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STRUCTURAL AND FUNCTIONAL FEATURES OF HEALING OF TIBA FRACTURES USING APIPHONOPHORESIS

A. I. Gozhenko[1], K. V. Pavlenko[1], V. Yu. Chivga[2]

[1]Ukrainian Research Institute of Transport Medicine
[2]International Humanitarian University

Abstract

Modernity is defined as an "epidemic of trauma". Every year, up to 2 million adults and 300 thousand children, residents of Ukraine, receive various injuries. Since injuries and their consequences are the cause of disability in 89% of the working population, the problem of trauma, including bone fractures, becomes not only a medical but also a social problem. In this regard, the treatment of injuries in general and fractures in particular remains very relevant.

The aim of the work was to compare the results of restorative treatment of fractures of tubular bones using apiphonophoresis with a variable dose of bee venom and apiphonophoresis with a fixed dose of "Apizarthron". The materials of this study were the data obtained in the study of 60 white male rats of the Wistar line of outbred breeding, weighing 180-200 g.

Our studies have established that apiphonophoresis, performed in a course, accelerates the process of restoring bone integrity in fractures. This is due to the increase in the activity of osteocytes, periosteocytes, and the protein-synthesizing functions of these cells.

Key words: fracture; bee venom; phonophoresis.

Introduction. Modernity is defined as an "epidemic of trauma". Every year, up to 2

million adults and 300 thousand children, residents of Ukraine, receive various injuries [1, 3]. Since injuries and their consequences are the cause of disability in 89% of the working population [3], the problem of trauma, including bone fractures, becomes not only a medical but also a social problem. In this regard, the treatment of injuries in general and fractures in particular remains very relevant

Traditional fracture treatment technologies are quite time-consuming, so in addition to increasing economic costs, the possibility of complications increases. That is why there is a need to develop technologies that would not only improve the quality of medical services, but also accelerate the regeneration process. One of the directions of such research may be the use of natural healing resources. An effective and fairly common natural factor that can stimulate regeneration is bee venom [1, 3, 5, 7, 8]. In our previous works [6] we have established that ultraphonophoresis with a variable dose of native bee venom not only accelerates the healing of fractures of tubular bones, but also makes possible the healing of complicated, variable fractures. However, this technology has some complications: collection, purification, storage of the venom in conditions preserving its medicinal qualities; dosage of venom in the manufacture of its oil mixtures; difficulty in taking into account the dose of venom during each procedure.

Based on the above, the aim of our work was to compare the results of restorative treatment of fractures of tubular bones using apiphonophoresis with a variable dose of bee venom and apiphonophoresis with a fixed dose of "Apizarthron".

Materials and methods

The materials of this study were data obtained from the study of 60 white male Wistar rats of outbred breeding, weighing 180-200 g. According to the objectives of the work, the rats were ranked into 3 groups. Group I (20 rats) consisted of animals that had a fibula fracture and observed its healing without external interventions. Group II (20 rats) consisted of rats that underwent phonophoresis with bee venom from the second day of the fracture. The course of apiphonophoresis consisted of 9 procedures that were performed every other day, the duration of the procedure was from 1 to 3 min. power flux density 2 W/sec2; dose of venom 1-3 procedure 0.3 mg (1 bee); 4-6 - 0.6 mg (2 bees); 7-9 - 0.9 mg (3 bees). Group III (20 rats) consisted of rats that underwent a course of phonophoresis with "Apizarthron" from the second day of the fracture. The number of procedures per course was 9 every other day, the duration of the procedure was 1 - 3 min.; power flux density was 2 W/sec2, the dose of poison was 0.3 mg.

A fibula fracture was simulated under non-butalic anesthesia by transecting the bone

with side cutters, after which the wound was covered with streptocide and sutured tightly. The condition of the injured limb was visually assessed (swelling, position, use of the limb). In the first group, animals were withdrawn from the experiment on days 3, 7, 14, and 21 after the fracture. In groups II and III, on days 7 and 14.

The animals were removed from the experiment by decapitation under Nembutal anesthesia. The experimental paw was removed, after fixation in 4% paraformaldehyde, decalcification was performed with 5% nitric acid. After that, the tissues were passed through alcohols of increasing concentration and embedded in celloidin. From the blocks obtained, we made microtome sections 7 μm thick, which were stained with hematoxylin-eosin and Van Gieson. The preparations were examined using a light microscope with an assessment of the condition of the periosteum, vessels, the regeneration variant, and the density of the bone substance.

