STIMULATION OF REPARATIVE PROCESSES IN BONE DEFECTS IN SHEEP USING AD TYPE BIOPHYTOMODULATORS


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Summary

Healing of bone lesions occurs in a longer period compared to soft tissue. Bone regeneration can be finalized as early as 5-6 weeks unless disturbance occurs. In our experiment we sought to histologically evaluate the stimulation of bone healing using AD biophytomodulators (DIEE), in tibial bone defects in sheep. We caused experimental tibial bone defects in Turcana breed ewes, aged one year, at three of them we applied biophytomodulators to the skin and three of them were the control group. We then harvested tissue from the defect after 7, 14 and 21 days. There were made histological slides stained by Goldner’s Trichrome method and examined under an optical microscope. The results obtained reveal the presence of newly formed bone tissue at 14 days for treated samples, at 21 days the bone tissue was more extended, while at the witness there was identified only healing based on connective tissue.

Keywords: sheep, bone stimulation, biophytomodulator.

Introduction

Bone wound healing is achieved through osteogenesis, a stadal process that is triggered immediately, the mechanical injury generating impulses to the cortex, which will command changes that occur in the fracture site.

Fracture repair is done through formation of new tissue in the fractured area, with subsequent appearance of a callus, which originates in the periosteum and to a lesser extent in endosteal cells. Periosteum and endosteum respond by increased proliferation of osteoprogenitor cells and in internal osteogenic areas, fibroblasts together with blood capillaries proliferate and invade the clot forming granulation tissue that will unite the bone fragments (Pușcașiu Dana et al., 1999). Angiogenesis is an essential phenomena for encondrale ossification (Hausman et al., 2001).

Osteoprogenitor cells will differentiate in the less vascularized surface areas in chondroblasts that will build a hyaline cartilage. In deep areas in the vicinity of the vascularized bone, the osteoprogenitor cells differentiate in osteoblasts that will produce the bone matrix. This way, a temporary osteocartilaginous callus is organized and it will gradually be replaced by bone tissue, which will become the permanent bone callus (Pușcașiu Dana et al., 1999). Calcium salts that impregnate the fracture callus come from decalcification of fractured bone fragments and through blood circulation from dietary intake (Wilson, 2002). The cause of bone fragments decalcification is an active hyperemia of vessels in the fracture site (Crisan et al. 1957).

The callus forming process may be influenced by using different methods of stimulation that lead to shortening of the period of bone healing. One such method is using type AD biophytomodulators. DIEE (Device for energetic loading and balancing) was patented by physicist Ancu Dincă. Quantifying of these processes can be done through various methods, the histopathology being most reliable.

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Materials and methods
The study included six, one year old, ewes, Țurcana breed, from a single source, and so benefited from the same growing conditions and care. They underwent experimental tibial bone defects of the same size (Fig. 3-4) on the tibial crest (Fig. 1.) Surgeries were conducted under general anesthesia and removal of bone fragments was performed using a drill (Fig. 2.).

Suture was performed in two layers: periosteum and muscle in continuous suture using absorbable material, chromic catgut and the skin was sutured in separate points using nonabsorbable material, surgical silk. After suture, incisions were sprayed with spray containing oxytetracycline.

After the surgeries, the animals were randomly divided into two groups: group I (control) and group BF. Animals in group BF were applied, laterally to the incision one DIEE biophytomodulator, fixed in 4 points with nonabesorbable material (Fig. 5.). After surgery, both groups were housed, cared for and fed identically.

Evaluation of results was done by harvesting the callus in the defects, with histological examination done at 7, 14 and 21 days after the surgeries. Harvested samples were fixed for 24 hours with Heidenhain-Susa mixture, then dehydrated with ethyl alcohol, clarified with butyric alcohol (n-butanol) and included in paraffin. Five μm thick sections, stained with Masson Trichromic method, modified by Goldner, were examined under a microscope Olimpus BX41, equipped with digital camera.

Results and discussions
At first harvest, 7 days after the surgeries, in witness M1 (Fig. 6.) we noted ongoing repair processes, but in relatively early stage, a large number of active fibroblasts and fine collagen fibers.
At 14 days after surgery, when the second harvest was done, in the control group (M2, Fig. 8.) repair processes have evolved since the prior harvest, with greater numbers of fibroblasts and neoformation capillaries, but in BF2 (Fig. 9.) connective tissue is consolidated and bone trabeculae are present in different stages of organization and consolidation.

In the sample treated with DIEE biophytomodulator (BF1, Fig. 7.), repair processes are present in a more advanced stage, compared to the control group, with significantly higher number of newly formed capillaries.
At the last harvest, after 21 days, the control (M3, Fig. 10.) has repair processes represented only by relatively well-vascularized connective tissue. On the other hand, in BF3 (Fig. 11.) bone proliferation is well underway, there is noted the presence of a significant number of osteoblasts and thicker bone trabeculae with tendency of expansion.

**Fig. 10.** M3 after 21 days
Goldner’s Trichrome ob. 10X

**Fig. 11.** BF3 after 21 days
Goldner’s Trichrome ob. 10X

**Conclusions**

Reparative processes in BF group runs with a higher speed compared with controls. At 7 days, the control group shows a smaller number of neoformation vessels and fibroblasts compared to the group with DIEE biophytomodulators. At 14 days we already identified the presence of newly formed bone in the BF sample, aspect that we didn’t find in the control group. At 21 days, bone tissue expanded in the BF group, while in the control group, the healing is done strictly based on connective tissue.

**References**

