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Comminuted tibial fracture healing by the use of the mineral complex Micellate (experimental study)

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Purpose Experimental study of the effect of *Micellate* on the processes of reparative regeneration on the model of comminuted tibial fractures. **Materials and methods** The paper analyzes the results of studies carried out on 20 mongrel dogs in whom comminuted tibial fractures were modeled. To stimulate osteogenesis, a complex of minerals called *Micellate* was used. **Results** It was found that *Micellate* acts selectively and restores the lack of the bone tissue mineral component. The regenerate remodels into an organotypic bone in a shorter time. **Conclusion** After a detailed study of the mechanisms of action and clinical trials, the preparation might find application both in the correction of osteopenia and osteoporosis that develop in the process of fracture consolidation, and for the prevention of fractures caused by the loss of the mineral component of bone tissue as a result of metabolic disturbances.
Keywords: tibia, comminuted fractures, osteogenesis, stimulation, Micellate

INTRODUCTION

The steady increase in the intensity of human life due to the rapid development of technological progress inevitably leads to a growth in the number and severity of injuries to the skeleton. Among such injuries, a special place is taken by comminuted fractures accompanied by the formation of several bone fragments with a significant destruction of the medullary canal content. They represent great difficulties in terms of reduction and fixation [1, 2]. One of the negative factors in their case is a sufficiently long period of consolidation, which in turn, in any variant of osteosynthesis, limits the regular adequate load on the limb that results in a loss of bone minerals, calcium and other elements, and metabolic disorders [3, 4, 5]. When an adequate load recovers, bone mineral content restores. However, this does not always happen. Even with a successful, at a first glance, consolidation, bone quality may be significantly affected [6].

In view of the foregoing, there arises an issue of how to correct the condition accompanied by a decrease in the mineral bone tissue component both at the consolidation stage and in the long-term postoperative period. To date, a large number of drugs and biologically active additives have been developed and used to correct osteopenia and osteoporosis of any genesis [7]. However, the problem cannot be completely solved with them due to both objective and subjective reasons. Decrease in the consumption of dietary calcium by the population, an imbalance in the composition of preparations, the forms of the elements in medical and dietary supplements with their low-grade digestibility (only 5-10 %) cannot compensate for the needs of the body. All this necessitates the search for new approaches to solving this issue. One of the new generation supplements that provide maintenance of the organism's mineral homeostasis is a group of preparations of carbonate salts of various minerals in the micellar form (microparticles measuring 10^{-8} or less with ultrahigh sorption capacity) produced under the general name *Micellate*.

The study was **aimed** at the experimental investigation of the Micellate effect on reparative regeneration on a canine model of comminuted tibial fracture repair.

MATERIAL AND METHODS

Two series of experiments (control and pilot) were performed using 20 mongrel dogs of both sexes aged from 2 to 10 years. The *Micellate* preparation was used in two forms: O (micellate of calcium carbonate with salts of elements in the proportions inherent in sea water) and A (micellate of calcium carbonate, additionally enriched with magnesium, iron, zinc, chromium in microdoses). The animals of the pilot series were prescribed the preparation according to the protocol:

a) drops under the tongue: 2–3 drops of brand A, twice a day in the morning and in the evening 15–20 minutes before the feeding during the first 15 days of fracture management;

b) supplement to drinking water: 5–7 drops of brand A per 1 liter of drinking water thoroughly mixed, administered throughout the entire experiment, starting from the first day;

c) applications: 3–4 drops of brand O applied to the

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wet surface of the skin (cleared from the hair) in the fracture region, then the site was wrapped in a plastic cover for 15–20 minutes; remnants of the solution after the application were removed with a damp cloth. The course was 10 days; drops were applied twice a day during the first five days and once a day in the last five days.

d) treatment of wire insertion points: one drop of grade A applied to the entrance and exit points of each wire immediately after its drilling, and further on once a week for a month.

