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ORIGINAL ARTICLE

Histomorphometrical Evaluation of the Effect of Different Amounts of 1, 25(OH) 2 D3 on Bone Healing in Rabbit Ulna

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Ahmad Asghari, Mohammad Mehdi Dehghan, Aliasghar Gharachorlou; Histomorphometrical Evaluation of the Effect of Different Amounts of 1, 25(OH) 2 D3 on Bone Healing in Rabbit Ulna.

ABSTRACT

Vitamin D (calciferol) comprises a group of fat soluble seco-sterols found naturally only in a few foods. Lack of this vitamin causes some disorders in bone metabolism. In this study, 32 adult female New Zealandian white rabbits were selected and divided into four equal groups randomly. After the induction of general anesthesia, a full thickness defect with 1 mm width was created in the mid shaft of ulna bone of both right and left fore limbs in all groups. The animals in first, second and third groups received the amount of 2500 IU, 5000 IU and 10000 IU vitamin $_{D3/kgBW}$ via intramuscular injection, respectively. Then, the same dose was repeated for them once in a week during 50 days. The animals in the fourth group received equal volume of normal saline as same as above regimes. 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 2 was equivalent to 82.961.65 which was higher among other groups. Considering the results of this study, the 1, 25-dihydroxyvitamin D3 has an effective role on repairing bone defect; but in high dose, it can prevent mineralization of bone.

Key words: vitamin D, fracture healing, Ulna, rabbit

Introduction

The two major physiologically relevant forms of vitamin D are D2 (ergocalciferol) and D3 (cholecalciferol).

Vitamin Dis photosynthesized in the skin of vertebrates by the action of solar ultraviolet (UV) B radiation on 7-3 dehydrocholesterol [9]. Vitamin D plays major role in the regulation of mineral homeostasis and affects bone metabolism. Vitamins are organic compounds needed in small quantities for the operation of normal body metabolism, and cannot be produced by the body's own cells. Vitamin D is not actually a vitamin. It is then hydroxylated once to form 25-hydroxyvitamin D3 (25D), and then a second time to form 1, 25-dihydroxyvitamin D3, which is generally regarded as the active form of the vitamin. Adequate vitamin D levels are essential for good bone health [18]. Vitamin D deficiency is characterized by inadequate mineralization or by demineralization of the skeleton. Among young animal's, vitamin D deficiency is a common cause of bone deformities known as rickets. Vitamin D deficiency in adults leads to a mineralization defect in the skeleton, causing osteomalacia, and induces secondary hyperparathyroidism with consequent bone loss and osteoporosis. Potential roles for vitamin D beyond bone health, such as effects on muscle strength, the risk for cancer and for type 2 diabetes, are currently being studied [4]. Treatment of vitamin

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D deficiency can reduce the risk of falls and fractures in patients. 1alpha, 25-dihydroxyvitamin D3, the hormonal form of vitamin D3 that mediates calcium translocation in intestine and bone Calcium and phosphorus are required for a wide variety of biological processes [14]. The production of 25(OH) D in the liver and of 1, $25(OH)_2D$ in the kidney is tightly regulated. In the liver, vitamin D-25-hydroxylase is down-regulated by vitamin D and its metabolites, thereby limiting any increase in the circulating concentration of 25(OH) D following intakes or following production of vitamin D after exposure to sunlight. In the kidney, in response to serum calcium and phosphorus concentrations, the production of 1, $25(OH)_2D$ is regulated through the action of parathyroid hormone (PTH)[7].

Materials and methods

In this study, 32 adult female New Zealand white and healthy rabbits with the weight of 2.5-3 kg ranging from 1-2 years old were used. They were randomly divided into four equal groups. Keeping as well as feeding conditions were the similar for all rabbits.

Preparing Surgical Anesthesia:

Beginning with a combined intramuscular injection of ketamine (50mg/kg) and xylazine (8mg/kg) rabbits were anesthetized and then the anterior limbs of animals were typically prepared for surgery. 10mg/kg meperidine was injected intramuscularly during induction and repeated 4 times during 12 hours. Normal saline 0.9% was injected with the rate of 5ml/kg/h during surgery.

Method of Surgery and Postoperative Care:

A cutting was created in the craniolateral surface of the anterior limb and then the ulna bone was exposed. After the induction of general anesthesia, a full thickness defect with 1 mm width was created by an electric oscillating saw in the mid shaft of ulnar bone of both right and left fore limbs in all groups. The remaining bone particles were deleted by suctioning out followed by suturing subcutaneous and skin. To prevent possible infections, 40,000 IU penicillin G Procaine, 4mg/kg gentamicin for 5 days and Ketoprofen 3mg/kg for 3 days were administered intramuscularly. After 12 days, the sutures were removed.

