Advances in Environmental Biology, 5(7): 1548-1553, 2011
ISSN 1995-0756

This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLE

Evaluation of the Effect of Different Amounts of Vitamin D3 in Bone Healing and Maintenance of Serum Vitamin D and ca Concentration in the Rabbit

[1]Ahmad Asghari, [2]Iraj sohrabi haghdost, [3]Aliasghar Gharachorlou

[1]Department of Surgery, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of pathology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[3]Department of Mycology, Tabriz branch, Islamic Azad University, Tabriz, Iran

Ahmad Asghari, Iraj sohrabi haghdost, Aliasghar Gharachorlou; Evaluation of the Effect of Different Amounts of Vitamin D3 in Bone Healing and Maintenance of Serum Vitamin D and ca Concentration in the Rabbit

ABSTRACT

Vitamin D is a derivative of cholesterol. 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 ulnar 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. Radiological evaluation was done after animal's surgery and 50 days after surgery. During 50 days, Serological evaluation was done for serum levels of vitamin D and calcium concentration. Radiological evaluation showed that bone repair in the second group was faster than other groups. Serologically, serum levels of vitamin D and calcium in third group was higher than 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

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 a fat-soluble steroid hormone formed from 7-dehydrocholesterol by the action of ultraviolet (UV) light on the basal layers of the skin. 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 [15]. Vitamin D deficiency is now recognized to be a worldwide problem with ageing, where it has been associated with falls and fractures, a principal cause of disability in the elderly. Treatment of vitamin 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. All studies of the effect of vitamin D metabolites on bone ceils in vivo in the vitamin D-deficient state are deterred by simultaneous changes in the Ca and P product of the extra cellular fluid. This makes it extremely difficult

Corresponding Author

Ahmad Asghari, Department of Surgery, Science and Research Branch, Islamic Azad University, Tehran, Iran
Dr.Ahmad.asghari@ gmail.com

to deduce whether the effects of the vitamin D metabolites on bone mineralization are consistent with some direct action upon bone cells or whether they are brought about by increases of the Ca and P product. Increasing the Ca and P product in vitamin D deficiency has been shown to normalize bone mineralization. Vitamin D functions to maintain calcium homeostasis together with two peptide hormones, calcitonin and parathyroid hormone (PTH). Vitamin D is also important for phosphorus homeostasis. Calcium and phosphorus are required for a wide variety of biological processes [11,15].

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

Radiographic Evaluation: the processes of filling the bone defect for the formation of callus and Disappearance of fracture line were assessed with radiography during the days of 0 (day surgery), 14, 28 and 50 after surgery.

Laboratorial evaluation: the blood of animals on days 0, 10, 20, 30, 40, and 50 were taken to measure the amount of calcium and vitamin D. To measure the amount of Vitamin D, chemiluminescence method was used.

Results:

Radiographic Results:

Radiographic images were evaluated by semi-quantitative way. In this context, two important indicators were defined and then they were ranked from zero to four. In each of the images, the desired parameters were evaluated. Radiographic evaluations of two important indicators are shown in Tables 1 and 2.

Radiological evaluation showed that the defined indicators in the first and second groups had a better performance than those in the third and fourth groups.

Radiographic images taken on days 0 (surgery day), 14, 28 and 50 in different groups are presented in figures 2, 3, 4 and 5.

Laboratorial Results:

Laboratorial results shown in Tables 3 and 4.

