wounds are among the most severe forms of traumatic injury, characterized by a complex clinical course, a high rate of complications, and substantial disability in affected individuals. Against the backdrop of ongoing armed conflicts, widespread access to firearms, and an increasing number of terrorist attacks, the frequency of such injuries continues to grow, placing the issues of their diagnosis and treatment at the forefront of contemporary clinical practice.

Although a considerable body of research has been dedicated to gunshot injuries, numerous aspects of their pathogenesis, tissue morphological changes, and the recovery of maxillofacial structures remain poorly understood. This highlights the need for continued experimental and clinical investigation aimed at gaining a deeper insight into the mechanisms of tissue damage, as well as at developing more effective approaches to surgical correction and patient rehabilitation.

To study the ultrastructural changes in the skin and oral mucosa the following **tasks** were set:

1. To study the ultrastructural changes in the skin and oral mucosa after gunshot-induced jaw injury in an in vivo experimental rat model.
2. To study the ultrastructural changes in the skin and oral mucosa after gunshot-induced jaw injury in an in vivo experimental rat model.

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.

Results of the Study

It was established that, following a gunshot injury, the examined tissues exhibited more pronounced manifestations of productive inflammation than after a mechanical fracture. The gunshot wound affected wider areas with destructive and necrotic changes in the soft tissues of the rats compared to mechanical trauma. These alterations were characterized by an accumulation of numerous histiogenic cells, primarily macrophages and mast cells. Moreover, some mast cell granules showed signs of histamine release, suggesting a reaction to an allergic component, possibly to gunpowder residues in this experimental setting.

Additionally, after the gunshot injury, the tissues experienced extensive and severe damage, including destruction and necrosis of both connective tissue cells and collagen fibers. At the same time, the affected area demonstrated an increased number of fibroblasts actively engaged in protein synthesis directed toward collagen production and the formation of collagen fibrils compared with mechanical fracture.

On the 7th day after the gunshot injury, the soft tissues of the rats showed productive inflammation in the proliferative phase, accompanied by elements of reparative regeneration. By contrast, on the 7th day after a mechanical fracture, the intensity of productive inflammation in the soft tissues was significantly lower than that observed after the gunshot wound.

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

Relevance

The challenge of managing gunshot wounds and their long-term consequences has historically been, and continues to be, one of the most demanding and urgent problems in military and field surgery. Since the advent of gunpowder, firearms have undergone constant evolution, with successive generations of projectiles being engineered to deliver ever-greater destructive force.

Recent fundamental research in wound ballistics and bone tissue regeneration has shed light on the specific impact of high-energy projectiles on bone, revealing destruction at the micro- and ultrastructural levels, as well as a potential influence on the rate and quality of bone consolidation [1,2]. Nevertheless, the precise nature of ultrastructural changes occurring in bone tissue during gunshot fractures remains incompletely understood [3,4,5,6].

The patterns of firearm injuries, the development of associated complications, and the clinical course of wound-related conditions with high mortality rates all reflect the use of ammunition with diverse projectile characteristics in combat settings. In light of this, numerous authors stress the importance of studying wound ballistics as a prerequisite for accurately evaluating injury profiles, determining appropriate surgical strategies, and elucidating the mechanisms underlying firearm trauma. The widespread adoption of modern small arms firing various projectile types at high muzzle velocities, combined with their unstable in-flight orientation, has altered wound ballistic patterns and contributed to a marked increase in injury severity [7,8,9].

Aim of the Study

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

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

1. To study the ultrastructural changes in the skin and oral mucosa after firearm-induced jaw injury in experimental rats.
2. To study the ultrastructural changes in the skin and oral mucosa 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 [1].

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 through a graded ethanol series. Impregnation and embedding of the material were performed using a mixture of Epon–Araldite epoxy resins. Subsequently, ultrathin sections were contrasted according to the Reynolds method [2].