Vitamin D Injection:

The animals in first, second and third groups received the amount of 2500 IU, 5000 IU and 10000 IU vitamin D3/kgBW via intramuscular injection, respectively. Then, the same dose was repeated for them once in a week during 50 days. The animals in the fourth group received equal volume of normal saline as same as above regimes.

Evaluation Methods:

Histomorphometrical Evaluation:

After the rabbits' Euthanasia, the ulna bone was taken out and then the middle area of the ulna bone including created deficit was placed in 10 percent formaldehyde solution and was sent to the laboratory for preparing histopathological sections.

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.

Results:

Histomorphometrical Results:

Histomorphometry Assessment results on the different groups listed in Table 1.

There was a significant Difference between group 1 and groups 3 and 4. There was a significant Difference between group 2 and groups 3 and 4. There was no significant Difference between group 1 and group 2. There was no significant Difference between group 3 and group 4.

Histomorphometry Assessment results show that the percentage of bone formation in Groups 1, 2, 3 and 4 are respectively 67.09±1.87, 82.96±1.65, 39.63±3.70 and 45.83±7.91which indicates that the percentage of bone formation in group II is higher than other groups.

Discussion:

Fractures are one of the clinical problems in humans and animals too. Based on the fact that the skeletal system is related to peripheral nerve and surrounded by soft tissue such as muscle; it has a significant role in motor system from the physiological and anatomical point of view. Vitamin D metabolism also has been under intense Reviews. Erben Weber and Greeve found that one of the biggest issues in osteoporosis therapy is the effective

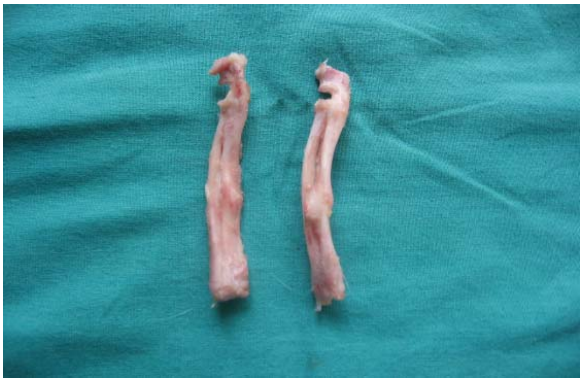

Fig. 1: Ulna bone removal after 50 days.

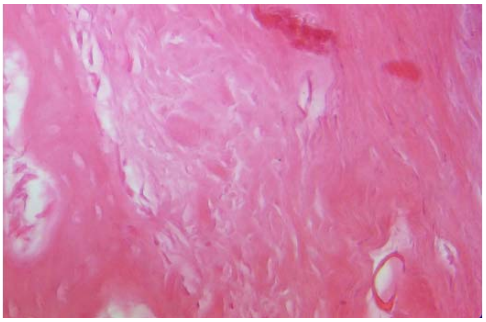

Fig. 2: Pictures.1 1 Active Periosteum composed of fibroblasts and abundant collagen filaments(Group 1).

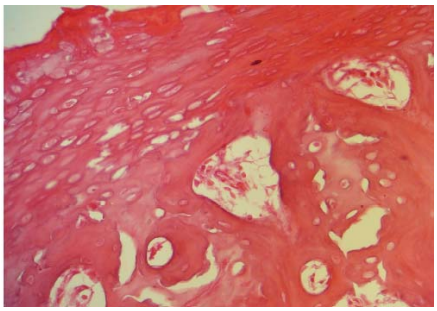

Fig. 3: Active osteoblasts, abundant hyaline cartilage with Endochondral ossification (Group 2).

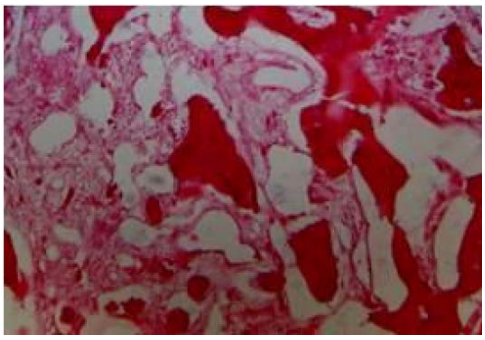

Fig. 4: The fibrous tissue between trabecula (Group 3).

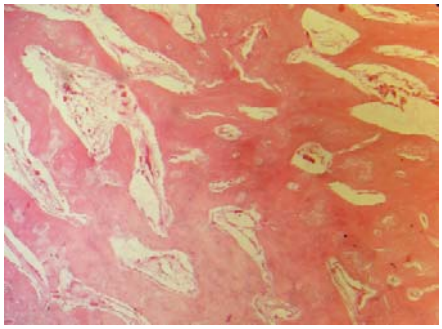

Fig. 5: Low hyaline cartilage being transformed to bone (group 4).