In the pilot series, antimicrobial agents were not used. In the control series, the animals were treated according to the generally accepted method: antibacterial therapy, treatment of the wire entry and exit points with antibacterial agents.

In dogs of both series, control (n = 10) and pilot (n = 10), impacted open tibial fractures with large and small fragmentation and complete displacement of fragments were produced with the help of a special device. Then the limb was splinted with a cast.

One day after the fracture, in the conditions of the operating room, a closed transosseous osteosynthesis of damaged bones was performed in anesthetized animals using the Ilizarov apparatus consisting of four supports. Each fragment (proximal and distal) was fixed with two pairs of crossed wires attached under tension to the corresponding supports. In all cases, a complete reduction of the fracture was achieved. Maintenance, feeding, care and euthanasia of the experimental animals were carried out in accordance with the European Convention for the Protection of Vertebrates Animals used for experimental and other scientific purposes.

Clinical, radiologic, histological and statistical methods of investigation were used in the work.

Clinical examination of the animals was carried out daily during the whole period of the experiment. Their general health, limb function, and the state of soft tissues in the fracture zone were assessed. The volume of soft tissues of the injured extremity was measured with a measuring tape and by the formula ($L= \pi \times (Dl + D2) / 2$, where $\pi = 3.14$, and D1 and D2 is the thickness of soft tissues of the tibia in AP and lateral projection determined by radiographs.

Radiographic studies were performed on the day of the operation, and on days 7, 14, 21, 28, 35, 42, 49, 65, 90 and 120 after surgery.

Histological studies were carried out using the light microscopy method with a large microscope (Opton, Germany). The preparations were stained with hematoxylin and eosin and Van Gieson.

RESULTS AND DISCUSSSION

The next day after the fracture modeling, limb swelling was observed in all the animals. The volume of soft tissues grew from 4 to 11 %. In both series, the maximum values were observed during the first seven days after the operation, gradually decreasing to the initial state in the control series by the 21st day of fixation and in the pilot series by the 14th day. Subsequently, atrophy of soft tissues developed and was observed in both series until the removal of the apparatus. It gradually recovered to the preoperative values by the period of three months after the apparatus removal. The limb support function recovered by the end of the first week after the operation and was retained for the entire experiment period.

Formation of crusts was observed around the wires by local application of the Micellate, more pronounced in the area of the wires closer to the fracture site. After their removal, the skin was practically unchanged, of normal color and tenderness. With regard to the healing of wound surfaces, this process took longer with the use of Micellate than with the standard therapy.

X-ray signs of fracture union in both series appeared by the 14th day of the experiment. However, there were no signs of bone fragments resorption by using Micellate in most cases while in the control dogs they were quite clearly seen.

After 30 days of the experiment, radiographs in the pilot series showed shadows of the regenerate in the fracture zone which approximated the intensity of the medullary cavity. The fragments were bridged by periosteal shadows. There were no signs of resorption in bone fragments (**Fig. 1 a**). At this stage, the apparatus was dismounted after a clinical trial and upon evaluation of the regenerate consistency.

In the control series, the fracture line was well visualized radiographically. There were extensive periosteal shadows on the fragments, from 2 to 7 mm wide, extending for 30-50 mm from the fracture zone, but not overlapping it. The union happened only after 49 days of fixation (**Fig. 1 b**).

Depending on the fracture type, bone union in the pilot series completed as follows: type A3 (transverse) after 21 days; type B2 (wedge-shaped) after 30-35 days, type C2 (segmental) after 35-49 days. In the control series, union of types B and C fractures occurred not earlier than 49 days. The average consolidation time in the pilot series was 40.1 days while in the control group it continued 49 days.

A month after the removal of the apparatus (**Fig. 2**), in both series of experiments, the fracture line on the X-rays was practically not seen. There were dense ampoule-like thickenings of the periosteum on the tibia (periosteal regenerate). The density of the regenerate was close to the density of the fragments.

In general, the radiographic manifestations were similar in both series. However, the total duration of the experiment in the pilot series was 19 days shorter.