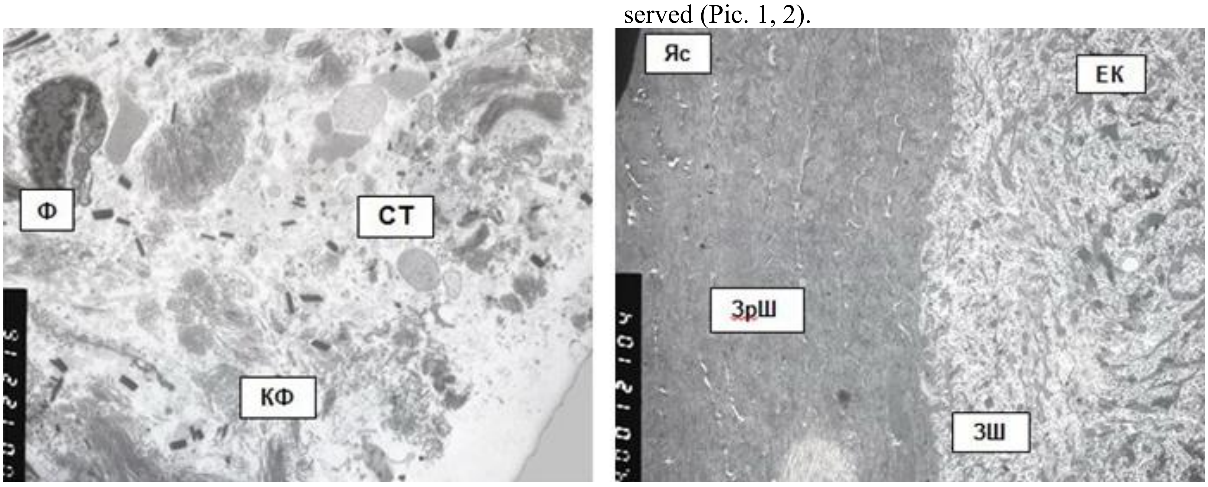
The specimens were examined and photographed using a PEM-100-01 transmission electron microscope (Ukraine). The study was carried out in the Electron Microscopy Group of the Laboratory for Pathological Anatomy and Electron Microscopy.

Results and Discussion

1. Examination of the condition of soft tissues in rats of the intact group

On the 7th day, in the connective tissue area of the skin of the intact rat, edema of the connective tissue ground substance was observed, along with a loose arrangement of collagen fibril bundles. Among these bundles, single erythrocytes, disrupted collagen fibril clusters, and spherical formations were identified, as well as fibroblasts with a well-developed granular endoplasmic reticulum, indicating enhanced collagen protein synthesis.

In the dense layer of the skin's connective tissue, slightly above the incision line, microvessels with dilated electron-lucent lumina and normal ultrastructure were found. Between tightly arranged collagen fibril bundles, large fibroblasts with evident signs of increased protein-synthesizing activity were also observed (Pic. 1, 2).

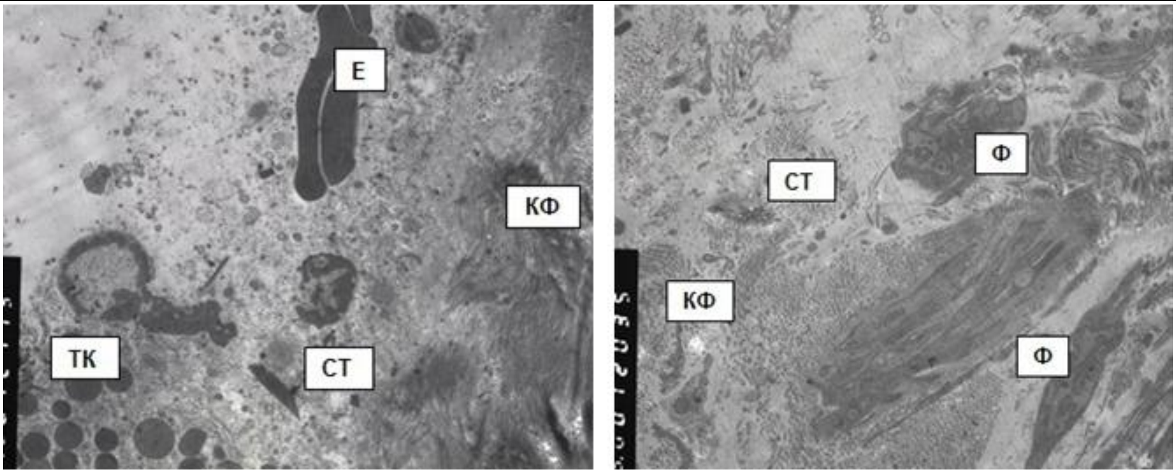


Pic. 1, 2. Ultrastructure of the connective tissue of an intact rat. ×3,000.
СТ — connective tissue; Ф — fibroblast; КФ — collagen fibrils; Яс — gingiva; ЕК — epithelial cell; ЗрШ — keratinized layer of the epidermis; ЗШ — granular layer of the epidermis.

2. Results of modeling non-firearm mechanical injury in rats

On the 7th day after mechanical injury, edema of the connective tissue ground substance was observed in certain areas of the soft tissues of the rat, along with remnants of cellular debris, isolated damaged cells, erythrocytes, and clusters of mast cells. In the fracture zone, homogenization of the collagen fibrils in the dense connective tissue was noted.

