

Original Research Article

Does a single high dose of vitamin D3 have an effect on fracture healing? Animal study

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Received: 01 October 2016

Revised: 11 October 2016

Accepted: 13 October 2016

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ABSTRACT

Background: Vitamin D has an important role in the regulation of calcium by stimulating intestinal absorption of calcium and phosphorous and osteoclastic resorption of bone thus being critical building bone. Given the high prevalence, severity, and cost of osteoporotic fractures, prevention strategies that are effective, low in cost and well tolerated are needed. One promising prevention strategy may be vitamin D supplementation. This animal study was done in order to establish the role of Vitamin D in fracture healing among rabbits.

Methods: 8 rabbits were involved in the study with 4 in the control group & 4 in the test group. A fracture was surgically induced on both the groups following which a single high dose of vitamin D was administered intra muscular injection to the test group and normal saline for the control group. Biomechanical properties of the fractured bones such as energy absorbed until fracture (EAUF), maximum load (N) and diameter at the fracture site (D) were assessed 12 weeks later.

Results: Mean value of EAUF for test group was 401.8 N-mm and control group was 404 N-mm. Mean value of maximum load in control group is 228.8 N and for test group is 186.9 N.

Conclusions: From the above results, the authors did not find any significant improvement in fracture healing in test group. Hence, further investigations are to be needed to prove the role of vitamin D3 in fracture healing.

Keywords: Animal study, Biomechanical study, Vitamin D3, Fracture healing

INTRODUCTION

Many substances appear to influence fracture healing: Biphosphonates, hormones (vitamin D) and growth factors and others. Their effects are due to different processes affecting bone healing such as calcium absorption, angiogenesis, collagen deposition, osteoblast stimulation and bone remodelling.¹ The effects of most of the factors on fracture healing have not yet been absolutely verified.

Vitamin D3 (Cholecalciferol) is known to be one of the hormones promoting fracture healing.² In the past, many studies on the effects of vitamin D3 on fracture healing were undertaken, frequently in vitamin D

depleted animal models and with different protocols. However, the mechanism of action of vitamin D3 is not clearly defined.

In this animal study we plan to compare the biomechanical properties of the surgically induced fractures between the test group and control group in order to establish an association between vitamin D and fracture healing.

METHODS

This animal study was conducted in Center of Excellence of Forensic Toxicology (CEFT), Sri Ramachandra Medical University, Chennai and Center Leather

Research Institute (CLRI) Chennai during the time period of November 2015–March 2016. After obtaining necessary clearance documents from animal’s ethics committee.⁸ New Zealand white rabbits with similar characteristics and features of same breed, sex, age and weight were approved for the study of which 4 were taken under the test group and 4 were taken under the control group with different names given in Table 1 and 2.



Figure 1: Rabbits housed at CEFT.

According to the protocol the rabbits were anaesthetized under sevoflurane, right mid shaft of tibia was selected as the site of fracture and the right tibia of each rabbit were exposed via a lateral skin incision under ketamine hydrochloride and osteotomized using gigli saw. Fracture was surgically reduced and fixed with a K wire in control group. A single high dose vitamin D3 (50,000 IU/Kg) intramuscularly to the test group immediately.

Following fracture fixation and equal amount of normal saline was injected intra muscularly in the control group. Twelve weeks post operatively all the 8 rabbits were euthanized and the healed right tibia bones were taken for biomechanical analysis in CLRI using a three point bending configuration using the Instron 3369 material testing machine.

The collected data were analysed with IBM SPSS statistics software 23.0 version. To describe about the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean and SD were used for continuous variables. To find the significance in categorical data Chi-Square test was used. In all the above statistical tools the p-value is considered as significant if the value is <0.05

RESULTS

The biomechanical properties were studied with the following parameters maximum load (N), energy absorbed until fracture (EAUF) and diameter of fracture (D) site were assessed in both control and test group. The mean value of EAUF in the test study was 401.8225 and that of the control group was 404.015; the mean value of

other biomechanical parameters such as maximum load in the test group is 186.9625 and the control group is 228.875. Based on the above statistical analysis values, p-value was not significant.

Table 1: Biomechanical parameters assessed in control group.