Work with animals was carried out in accordance with the International Code of Medical Ethics (Venice, 1983), the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), and the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), Directive 2010/63/EU of European Parliament and Council on the protection of animals used for scientific purposes, Law of Ukraine "On the Protection of Animals from Cruelty" No. 440-ИХ dated January 14, 2020.

Results. Discussion

Observation of rats with untreated fractures of the tibia 3 days after the start of the experiment showed that they do not use the operated paw when moving around the cage. The damaged limb is enlarged in diameter, its temperature is elevated, the wound is protected by a dry scab, in some animals 1-2 drops of serous fluid are released from under the scab. Later, on the 7th and 14th days of the experiment, the volume of the limb gradually decreases, its temperature normalizes, the rats begin to use it episodically. On the 21st day after the fracture, the rats use the limb to its full extent, its volume and temperature do not differ from the healthy limb. An epithelialized scar forms at the site of the surgical wound.

In cases where apiphonophoresis of the damaged limb was performed, on the 3rd day of the experiment, the rats used it episodically, the volume of the limb was increased, the temperature parameters were similar to the undamaged limb. The wound was covered with a dry scab. After 7 days, in rats of groups II and III, the volume and temperature indicators of the experimental limb were equal to the similar parameters of the healthy limb. The rats used the limb when moving around the cage quite actively. The wound was almost completely

epithelialized. After 14 days after the start of the experiment and 7 sessions of phonophoresis, the appearance and volume of movements of the experimental limb fully corresponded to the healthy one. No peculiarities in the course of fracture repair in rats depending on the dosage of bee venom were established.

Histological studies performed in the process of untreated fracture established the following. On the 3rd day of the experiment, the soft tissues around the fracture are edematous and dispersed. In them, as well as in the fracture zone and bone cells near the fracture, an accumulation of lymphocytes is observed. In the fracture zone, eosinophilic inclusions are observed. The periosteum is swollen and widespread. On the 7th and 14th days of observation, the edema decreases and disappears gradually. The composition of cells in the fracture zone changes. On the 7th day, the presence of histiocytes is determined, which on the 14th day become the only type of cells observed in the fracture zone. These cells, together with eosinophilic homogenate and protein inclusions, fill the entire fracture space. In addition, the fracture zone from the fragments includes "tongues" of dense bone substance, in which separate nests containing 1-2 osteocytes with large light-colored nuclei are determined. On the 14th day of the experiment, these "tongues" filled the fracture space and connected both fragments. Histiocytes were observed in the bone foci located next to the fracture. On the 21st day after the fracture, there was no swelling and infiltration of tissues around the fracture, but there was a coupling of fibrous fibers. The dense substance of the bone is continuous, with numerous nests, in which 2-4 osteocytes with large nuclei are observed. The bone canal is narrowed and there are disordered beams in it. The histological picture of the fracture in rats that received a course of apiphonophoresis was different from the above. 7 days after the fracture (3 sessions of apiphonophoresis with an increasing dose of poison) a slight swelling with single lymphocytes is observed around it. In the periosteum there are cells with enlarged, juicy-colored nuclei. The dense substance of the bone is continuous, in the fracture zone it has a bright eosinophilic color, at a distance it has a terracotta color. Numerous cells (osteocytes) enter this substance from the periosteum.

On the 14th day of the experiment (3 sessions of phonophoresis with 0.3 mg of bee venom and 3 sessions with 0.6 mg of venom), the tissues around the fracture are of normal appearance. The periosteum is solid, the cells in it have enlarged nuclei. The dense substance of the bone is solid of normal color with a small number of nests of osteocytes of normal appearance. In the bone canal, massive outgrowths extend from the dense substance, which elsewhere merge into fields. The outgrowths consist of fibrous fibers, homogeneous substance and nests of osteocytes. In the cavity of the canal, there are accumulations of histiocytes (Fig.

1).

