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Histomorphometrical Evaluation of Androgenic Steroid on Tibial Bone Defect Healing in Rabbit

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ABSTRACT

Androgens have proliferative effects on osteoblasts and increase fracture healing by systemic and local stimulation of bone formation. The aim of the present study was to evaluate if the systemic stimulation by androgens leads to increased bone-defect healing. Ten mature, healthy, white Newzealand rabbits were selected and all rabbits were castrated with scrotal approach. The rabbits were divided into two groups. After general anesthesia, a hole in size of 1×4 mm in diameter and depth was created in the middle of the left tibia shaft with low speed dental bit. Group 1 (5 rabbits) were injected with nandrolone 2 mg/kg body weight intramuscularly. Group 2 the 5 control rabbits received cyproterone acetate 5 mg/kg body weight orally. 50 days after surgery, the rabbits become Euthanasia. Then bone defect was evaluated by means of histomorphometrical factor. Histomorphometrical evaluation showed that the percentage of bone formation in group 1 was equivalent to 54.30±0.28 which was higher among other group. The results show that androgenic steroid such as nandrolone can increase the bone healing in middle shaft of tibia defect in rabbit.

Key words: Androgens, Cyproterone Acetate, Nandrolone, Rabbit.

INTRODUCTION

Bone is a specialized form of connective tissue that functions as an integral part of the locomotors system. Bones act as lever arms during motion, provide resistance to the effects of gravitational force on the body, and provide protection and support to adjacent structures. Bone also serves as a reservoir of mineral for systemic mineral homeostasis. Normal bone healing is an ongoing process, which can be affected by various factors. Fracture repair and bone healing can be promoted by administration of some drugs, among which are growth factors that parathormone hormone and anabolic steroid hormones [1, 2]. Sex steroids play an essential role in the maintenance of bone health throughout life, and the mechanisms by which these effects are mediated in a subject of much controversy. Osteoblast cells appear to be stimulated by androgens in vitro; however their use in vivo is limited due to the virilizing side effects as well as alteration in the lipoprotein profiles. Androgenic-anabolic steroids are synthetic derivative of the male hormone testosterone. Nandrolone decanoate is an androgenic anabolic steroid that is used for prevent of osteopenia [3], increasing bone mass [4, 5], treatment of osteoporosis [6], in different animal and human cases. Despite the adverse effect of steroid hormones. They can be used to stimulate fracture healing without untoward side effects. The effective mass of anabolic steroids on osteogenesis is demonstrated in

combination with exercise training. Cyproterone acetate (**CA**)**,** an antiandrogenic compound, was used in order to investigate the role of testosterone in bone growth processes [**7**].

MATERIALS AND METHODS

Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health Publication no. 85-23, revised in 1985) and our Ethical committee on animal care approved the protocol. In this study, 10 New Zealand 35-36 week old and weighing 2.5-3 kg male rabbits were used, and divided into two groups (I and II) of 5 rabbits each, according to the procedure performed. All rabbits were kept in individual cage during the whole experimental period, under strict hygienic conditions and fed with standard ration for rabbits and water ad labium. All rabbits were castrated with scrotal approach.

1) Anesthesia

Under intramuscular Diazepam (1mg/kg) premedication and intravenous Ketamine hydrochloride (35mg/kg) and Xylazine (5mg/kg) general anesthesia.

2) Tibial defect

The diaphysis of left tibia was exposed by longitudinal skin incision on the medial aspect of hind limb; muscle and fascia were carefully dissected. The space between extensor and flexor muscles groups was dissected, providing a wide view of tibial bone and a hole of 1×4 mm in diameter and depth was created in the middle of the left tibia shaft with low speed dental bit. The cavity then was washed carefully with a physiological saline solution. Muscle, fascia and skin were closed by routine suturing. All of the rabbits were castrated by scrotal approach at last.

3) Drug administration

The rabbits were randomly divided into two groups. Group 1 (5 rabbits) were injected with Nandrolone 2mg/kg body weight intramuscularly. Immediately after surgery and followed by once a week for 8 weeks. Group 2 the 5 control rabbits received cyproterone acetate 5mg/kg orally every other day until the 8 weeks post operatively.