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 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 [6,8,16]. Omeroglu reported that high doses of vitamin D had positive effects on biomechanical parameters in fracture healing [14]. Heng reported that Vitamin D has a crucial role in osteoblastic differentiation of embryonic stem cells and their final maturity [10]. Gupta and et.al. found that Adding vitamin D to the culture of stem cells isolated from adipose tissue for osteoblastic

Table 1: Formation of external callus

Absence of external callus	0
Starting observable external callus	+1
Creating external callus Bridge with clearly visible fracture line	+2
Creating external callus Bridge with partially visible fracture line	+3
Completed Callus formation and Complete disappearance of fracture line	+4

Table 2: Disappearance fracture line

Full visible fracture line (gap)	0
Disappearance of fracture line, about 25 percent	+1
Disappearance of fracture line, about 50 percent	+2
Disappearance of fracture line, about 75 percent	+3
Complete disappearance of fracture line	+4

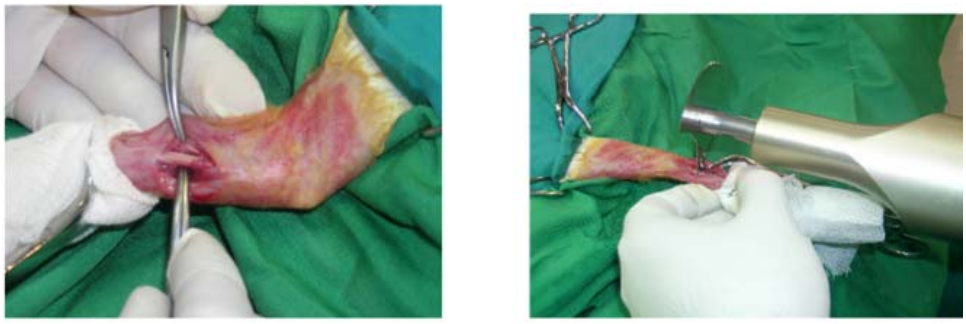

Fig. 1: Creating a full thickness defect by an electric oscillating saw.

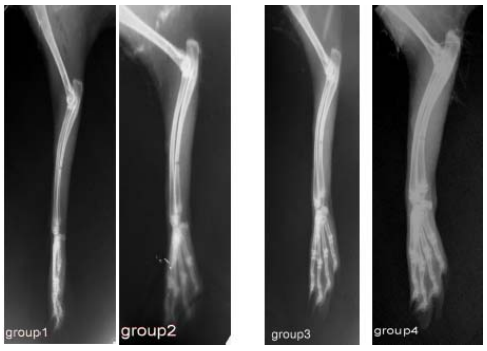

Fig. 2: (Surgery day).

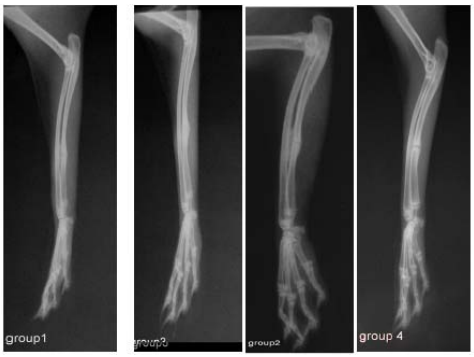

Fig. 3: (14th day).

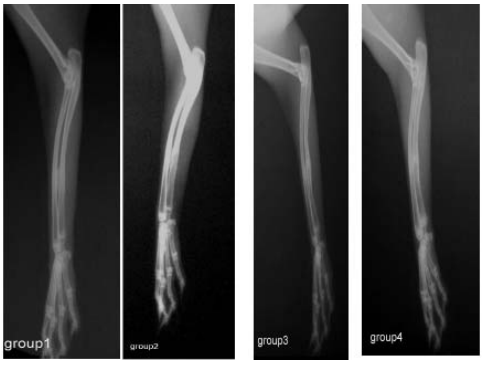

Fig. 4: (28th day).

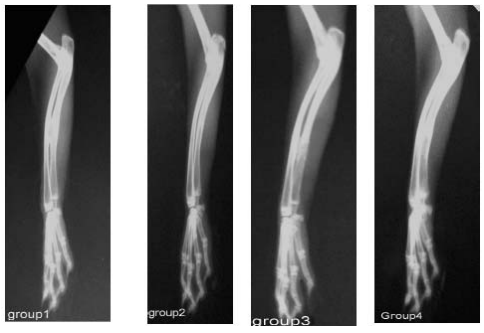

Fig. 5: (50th day).

differentiation of these cells is essential [9]. 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 (Vitamin d receptor) are required [12]. 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

Table 3: The amount of vitamin D in different groups from the days of 0 - 50 (Pg / ml).