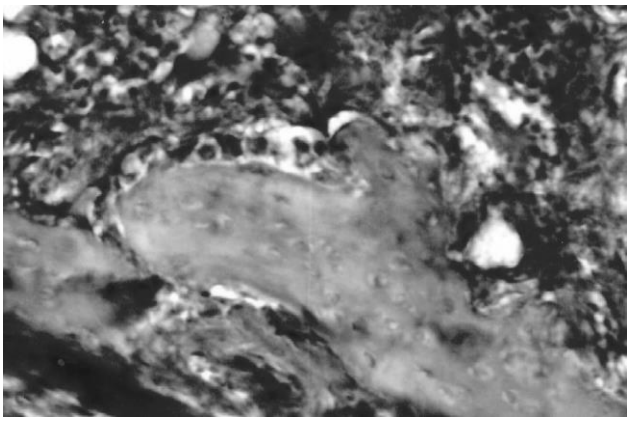

Fig. 1. Rat tibia. Fracture area treated with phonophoresis with varying doses of bee venom. Outgrowth from dense substance in the cavity of the bone canal with fibrous fibers and osteocytes. Around the accumulation of histiocytes. Van Gieson. x200.

Histological studies of the bone in rats that received a course of phonophoresis with "Apizarthron" (a fixed dose of bee venom) from the second day of the fracture did not reveal significant differences from the picture observed in rats with a change in the dose of venom (Fig. 2).

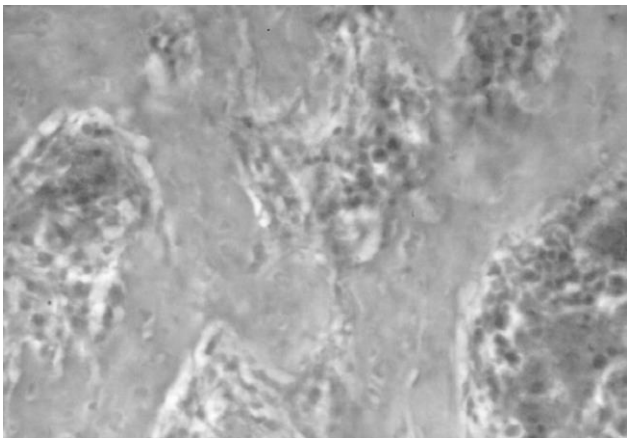

Fig. 2. Seventh day of fibula fracture in rats. Phonophoresis with "Apizartron". Beams entering the fracture zone, fibrous fibers of histiocytes. Van Gieson. x200.

7 days after the fracture and 3 sessions of apiphonophoresis, a slight edema of the surrounding tissues and the presence of single lymphocytes are observed. In the periosteum, the cells are disorganized, their nuclei are enlarged and moderately colored. Fibrous fibers are present. Between the fragments, merging with them, a homogeneous moderately eosinophilic mass is located. From the side of the bone canal and periosteum, histiocytes and round-

nucleated cellular elements penetrate this mass. In addition, "tongues" penetrate this mass of homogeneous substance from the side of the dense bone substance of the fragments, which consist of coarse fibers, in which nests of osteocytes (2-3 cells) and individual osteons are located. The sizes of these "tongues" are different.

On the 14th day of the experiment (6 sessions of phonophoresis with "Apizartron"), the tissue around the fracture does not show the usual appearance of swelling or the presence of lymphocytes. The periosteum and dense bone tissue are continuous. Beams of coarse fibrous fibers, nests of osteocytes, and areas of dense bone substance enter the bone canal from it. Osteocytes with enlarged, pale-colored nuclei. The beams intersect randomly.

Conclusions

Thus, our studies have established that apiphonophoresis, performed in a course, accelerates the process of restoring bone integrity in fractures. This is due to the increase in the activity of osteocytes, periosteocytes, and the protein-synthesizing functions of these cells.

1. As a result of activation, fracture closure occurs on the 7th day of the process and bone integrity is restored on the 14th day of the process.
2. The nature and duration of the fracture healing process does not depend on the increase or stability of the dose of bee venom. We believe that the bioactive components of bee venom act stably and this action is not dose-dependent.

The obtained data determined the main mechanisms of the biological action of bee venom on the processes of osteogenesis. Considering that the absence of dose-dependence of the action of bee venom was established, further developments may help improve medical technologies for accelerating the healing of bone tissue defects, which would eliminate complications associated with the peculiarities of the action of bee venom.

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