BIOCHEMICAL STUDY OF THE INFLUENCE OF AD TYPE BIOPHYTOMODULATORS IN HEALING OF BONE DEFECTS IN RATS

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Summary

The aim of the study was to investigate the effect of AD biophytomodulators on bone fracture healing and biochemical indices of bone turnover. Adult male white Wistar rats with a drilled hole in the femur shaft were exposed to the action of the AD biophytomodulators. The control group also underwent surgery, but has not received any postoperative treatment. The surgeries were done under neuroleptanalgesia. The serum values of alkaline phosphatase and osteocalcin were determined preoperatively and 14 days after surgery. The values of alkaline phosphatase had a statistically significant increase (p<0.05), postoperatively, in the animals treated with AD-DIEE type biophytomodulators. Also in this group, postoperative osteocalcin values showed an average which was higher than the one of the control group. These data suggest that AD biophytomodulators may be an adjuvant to the dynamics of bone regeneration. Further study is necessary, because the mechanisms of interaction of biophytomodulators and bone fracture healing remain to be clarified.

Key words: rat, bone, healing, stimulation, biophytomodulator.

Introduction

Finding new ways of enhancing bone tissue healing and shortening post-fracture recovery time is a major challenge for researchers in the fields of orthopedics and maxillofacial surgery. Turk (2001) showed that post-osteotomy exposure of rabbits to a low-frequency magnetic field determines a growth in the density of the newly formed callus. Lo YiChen et al. (2010) highlighted the positive effect of betulinic acid on MC3T3-E1 osteoblasts, inducing higher alkaline phosphatase, osteocalcin and osteopontin levels.

Peng LiHua et al. (2010) showed the beneficial effects of a mix of six plants (Radix Dipsaci, Ramulus Sambucus Williamsii, Rhizoma Notoginseng, Flos Carthami, Rhizoma Rhei and Fructus Gardeniae) on fracture healing. They assessed both the properties of the newly-formed callus and the activity of serum alkaline phosphatase. This study presents the first scientific evidence of the efficacy of a herbal paste in the promotion of bone healing.

Gerova and Galunska (2010) list a series of biochemical markers relevant to bone turnover, among which: alkaline phosphatase, osteocalcin, procollagen propeptides, hydroxyproline, tartrate-resistant acid phosphatase, pyridinoline crosslinks, collagen telopeptides, cathepsin K.
Bone alkaline phosphatase, produced by osteoblasts, is the only enzyme with practical relevance for bone tissue pathology and its serum value rises when an osteoblastic reaction, associated with bone tissue production or repair occurs. The range of normal values is large, up to 300 iu/l, in most species (Kerr, 2002).

Osteocalcin is a non-collagenous protein found in bone and dentin. Osteocalcin is produced by osteoblasts, has a metabolic role and is pro-osteoblastic. Also, it is involved in bone mineralization and calcium homeostasis. In vitro, osteocalcin can stimulate CyclinD1 and Insulin expression in pancreatic β-cells and Adiponectin, an insulin-sensitizing adipokine, in adipocytes. In vivo osteocalcin can improve glucose tolerance (Lee et al., 2007).

Giannetto et al. (2010) proved that circadian rhythm affects osteocalcin levels in equines. Al-Sobayil (2010) studied circadian rhythm of bone markers alkaline phosphatase and osteocalcin in serum of dromedary camels, concluding that only the levels of serum osteocalcin change throughout the day. Both scientists suggested establishing certain times for the collection of serum samples when assessing the osteocalcin levels.

The purpose of this study is to assess the impact of AD-DIEE biophytomodulators on healing of bone defects in rats, by assessing serum levels of osteocalcin and alkaline phosphatase. These devices have been patented by the physicist Ancu Dinca (RO119756-2004). Operation of this device would be based on the capacity of some plants to generate specific effects of resonance. Thus for the production of these modulators a mix of 20-40 plants has been used. Oana et al (2010) demonstrated that these biophytomodulators have a positive influence on the healing of bone defects in sheep.