Histological study at the light-optical level revealed certain differences between the pilot and control series. Thus, in the control series, the fragment union was of bony-fibrous-connective tissue-cartilaginous type at the end of the fixation period (**Fig. 3 a**). In the intermediate region of the regenerate, hyaline cartilage with sections of fibrous connective tissue prevailed (**Fig. 3 b**).

The periosteal region was represented by areas of

medium-size woven cancellous bone interrupted by small islands of fibrous connective tissue (**Fig. 3 c**). The periosteal strata formed ensured the organics of fragmental bridging. In the endosteal region, areas of loose fibrous connective tissue, fibrous and hyaline cartilage, as well as big-size woven cancellous bone were observed (Fig. 3 d). In the intertrabecular spaces, a gelatinous and red bone marrow continued to form by this period. Significant porosity of bone fragments was noted. The periosteal bone strata extended to 1.7-3.2 cm along the fragments in the proximal and distal directions.

In the pilot series, at the end of the fixation period, bone or predominantly bone union was observed in the fracture zone, with small portions of fibrous and cartilaginous components that was ensured by the junction of the periosteal strata and the formation of endostal bone callus (**Fig. 4 a**). By complete bone union, a compact small-sized woven spongy bone was observed in the intermediate zone (**Fig. 4 b**), a spongy bone of a fine and medium-size woven structure in the periosteal region (**Fig. 4 c**). The endosteal region of the regenerate consisted of a network of trabeculae formed by the reticulofibrous bone tissue. In the intertrabecular spaces, the red bone marrow continued to be formed (**Fig. 4 d**).

By the predominantly bone union formation, the periosteal envelope was represented by a small- and medium- size woven spongy bone with the inclusion of hyaline cartilage. In the intermediate zone, a large and medium-sized woven network of trabeculae of the reticulofibrous structure was defined. In the endosteal parts, rough fibrous bone trabeculae were formed with small areas of loose connective and cartilaginous tissue.

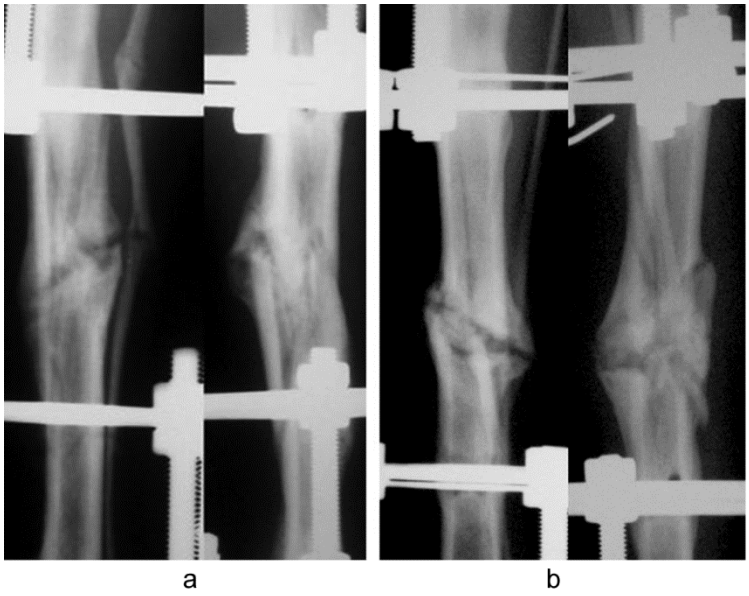

Fig. 1 Fragments of the radiographs of tibial bones of dogs at the end of the fixation period: a) 30 days fixation in pilot series, b) fixation for 49 days in control series