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

Percentage of bone formation (Mean±SE)	Rate of bone formatio n Groups
67.09±1.87	1
82.96±1.65	2
39.63±3.70	3
45.83±7.91	4

treatment of bone tissue in a skeletal structure with the features of osteopenia. A number of empirical studies on mice have shown that vitamin D not only can prevent estrogen deficiency but also can cause effective bone formation [8,11,19]. Omeroglu reported that high doses of vitamin D had positive effects on biomechanical parameters in fracture healing [17]. Heng reported that Vitamin D has a crucial role in osteoblastic differentiation of embryonic stem cells and their final maturity [13]. Gupta and *et al*. found that Adding vitamin D to the culture of stem cells isolated from adipose tissue for osteoblastic differentiation of these cells is essential [12]. Researches show that for actively absorption of calcium, growth of long bones and osteoblast as well osteoclasts activities, both 1, 25, vitamin D and VDR

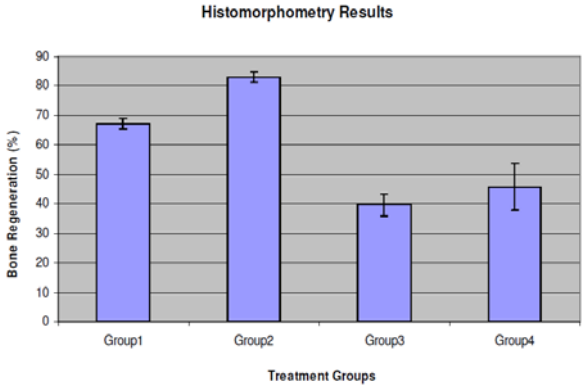

Chart 1: Histomorphometry Results.

(Vitamin d receptor) are required [15]. Studies show that vitamin D3 or its metabolites increased the strength of the callus. It is concluded that 24, 25(OH) 2D3 is essential for bone formation in addition to the known active vitamin D metabolite 1, 25(OH)2D3. Repletion with the combination of 24, 25(OH)2D3 and 1 ,25(OH)2D3 produced the most marked results, in that the callus was even stronger than replete with vitamin D3 by alone [5]. To investigate the effect of 25-OH-vitamin D supplements (calcidiol) on fracture healing in the female elderly rats, a positive correlation was found between blood levels of 25-OH-vitamin D at death and the mechanical strength of the callus. Thus, the administration of 25-OH-vitamin D after the experimental fracture significantly improved the mechanical strength of the fractured bone [6]. Beresford suggests that 1, 25-(OH)2D3 is an important modulator of the growth and differentiation of human bone cells in vitro. He is also consistent with the possibility that 1, 25-(OH)2D3 has direct effects on bone formation in vivo (2). In other study it is shown that at low levels (1ng/day) 1, 25-(OH)2D3 sustained a healing response equivalent to that of 25-hydroxyvitamin D3 (100ng/day) or the parent vitamin. The effects of 1,25-(OH)2D3 on bone did not correlate with changes in plasma Ca or inorganic phosphorus; It is concluded that 1,25-(OH)2D3 can effectively heal the bone lesions of vitamin D deficiency, but that, at high concentrations, the sterol can inhibit mineralization [10]. This is also true in the present study. High doses of vitamin D has no positive effect on fracture healing. Group II with 5000 IU/Kg dose of vitamin D shows better results than the other groups. In other study it is shown that vitamin D plays a central role in the regulation of mineral homeostasis. The treatment of osteoprosis with vitamin D has been studied .In addition, a reduction of fracture risk by treatment with vitamin D has been reported [16]. In this study, the third group received the higher amount of vitamin D, but in the second group an advanced form of bone mass is observed among other groups. Studies show that vitamin D improves the process of bone mineraling through the stimulation of intestinal absorption of calcium and phosphorus via maintaining serum levels of these elements. In addition, vitamin D plays an important role in regulating bone cells activity such as osteoblast, osteoclasts, and in maintaining the dynamic state of bone [1]. According to researchers anabolic vitamin D activity in osteoblast is confirmed; in fact vitamin D stimulates osteoblasts to produce several factors participating in bone formation, Like alkaline phosphatase, collagen type I, insulin-like growth factor I, osteocalcin, and stimulating activities Adenylatecyclase [3]. Considering the results of this study, the 1, 25-dihydroxyvitamin D3 has an effective role on repairing bone defect; but in high dose, it can prevent mineralization of bone.

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