4) Histomorphometrical Evaluation

All rabbits were euthanized with an intravenous injection of an over dosage of thiopental sodium, causing a quick and painless death, at 50 postoperative days. The tibia bone was taken out and then the middle area of the tibia bone including created deficit was placed in 10 percent formaldehyde solution and was sent to the laboratory for preparing histopathological sections (H & E). Some photographs were taken from all sections by zooming X40. The images were analyzed with the Sigma Scan Pro 5 software. For each image, first the number of whole image's Pixels was recorded. The areas of bone formation (which obviously had similar color characteristics) were selected and the number of Pixel, were calculated. Percentage of bone formation was obtained from the ratio of bone tissue's Pixel to the whole image's pixel. To increase the accuracy of evaluation, five sections of each sample were chosen and again by the same way the number of total bone's Pixel was measured and compared with previous results.

5) Post Operative Care

Antibiotics (penicillin G procaine 40000 IU/kg IM, bid), dexamethasone (0.6 mg/kg, IM) and analgesic such as tramadol hydrochloride (5 mg/kg, IM, bid) were administered for 3 post-operative days. No operative or postoperative complications were encountered. All of the rabbits tolerated surgery well and survived until the final experimental time. No wound opening or infections were observed.

6) *Statistical analysis*

Statistical evaluation of data was performed using the software package SPSS 16. Data are expressed as the mean±SEM for each group. Statistical differences between groups were evaluated with ANOVA followed by the Tukey test to analyze histomorphometric data among groups I and II. The significant level was set at $p<0.05$.

RESULTS

Histomorphometry evaluation results obtained, 50 days after surgery, indicating that percent of bone formed in group I significantly more group II ($p<0/05$) (figure 1, 2). Histomorphometry Assessment results show that the percentage of bone formation in Groups I and II is respectively 54.30±0.28 and 32.91±1.2 which indicates that the percentage of bone formation in group I is higher than other group (table 1).

Table 1: Histomorphometry Assessment Results (percentage of bone formation)

Groups	Percentage of bone formation (Mean±SE)
I	54.30±0.28
II	32.91±1.2

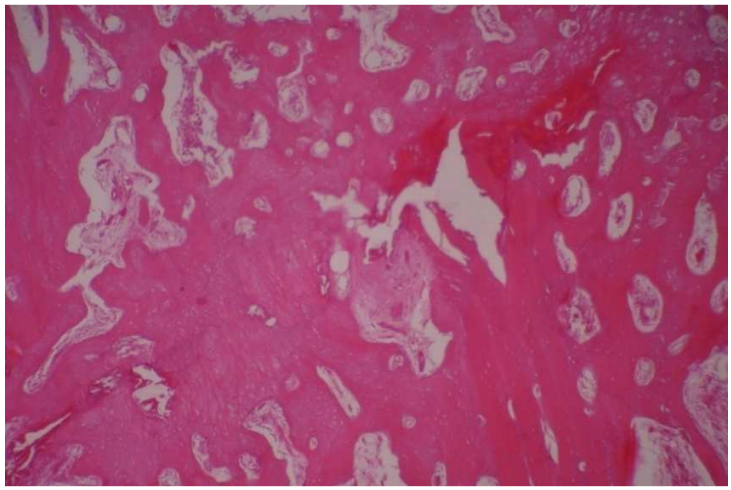

Figure 1. Healing site in group I. Active osteoblasts, abundant hyaline cartilage with Endochondral ossification and defect is filled with newly formed bone

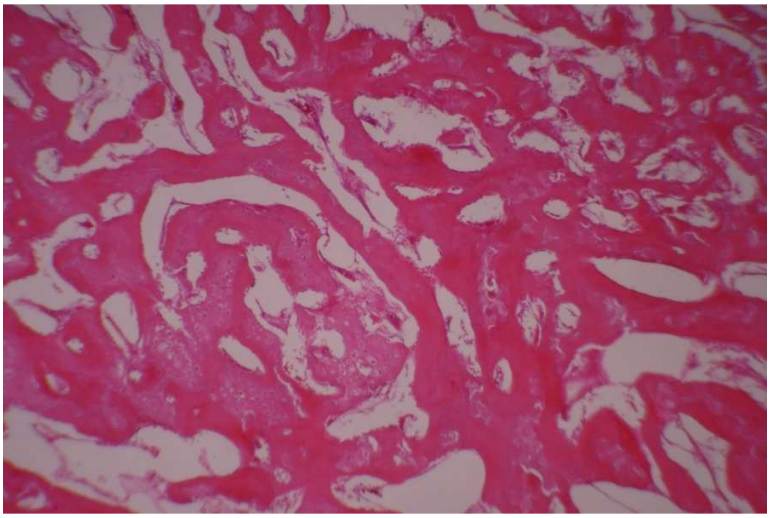

Figure 2. Healing site in group II. Matrix composed of fibroblasts and Low hyaline cartilage being transformed to bone