Adjacent to the fracture site, fragments of collagen fibril bundles and large electron-lucent regions containing fibroblasts actively synthesizing collagen fibrils were observed (Pic. 3, 4).

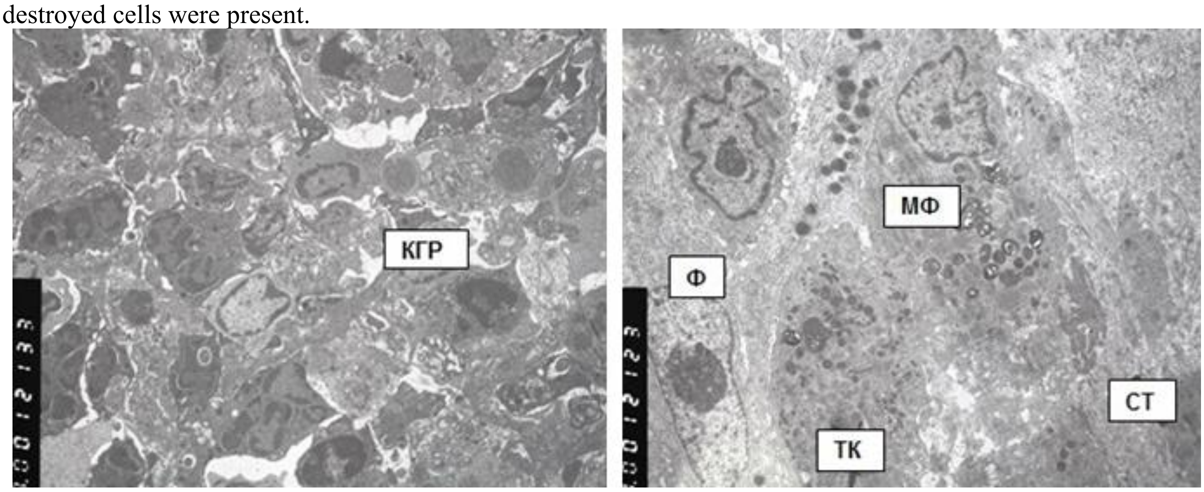


Pic. 3, 4. Ultrastructure of the connective tissue beneath the gingiva of a rat on the 7th day after mechanical injury. ×3,000.
CT — connective tissue; ТК — mast cell; E — erythrocyte; КФ — collagen fibrils.

3. Results of modeling firearm injury in rats

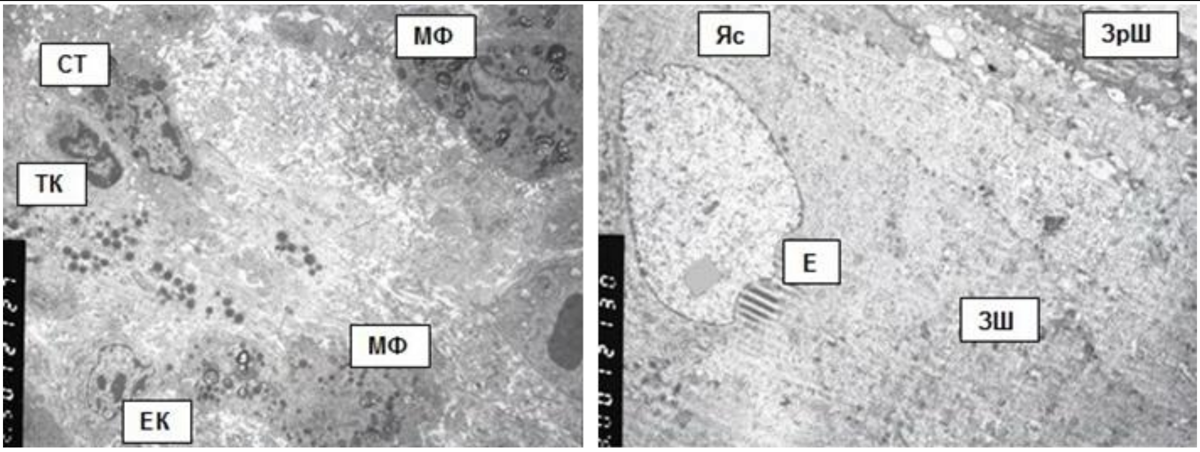
On the 7th day after the gunshot wound, accumulations of histiocytic-type cells were observed in certain areas of the soft tissue within the wound region, many of which were in a state of destruction and necrosis. Among these, leukocytes and macrophages predominated. Between them, cellular debris and remnants of destroyed cells were present.