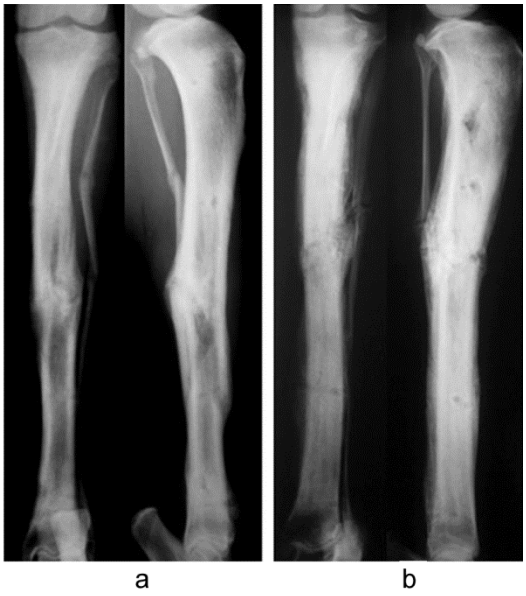

Fig. 2 Roentgenograms of the canine tibiae: a) pilot series, fixation for 30 days and without apparatus 30 days (total duration of the experiment 60 days); b) control series, fixation for 49 days and without apparatus 30 days (total duration of the experiment 79 days)

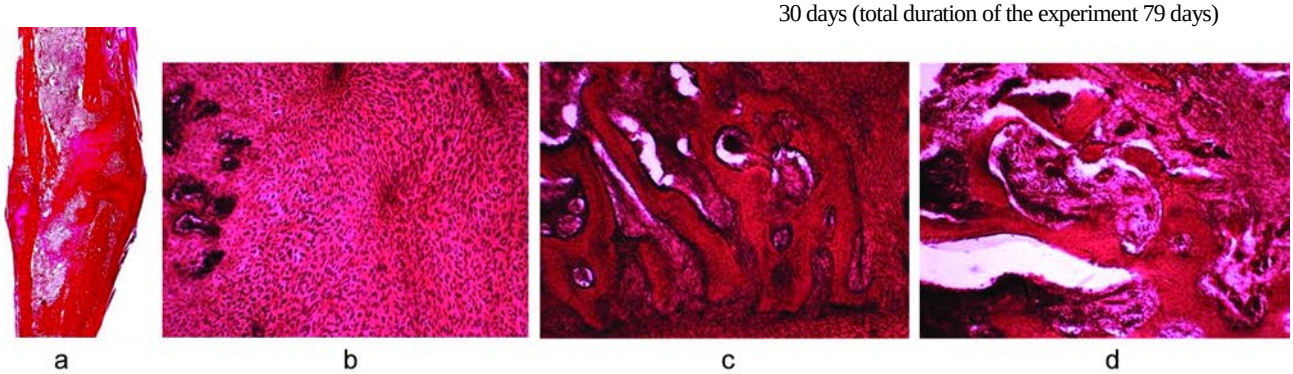

Fig. 3 Features of the histostructural composition of the diaphysial regenerates in the control series at the end of the fixation period: a) histotopogram of the longitudinal section of the regeneration of the tibial diaphysis (control). Van Gieson staining, magnification = 1.5; b) areas of fibrous and hyaline cartilage in the intermediate region; c) periosteally formed reticulofibrous bone tissue; d) areas of gelatinous and red bone marrow in intertrabecular spaces. Staining with hematoxylin and eosin, magnification: b – 100 ×; c, d – 40 ×