Days	Groups	N	Mean	Std. Deviation	Interval
0	1	8	38/0[a]	7/1	32/1-44/0
	2	8	35/9[a]	5/6	31/2-40/6
	3	8	35/9[a]	4/7	32/0-39/9
	4	8	34/2[a]	4/6	30/4-38/1
10	1	8	56/5[a]	10/0	48/2-64/9
	2	8	67/4[b]	8/9	59/9-74/9
	3	8	88/9[c]	6/6	83/4-94/4
	4	8	35/6[d]	3/9	32/3-38/8
20	1	8	70/2[a]	11/6	60/4-79/9
	2	8	85/8[b]	6/9	80/0-91/6
	3	8	116/6[c]	6/9	110/8-122/3
	4	8	36/2[d]	4/4	32/5-39/8
30	1	8	82/7[a]	12/9	71/9-93/4
	2	8	106/2[b]	7/8	99/6-112/7
	3	8	141/4[c]	6/3	136/2-146/7
	4	8	37/3[d]	4/7	33/4-41/3
40	1	8	94/1[a]	12/8	83/4-104/9
	2	8	119/6[b]	7/0	113/7-125/5
	3	8	149/8[c]	0/5	149/4-150/2
	4	8	35/9[d]	4/4	32/2-39/6
50	1	8	106/7[a]	12/7	96/1-117/3
	2	8	136/2[b]	6/5	130/8-141/6
	3	8	150/0[c]	0/0	150/0-150/0
	4	8	35/5[d]	4/3	31/9-39/0

Similar non-Latin characters have statistically significant differences from each other. (P <0.05).

Table 4: The amount of calcium in different groups from the days of 0 - 50 (Mg / dl).

Days	Groups	N	Mean	Std. Deviation	Interval
0	1	8	15/8[a]	1/6	14/4-17/1
	2	8	16/8[a]	2/5	14/7-19/0
	3	8	15/9[a]	3/7	12/7-19/1
	4	8	15/5[a]	2/9	13/1-18/0
10	1	8	16/5[a]	1/7	15/1-18/0
	2	8	18/8[a]	2/5	16/7-21/0
	3	8	26/3[b]	4/1	22/8-29/8
	4	8	23/2[b]	3/2	20/5-25/9
20	1	8	17/4[a]	2/0	15/7-19/1
	2	8	20/3[a]	2/9	17/9-22/8
	3	8	34/9[b]	4/7	31/0-38/9
	4	8	20/9[a]	2/2	19/0-22/7
30	1	8	18/2[a]	2/0	16/6-19/9
	2	8	21/6[a]	2/9	19/2-24/0
	3	8	40/1[b]	5/9	35/2-45/0
	4	8	20/1[a]	1/9	18/5-21/6
40	1	8	19/1[a]	1/9	17/5-20/7
	2	8	22/9[a]	2/9	20/4-25/4
	3	8	41/3[b]	5/5	36/7-45/9
	4	8	20/5[a]	3/3	17/7-23/2
50	1	8	19/8[a]	1/8	18/2-21/3
	2	8	24/3[a]	2/8	22/0-26/7
	3	8	47/1[b]	3/9	43/8-50/3
	4	8	20/2[a]	3/6	17/1-23/2

Similar non-Latin characters have statistically significant differences from each other. (P <0.05).

than replete with vitamin D3 by alone [4]. 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 [5]. 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 [7]. 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 [13]. In this study, the third group received the higher amount of vitamin D and serum calcium levels also were higher than other groups, 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.

Acknowledgment

The authors are grateful to Dr Abedi who assisted in the operation of Rabbitss. Special thanks are due to dr Sorory for preparation of radiographic images. The authors thank Dr Safi for laboratory results.