Rabbits	EAUF [N-mm]	D (mm)	N
Adam	205.5	8.56	235
Arkam	969.7	11.8	272
Eureka	154.6	11.1	148
Pele	286.0	6.95	259
Mean	404	9.1	228.8
SD	381	2.28	55.5

Table 2: Biomechanical parameters assessed in test group.

Rabbits	EAUF [N-mm]	D (mm)	N
Maradona	329.7	14.2	184
Zidane	116.10	8.75	162
Leo	123.85	9.27	198
Cristiano	1037.55	10.0	201
Mean	401.8	10.6	186.9
SD	435.2	2.48	17.9
p-value	0.886	0.686	0.343



Figure 2: X-ray at 12 weeks post-operatively.

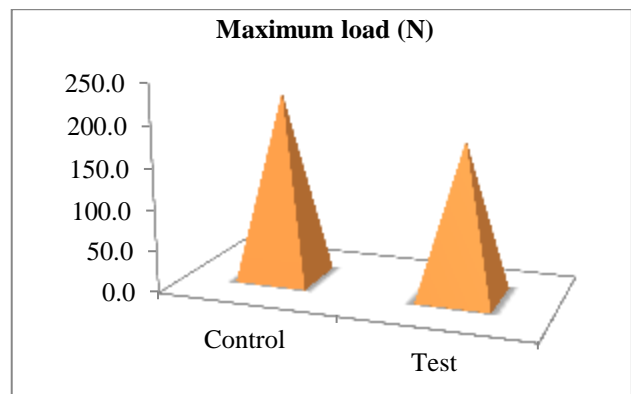


Figure 3: Histogram comparing maximum at fracture site between the two groups.

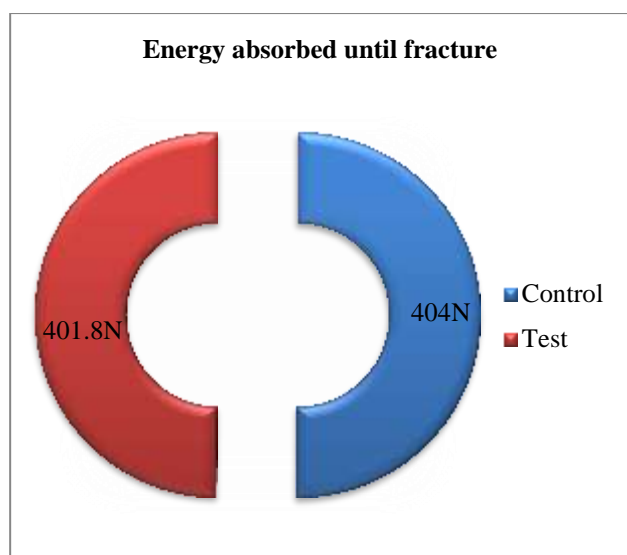


Figure 4: Doughnut chart depicting EAUF between the two groups.

DISCUSSION

This study determined that a single high dose of vitamin D3 was found not to increase the biomechanical properties of the bone such as EAUF and maximum load thus not affecting fracture healing but the diameter at the fracture site was found to have increased in the test group compared to that of the control group. A similar study was done in Ankara Turkey by Omerglu et al, among 20 New Zealand white rabbits in which biomechanical analysis was carried out 6 weeks post operatively using the Lloyd LS500 material testing machine.² A study done from University of Oslo, Norway on rats showed experimental osteoporosis induced by ovariectomy and vitamin D deficiency does not markedly affect fracture healing in rats.³ Whereas a study conducted by Fu et al showed that dietary supplement improves fracture healing.⁴ A similar study done in rabbits showed impaired fracture healing similar to the results obtained in our study but in case of rats it has shown to have a stronger fracture callus.^{5,6}

Though vitamin D is a secosteroid hormone essential for calcium absorption and bone mineralization which is positively associated with bone mineral density, bone health and strength of the fracture callus but no significant improvement was observed in the study.⁷ However, Kudo et al found no stimulating effect on osteoclast bone resorbing activity.⁸ On the other hand vitamin D may also inhibit osteoclastogenesis by down-regulation of RANK expression.⁹ Braun et al found that supplementation with a vitamin D analog, 1 alpha-(OH)D3, decreased bone resorption.¹⁰ However none of the studies were definitely conclusive.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Hussain AZ, Jambu N, Lourdes K. Does a single high dose of vitamin D3 have an effect on fracture healing? Animal study. *Int J Res Orthop* 2016;2:260-2.