**Material and methods**

The biological material used in this experiment consisted of 20 white Wistar, male, 6 months old, clinically healthy, average weight 230 g, rats. For the site of the bone defects the femoral shaft was chosen (Figure 1); the animals underwent neuroleptanalgesia, which was induced using xylazine 8mg/kg and ketamine 40 mg/kg, administered intraperitoneally. Bone defects (drilled holes) were inflicted using a dental drill of 1.6 mm diameter, adapted to a dental 3500 rpm micromotor. Irrigation with saline (Figure 2) was used to avoid overheating. The defect was drilled through the whole thickness of the compact (Figure 3), reaching the medullary canal. The skin was then sutured, using 4-0 surgical silk (D-tek Sutures, Demophorius Limited) (Figure 4).

**Figure 1.** Highlighting the point where the bone defect will be drilled  
**Figure 2.** The use of a dental drill to inflict the femoral defect, irrigation with saline
The animals were randomly divided in two equal groups. The animals in the first group were treated postoperatively with AD-DIEE type biophytomodulators, and the ones in the second group, the control group, did not receive any treatment.

To assess the values of serum alkaline phosphatase and osteocalcin, blood samples were collected from animals the day before surgery and 14 days postoperatively. To prevent changes induced by circadian rhythm, blood samples were collected at the same time of the day in both instances.

Osteocalcin was dosed using the commercial research kit produced by Life Science Inc. USCN., Wuhan, China, which is based on an ELIZA method. Alkaline phosphatase was assayed by a spectrophotometric method.

For statistical calculation, determination of the "p" index was conducted using GraphPad In Stat program (two-tailed t-test). Statistical interpretation of the data and of the value of "p" was made following the scheme: p ≤ 0.05 - statistically significant results, p ≤ 0.01 - statistically distinct significant results, p ≤ 0.001 - statistically highly significant results.

Results and discussion

![Figure 5](image5.png)

**Figure 5.** Postoperative changes in mean values of alkaline phosphatase (U/l) in the group treated with biophytomodulators and control group

![Figure 6](image6.png)

**Figure 6.** Postoperative changes in mean values of osteocalcin (ng/ml) in the group treated with biophytomodulators and control group
Preoperative alkaline phosphatase levels were within physiological limits. During the bone defect healing process, we see an increase in the average values of alkaline phosphatase at 14 days after surgery (Figure 5), both in the treated group and control group. In the animals treated with AD-DIEE type biophytomodulators we have a statistically significant increase in alkaline phosphatase levels ($p <0.05$).

Mean osteocalcin values measured at 14 days postoperatively are also higher compared to preoperative measurements. We have a stronger increase of osteocalcin level in the group treated with AD-DIEE biophytomodulators compared to the control group (Figure 6).

The healing of the bone defect created in the femoral shaft follows the pattern of "stable fracture repair", where woven bone is visible microscopically as early as 36 hours after injury. Depending on the mechanical forces acting at the site, the newly-formed callus eventually can be reduced in size by osteoclasts until the normal shape of the bone is restored. This process, however, might take years to complete (McGavin and Zachary, 2006). At 14 days post-intervention, when the biochemical tests were done, the osteoblastic activity is in progress, in the primary callus production phase. The bone markers assessed in this study are in direct relation with this process, our results indicating that the osteoblastic activity is higher in the group treated with the AD-DIEE biophytomodulators. The results obtained (biochemical tests) need to be continued with histopathological tests, densimetry of the newly formed callus and mechanical tests.

**Conclusions**

Biochemical tests revealed a higher postoperative increase in the values of investigated bone markers in the animals treated with AD-DIEE type biophytomodulators compared to values obtained in the control group. These higher values could be associated with a more intense osteoblastic activity in the treated group, which was stimulated by the AD-DIEE biophytomodulators.

These data suggest that AD biophytomodulators may be an adjuvant to the dynamics of bone regeneration, but further study is necessary in order to elucidate the mechanism through which they enhance bone healing.

**References**


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