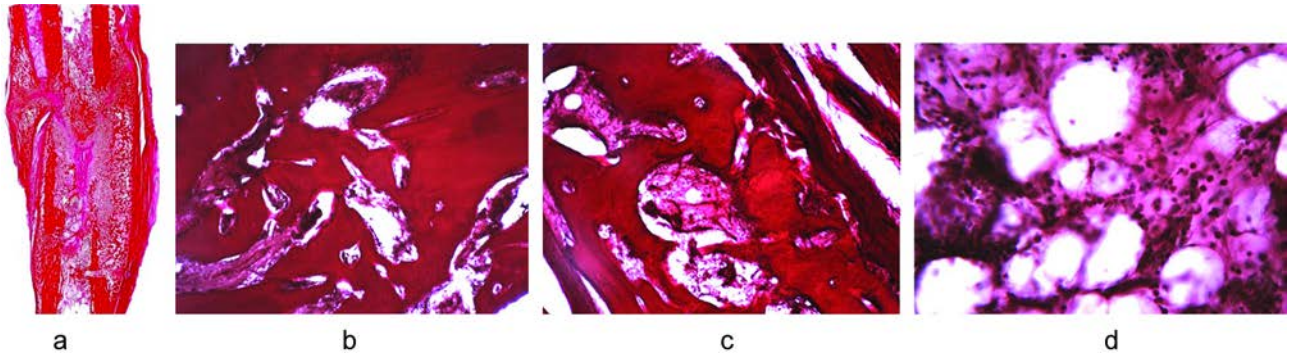

Fig. 4 Features of the histostructural composition of the diaphysis regenerates in the pilot series by the end of the fixation period: a) histotopogram of the regeneration of the tibial shaft; b) medium-size woven spongy bone in the intermediate region; c) periosteally formed rough fibrous bone tissue; d) area of red-and-yellow bone marrow in the bone marrow cavity of the injury zone; a – Van Gieson staining; magnification × 1.5; b, c, d – staining with hematoxylin and eosin; magnification: b, c – 40 ×, d – 100 ×

Thirty days after removal of the apparatus in the control series, in all the cases the formation of a continuous cortical plate and bone marrow cavity began (**Fig. 5 a**). It should be noted that a marked porosity of the cortical plate was noted in all the cases. The intermediary zone consisted of sections of fine and medium-size woven spongy bone (**Fig. 5 b**). The bone callus, formed in the intermediate and periosteal areas, included bone splits and fragments. Their gradual resorption, in turn, induced the process of osteogenesis in the adjacent areas. The bone marrow cavity in the union zone was filled with a red-and-yellow bone marrow with fragments of a rare trabecular network, capillaries of the sinusoidal type, full-blooded arteries of a small diameter, venules and arterioles.

In the pilot series, bone consolidation (**Fig. 6 a**), formed by a compact lamellar bone tissue, was observed between the fragments in this period (**Fig. 6 b**). The cortical plate was continuous at all extension. The bone cavity was filled with a yellow bone marrow with red marrow foci in the fracture zone (**Fig. 6, c, d**).

Three months after removal of the apparatus, in the control series animals, the area of the injury was represented by bone tissue which was at the stage of organotypic rearrangement, with a cortical plate of different degree of maturity and discontinuous in several areas (as a result of resorption of loose fragments). On the endosteal surface, narrow chains of trabeculae undergoing osteoclastic resorption were detected.

In pilot series animals, the continuous cortical plate had a lamellar structure. The consolidation zone was represented by the bone tissue of the organotypic structure.