DISCUSSION

The stimulation of healing of bone defects with androgens leads to a significantly higher bone content inside the defects. In clinical application, androgens may be a possibility to increase bone formation, especially in elderly patients. Furthermore, it may be possible to shorten postoperative rehabilitation because of the effects of androgens on muscles [8]. Nandrolone decanoate makes increase in bone mineral content [9]. A result of study on ovariectomized cynomollgus monkeys demonstrate that cortical bone formed 1 or 2 years after ovariectomy has a

higher phosphate content, a lower carbonate content, and more nature collagen cross-links (nonreducible cross-link/reducible cross link ratio) than that formed in sham-operated control. Treatment with a nandrolone decanoate reverses most of the ovariectomy induced chemical changes in the cortical bone to the level of the ovary intact controls, but had a little effect on the trabecular bone. This result demonstrated that bone newly synthesized after ovariectomy is chemically different from healthy bone within specific bone region, which many contribute to reduce bone quality in osteoporosis [10, 11, 12]. Another study demonstrated the changes in bone composition that occur with nandrolone treatment, however, are less well characterized [4]. Nandrolone treatment increased bone mass, it could not reverse the decrease in bone strength due to ovariectomy. The results demonstrate that ovariectomy and nandrolone treatment did not affect the degree of mineralization as defined by the phosphate/protein ratio, but acid phosphate content in cortical and subchondral bone was increased by ovariectomy, suggesting this bone to be less mature due to increased remodeling that occurs after ovariectomy [4, 10, 12]. In the subchondral and cortical bone regions, ovariectomized monkeys showed a lower total carbonate content than sham controls, specifically due to the decrease in labile carbonate content [4, 3]. In the trabecular region, no change of carbonate content was observed. Treatment with nandrolone decanoate was found to restore the loss in carbonate [3, 4, 10, 12]. Body weight increased over 50% with administration of nandrolone [3]. In another study, two groups were studied which group 1 was treated with estrogen-progesterone and group 2 was treated with estrogen-progesterone plus nandrolone decanoate that the cancellous bone density showed in 6 month an increase of 21% in group 1 and 29% in group 2 to subsequently stay at that level. All these changes from the basal level were highly significant but there were no significant differences between the two groups [13, 14]. In another study was showed the effects of 20nandrolone decanoate (ND; 50 mg IM every three weeks) on calcium metabolism and forearm bone density were studied in a randomized trial in 35 women receiving long-term therapy with corticosteroids (CST) for rheumatic disease. In conclusion, nandrolone decanoate therapy may be used in the prevention of CST-induced osteoporosis. It also seems to exert mild inhibition of bone resorption without affecting or even stimulating bone formation [6]. Also was showed that bone formation is clearly decreased during estrogen-progesterone therapy, it is not affected by long term therapy with anabolic steroids [14]. Another study's results were showed that treatment with nandrolone decanoate does increase the bone mineral content; however, this may not be due to a direct increase in bone formation. The mechanism theoretically is a combination of decreased bone resorption and increased muscle mass, which both play a beneficial role in conserving bone. Loss of bone mineral density, anemia, and hair changes also may occur in unusual and excessive consumption of androgens [15]. Principally, antiandrogens affect all androgen-dependent organs and functions as for instance accessory sexual glands, spermatogenesis, skin and skin appendages, libido and potency, male sexual differentiation, longitudinal bone growth and bone maturation. Pharmacologically, it is important to distinguish between the steroidal antiandrogens of the cyproterone acetate type and the nonsteroidal pure antiandrogens (flutamide, anandron) [16]. Cyproterone acetate is antiandrogenic, it is a quite potent progestogen and it is antigonadotrophic. Based on pharmacological and biochemical backgrounds cyproterone acetate is used in the following indications: Androgen mediated disorders of the skin such as acne, hirsutism, alopecia, advanced prostatic carcinoma, precocious puberty and male hypersexuality [16]. Physiologic effects of estrogens, progestin megestrol acetate, medroxyprogesterone acetate including gynecomastia, changes in body composition (weight gain, reduced muscle mass, increase in body fat), and changes in lipids, are less commonly recognized as side effects of androgen deprivation therapy. In another study cyproterone acetate (CA), an antiandrogenic compound, was used in order to investigate the role of testosterone in bone growth processes. The mineralization processes of the bone matrix were almost completely blocked and the antlers persisted in growing throughout the whole year [15, 5].

According to studies and the results of this study seems despite that there are discrepancies, androgenic steroid such as nandrolone in bone defect can be cause of calcium deposit and faster bone formation.

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