In other areas of the gunshot injury site, leukocytes, erythrocytes, cells, and collagen fibrils in a necrotic state were identified, covering a large area. Numerous mast cells were observed in this region, some containing granules in a normal state, while others showed clear signs of degranulation (Pic. 5, 6).



Pic. 5, 6. Ultrastructure of the connective tissue beneath the gingiva of a rat on the 7th day after firearm injury. ×3,000.
КГР — histiogenic cells; СТ — connective tissue; Ф — fibroblast; МФ — macrophage; ТК — mast cell.

Deeper within the connective tissue, large macrophages, epithelioid cells, and fibroblasts were also observed, exhibiting signs of active protein-synthesizing processes directed toward the production of collagen fibrils. In the superficial layers of the skin epithelium, widening of intercellular junctions and accumulation of vacuoles within the cells were noted (Pic. 7, 8).



Pic. 7, 8. Ultrastructure of the connective tissue beneath the gingiva of a rat on the 7th day after firearm injury. ×3,000.
СТ — connective tissue; МФ — macrophage; ТК — mast cell; ЕК — epithelioid cell; Ш — skin; Е — epithelium; ЗШ — granular layer; ЗрШ — keratinized layer.

Conclusions

The comparative study of ultrastructural changes in the soft tissue (skin) seven days after firearm injury and mechanical fracture demonstrated that firearm injury causes more pronounced manifestations of productive inflammation than mechanical trauma. The gunshot wound affected wider areas with destructive and necrotic changes in the soft tissues of rats compared with mechanical fracture.

These changes were characterized by the accumulation of a large number of histiogenic cells, predominantly macrophages and mast cells. Moreover, some mast cell granules exhibited signs of histamine release, indicating a reaction to an allergic component — possibly, in this case, to gunpowder residue from the firearm discharge.

In addition, after firearm injury, the tissues underwent extensive and severe damage, including destruction and necrosis of both connective tissue cells and collagen fibers. At the same time, an increased number of fibroblasts actively engaged in protein synthesis directed toward collagen production and collagen fibril formation were observed compared with mechanical trauma.

On the 7th day after firearm injury, productive inflammation in the proliferative phase with elements of reparative regeneration was noted in the soft tissues of rats. On the 7th day after mechanical fracture, the intensity of productive inflammation in the soft tissues was significantly lower than that observed after firearm injury.

References

1. Huliuk AH. Eksperymentalna model vohnepalnykh poshkodzhen shchelep u shchuriv [Experimental model of gunshot injuries of the jaws in rats]. Certificate of copyright registration No. 119858. 2023 Jun 19.

2. Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol. 1963;17:208–212.

3. Nguyen TN, Meek G, Breeze J, Masouros SD. Gelatine backing affects the performance of single-layer ballistic-resistant materials against blast fragments. Front Bioeng Biotechnol. 2020;8:744. doi:10.3389/fbioe.2020.00744.

4. Velasco JM, Valderama MT, Margulieux K, et al. Comparison of carbapenem-resistant microbial pathogens in combat and non-combat wounds of military and civilian patients seen at a tertiary military hospital, Philippines (2013–2017). Mil Med. 2020;185(1–2):e197–e202. doi:10.1093/milmed/usz148.

5. Kim TK. T test as a parametric statistic. Korean J Anesthesiol. 2015;68(6):540–546.

6. Valentine KP, Viacheslav KM. Bacterial flora of combat wounds from eastern Ukraine and time-specified changes of bacterial recovery during treatment in Ukrainian military hospital. BMC Res Notes. 2017;10(1):152. doi:10.1186/s13104-017-2481-4.

7. Zarutskyi YaL, Khomenko IP, Verba AV, Burluka VV. Boiova khirurhichna travma. Voienno-polova khirurhiia [Combat surgical trauma. Military-field surgery]. In: Zarutskyi YaL, Bilyi VYa, editors. Kyiv: Feniks; 2018. p. 45–59.

8. Mishalov VD, Mykhailenko OV, Khokholieva TV, Petroshak OYu. Sudovo-medychna ekspertyza obiektiv pry vohnepalnii travmi: monohrafiia (vydannia dopovnene) [Forensic medical examination of objects in gunshot injury: monograph (expanded edition)]. Kyiv; 2019. 303 p.

9. Shcherbak VV, Tolmachev OO, Kundius OV, Abdurasulov AA. Metodolohiia provedennia balistychnoho eksperymentu na biolohichnykh imitatorakh tila liudyny [Methodology for conducting ballistic experiments on biological human body simulators]. Kryminalnyi Visnyk. 2015;24(2):131–132.