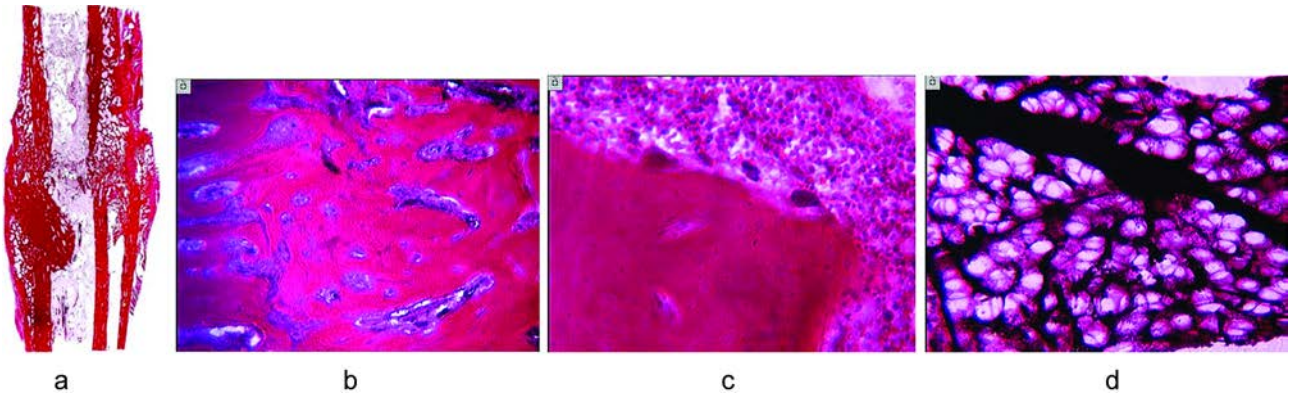

Fig. 5 Features of the histostructural composition of the diaphysis regenerates in the control series (I) 30 days after removal of the apparatus: a) histotopogram of the longitudinal sawn section of the regenerate of the tibial diaphysis; b) fine-sized woven spongy bone in the intermediate zone of the regenerate; c) osteoclastic resorption of bone fragment surface; d) red-and-yellow bone marrow and expanded microvessels in the medullary cavity in the fracture union zone; a - Van Gieson staining; b, c, d – staining with hematoxylin and eosin; a – magnification 1.5 ×; b – magnification 25 ×; c – magnification 165 ×

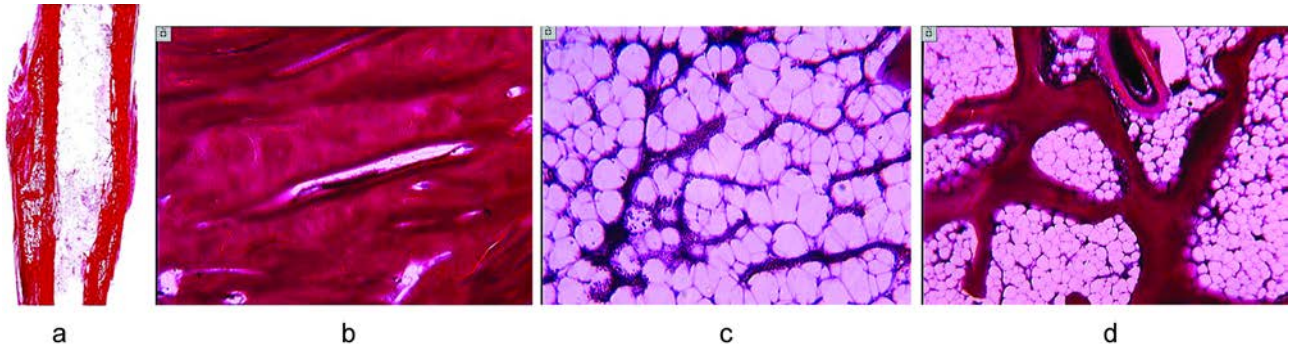

Fig. 6 Features of the histostructural composition of the diaphysis regenerates in the pilot series (II), 30 days after removal of the apparatus: a) histotopogram of the longitudinal section of the regeneration of the tibial diaphysis; b) newly formed lamellar bone in the intermediate region of the regenerate; c) yellow, with small areas of hematopoiesis, bone marrow formed in the medullary canal in the projection of the fracture; d) single bone trabeculae in the medullary cavity; a – Van Gieson staining; b, c, d – hematoxylin and eosin; magnification: a – 1.5 ×; b, c – 165 ×, d – 40 ×

CONCLUSION

The complex studies conducted by us confirm the expediency of using the preparation Micellate for healing of comminuted fractures of tubular bones. A balanced complex of minerals in an easily acquirable form acts selectively and substitutes the lack of a mineral component of the bone tissue precisely there when there is a need for it. After a detailed study of the mechanisms of action and clinical trials, the preparation might be used both for correction of osteopenia and osteoporosis that develop in the process of fracture consolidation (resorption of the ends of fragments and freely lying fragments), and for prevention of fractures caused by the loss of the bone mineral component due to metabolic processes disorders, especially in the elderly persons.

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