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European Journal of Experimental Biology, 2012, 2 (4):1315-1319

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Radiographic evaluation of androgen 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. Four round defect were holed on tibial shaft with similar distances. The diameter of the holes increased from 1 to 4mm respectively from distal to proximal part of the tibial diaphysis. 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. For evaluation of healing process, standard lateromedial and craniocaudal radiographic projection were made before and immediately after surgery and it was repeated at 15, 30, 45 and 60 days after surgery. There is no significant difference between two groups on 0, 15th and 30th days but there is significant difference on 45th and 60th days.

Key words: Androgens, Rabbit, cyproterone acetate, Nandrolone

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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 or right) tibia was exposed by longitudinal skin incision on the medial aspect of hind limb; muscle and fascia were carefully dissected. Four round defect were holed on tibial shaft with similar distances. The diameter of the holes increased from 1 to 4mm respectively from distal to proximal part of the tibial diaphysis. 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)Evaluation of healing process:
Standard lateromedial and craniocaudal radiographic projection were made before and immediately after surgery and it was repeated at 15, 30, 45 and 60 days after surgery. Radiographic evaluation of bone healing was done with considering the disappearance of radiolucency.

5)Statistical analysis:
Statistical differences between groups were evaluated with mann-whitney u-test to analyze data among groups. The significant level was set at $p<0.05$.

RESULTS

There is no significant difference in the amount of callus formation between group 1 and group 2 until 30^{th} day (figure 1, 2), but there is significant difference on 30^{th}, 45^{th} and 60^{th} day between two groups. In group 1 were filed 1mm and 2mm holes one on 45^{th} day (figure 3), and were filed 3mm and 4mm holes on 60^{th} day (figure 5). In group 2 were filed 2mm hole on 45^{th} day (figure 4), that there is significant difference in two groups on 30^{th} till 60^{th} day (figure 6), but there is no significant difference from zero till 30^{th} day. There is no significant difference between two groups on 0, 15^{th} and 30^{th} days but there is significant difference on 45^{th} and 60^{th} days.

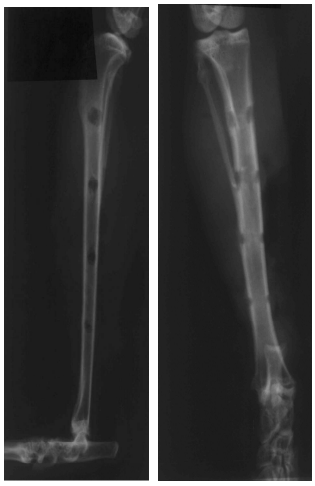

Fig 1. Survey radiography of group 1 on 30th day

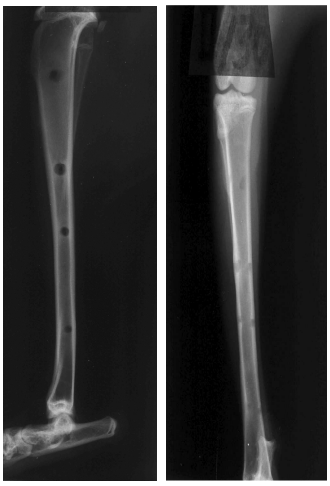

Fig 2. Survey radiography of group 2 on 30th day

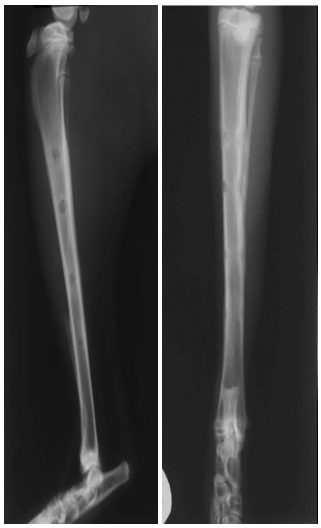

Fig 3. Survey radiography of group 1 on 45th day

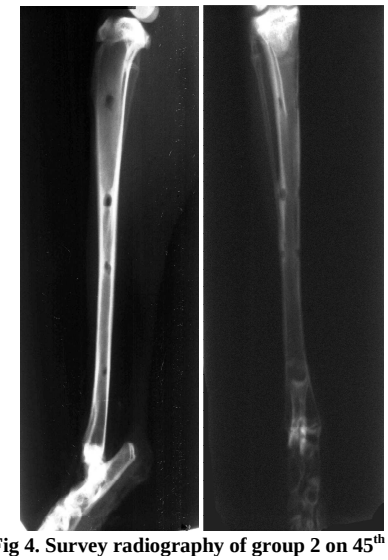

Fig 4. Survey radiography of group 2 on 45th day

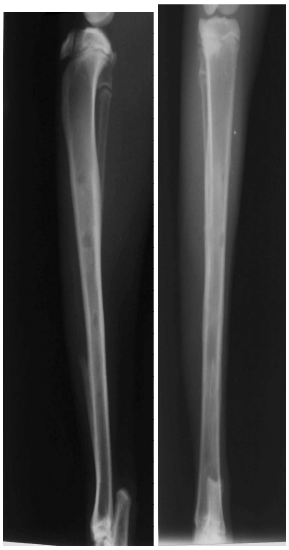

Fig 5. Survey radiography of group 1 on 60th day

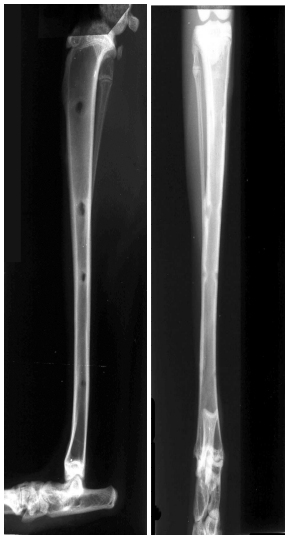

Fig 6. Survey radiography of group 2 on 60th day

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 1318nandrolone 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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