Reference

1. Anderson, P.H., R.K. Sawyer, *et al*., 2007. 25-Hydroxy vitamin D requirement for maintaining skeletal health utilizing a Sprague-dawley rat model. J of Steroid Biochemistry & Molecular Biology, 103: 592-595.
2. Beresford, J.N., J.A. Gallagher, R.G. Russell, 1986. 1, 25-Dihydroxy vitamin D3 and human bone-derived cells in vitro: effects on alkaline phosphatase, type I collagen and proliferation. Endocrinology, 119(4): 1776-85.
3. Chang, P.L., C.W. Prince, 1993. la, 25-dihydroxyvitamin D3 enhances 12-O-tetradecanoylphorbol-13-acetate-induced tumorigenic transformation and osteopontin expression in mouse JB6 epidermal cells. Cancer Res., 53: 2217-2220.
4. Dekel, S., R. Salama, S. Edelstein, 1983. The effect of vitamin D and its metabolites on fracture repair in chicks. Clin Sci (Lond)., 65(4): 429-36.
5. Delgado-Martinez, A.D., *et al*., 1998. Effect of 25-OH-Vitamin D on Fracture Healing in Elderly Rats.J Orthop Res., 16: 650-653.
6. Erben, R.G., H. Weiser, F. Sinowatz, W.A. Rambeck, H. Zucker, 1992. Vitamin D metabolites prevent vertebral osteopenia in ovariectomized rats.Calcif tissue Int., 50: 228-236.
7. Gallagher, J.A., M. Beneton, L. Harvey, D.E. Lawson, 1986. Response of rachitic rat bones to 1, 25-dihydroxyvitamin D3: biphasic effects on mineralization and lack of effect on bone resorption. Endocrinology, 119(4): 1603-9.
8. Greeve, P.C.M.J., L.F.M. Hampson, Van Zotphen, 1993. Legislation and animal experimentation. In: Principles of Laboratory Animal Science. (Van Zotphen, LFMV, Baumans A C, Beynen Eds). Elservier Science Publishers. Amsterdam, pp: 9-16.
9. Gupta, A., D.T. Leong, H.F. Bai, S.B. Singh, *et al*., 2007. Osteo-maturation of adipose-derived stem cells required the combined action of vitamin D3.betaglycerophosphate, and ascorbic acid.Biophys Res Commun., 362(1): 17-24.
10. Heng, B.C.T., L.W. Cao, P. Stanton, Robson and Bolsen, 2004. Strategies fordirecting the differentiation of stem cells into the osteogenic Iineage in vitro.Bone Miner Res., 19: 1379-1394.
11. Jian, S.C., N.S. Philip, *et al*., 2008. Hypovitaminosis D and parathyroid hormone response in the elderly: effects on bone turnover and mortality. Clinical Endocrinology, 68: 290-298.
12. Johannes, P.T.M.V.L., C.M.V.D.B. Gert-Jan, *et al*., 2001. 24, 25-dihydroxyvitamin D3 and bone metabolism. Steroids, 66: 375-380.
13. Lund, B., E. Charles, C. Egsmose, 1985. Changes in Vitamin D Metabolites and Bone Histology in Rats During Recovery from Rickets. Calcif Tissue Int., 37: 478-483.

14. Omeroglu, H., Y. Atep, O. Akkup, F. Korkusuz, A. Bicimoglu, N. Akkap, 1997. Biomechanical analysis of the effects of single high-dose vitamin D3 on fracture healing in a healthy rabbit model.Arch Orthop Traum Su., 116(5): 271-274.
15. Robert, B.R., W.S. John, B.M. Donald and J.M. Lawrence, 2001. Handbook of vitamins. Third edition. Marcel Dekker, Inc., pp: 51-113.
16. Weber, K., M. Goldberg, M. Stangassinger, R.G. Erben, 2001. 1 alfa-hydroxyvitamin D2 is less toxic but not bone selective relative to 1 alfa-hydroxyvitamin D3 in ovariectomized rats. bone Miner Res., 16: 639-651.