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Role of vitamin D3 in prevention of radiotherapy-induced epiphyseal ınjury

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ABSTRACT

BACKGROUND*: Vitamin D3 is a growth factor improving bone mineralization, and regulating osteoblastic activity and longitudinal bone growth. In this study, impact of vitamin D3 supplementation administered prior to fractionated RT in reducing RT-induced epiphyseal injury is investigated..*
MATERİAL-METHOD*: Six week old male Sprague-Dawley rats were enrolled to one of the four groups: Group 1 was assigned as control group (n=7); Group 2 received fractionated RT alone; Group 3 received 50000 IU/kg i.m. vitamin D3 injection alone; and Group 4 received 50000 IU/kg i.m. vitamin D3 injection prior to fractionated RT. Fractionated RT in the irradiated groups was delivered to distal femur and proximal tibia in the left legs of each rats to a total dose of 24 Gy in 3 fractions with the contralateral right leg as the nonirradiated control. Vitamin D3 injection in Group 3 was performed on the day before the RT. Bone growth was calculated according to the lengths of femur, tibia and total leg measured on the radiographs taken at the time and 6 weeks after the delivery of RT.*
RESULTS*: RT resulted in a mean percent overall limb growth loss of 56.2% ± 6.7% and a mean percent overall limb discrepancy of 12.7% ± 1.3%. Administration of 50000 IU/kg i.m. vitamin D3 before RT reduced the mean percent overall limb growth loss and the mean percent overall limb discrepancy to 28.5% ± 5.6% and 4.4% ± 3.3%, respectively. These values were significantly different compared with the groups receiving irradiation alone (P=0.001 for each).*
CONCLUSION: *In conclusion, these result demonstrated the potential for vitamin D3 administered before fractionated RT to significantly reduce the RT-induced epiphyseal injury.*
KEY WORDS*: Vitamin D3, radiotherapy, rat, epiphyseal injury, radioprotection.*

INTRODUCTION

In childhood cancers, the development of multi-drug treatment principles, the improvement of operation techniques and radiotherapy (RT), and the improvements in survival cause more children with cancer to reach adulthood (1,2). As a result, late effects due to the disease itself and its treatment have begun to be encountered in people with a prolonged life expectancy. RT, which is an effective treatment for skeletal and soft tissue sarcomas in childhood, may cause adverse effects on bone growth such as

deformity, shortness and asymmetry in the skeletal system due to radiation-induced epiphyseal plate damage, which may limit the amount of radiation that can be safely administered (2-4).

The success of cancer treatment is related to the therapeutic rate. The therapeutic ratio refers to the ratio of the dose that produces an antitumor effect in tumor cells to the dose that causes the same amount of damage to normal tissue. It is possible to increase the therapeutic rate in two ways: a) To increase the sensitivity of tumor cells to treatment, b) To reduce the negative effect on normal tissues.

Dose fractionation, hyperfractionation and the use of radioprotectant agents are recommended strategies to reduce the damage caused by RT on the epiphyseal plate (5-16). Studies have shown that hyperfractionation prevents bone growth loss more effectively than standard fractionation (9,10). However, clinical use of this method in young children is limited as it requires a large number of anesthetic sedation. The use of prophylactic radioprotectant before fractionated RT is an alternative method. In preclinical studies, radioprotectants such as amifostine and melatonin have been shown to significantly, if not completely, reduce bone growth retardation (17).

Many factors such as vascular changes and cellular changes after RT may play a role in the pathogenesis of growth plate changes. Cellular death and cytological changes of chondrocytes and osteoblasts, damage to vascular structures and decreased blood supply may result in growth loss.

Vitamin D3 is essential for normal bone development, calcium phosphorus homeostasis and mineralization. It plays an autocrine and paracrine role during endochondral bone development and contributes to longitudinal bone growth (18-20). It plays an important role in the regulation of growth, differentiation and functions in growth plate chondrocytes.

It has been shown to regulate osteoblast gene transcription, proliferation, differentiation and mineralization. It has been shown to increase neoangiogenesis by increasing the expression of vascular endothelial growth factor (VEGF) in chondrocytes at the time of vascular invasion and in osteoblasts (21,22). These effects suggest that vitamin D3 may reduce the damage caused by radiation in the growth plate and may be beneficial in providing the necessary blood flow for the repair of the damage. Vitamin D3, a growth factor, suggests that it can positively induce growth and protect bone and cartilage tissue after radiation-induced damage through growth activation. In addition, it has been reported that vitamin D3 has antineoplastic effects in breast, prostate, bladder, lung, colon, endometrium, kidney, ovary, pancreas, bone and brain carcinomas, soft tissue sarcomas, neuroblastoma and myeloid leukemia cells (23-25). This effect occurs through mechanisms such as suppression of cell proliferation, increased cell differentiation and apoptosis, and inhibition of angiogenesis. It has been shown that ionizing radiation and vitamin D3 have a synergistic effect in prostate cancer cells, and that the effects of RT and Vitamin D3 are added to each other and increase apoptosis in breast cancer cell culture (26). In human normal fibroblast cell culture, it was found that it did not have an additional effect on RT and did not increase apoptosis. These findings suggest that while vitamin D3 increases the sensitivity of cancer cells to radiation, it can provide protection in normal cells, thus increasing the therapeutic rate.

In this study, it was investigated whether vitamin D3 treatment given prophylactically in rats with epiphyseal plate irradiation reduces radiation-induced growth loss.

MATERIAL and METHODS

Study Groups and Experiment

This study was carried out by Baskent University Faculty of Medicine (BÜTF) Radiation Oncology Department in 2011. The study was approved by Baskent University Animal Experiments Ethics Committee (project no: DA10/26) and supported by Başkent University Research Fund.

Sprague-Dawley rats are an animal model for the study of skeletal growth effects, as the first 15 weeks of their life are the fastest period of their growth and are large enough to allow precise bone measurements while amplifying bone growth disorders (7,10). In the study, 28 Sprague-Dawley male rats, 6 weeks old, with an average weight of 74 g (53-101 g) were used. Rats were obtained from BÜTF Experimental Animals Research Laboratory. All rats were kept in plexiglass cages at a temperature of 20-22 0C, with 50-70% humidity, with the air changing 18 times per hour, and they were cleaned daily. Body weights of all rats were measured at the beginning of the study, every 72 hours, and at the end of the study. Before the study, 4 study groups of 7 rats were formed and animals in all experimental groups were numbered. 1st group control (group K), 2nd group RT only (group RT), 3rd group only 50000 IU/kg i.m. vitamin D3 (group VIT D), 4th group RT + 50000 IU/kg i.m. vitamin D3 (group RT + VIT D).

Rats were administered 50 mg/kg intraperitoneally (i.p.) ketamine and 7 mg/kg i.p. Anesthesia was provided by administering Xylazine. Vitamin D3 (Devit 3 ampoule, Deva İlaç, Istanbul, Turkey) treatment i.m. One day before RT, a single dose of 50000 IU/kg was administered to the third and fourth group rats. Control and RT control groups were also given i.m. 0.5 cc 0.9% NaCl was injected.

RT was applied to the rats in the second and fourth groups. Treatment areas of 3x3 cm were opened to cover the knee region (left femur distal ½ and left tibia proximal ½) of each rat under anesthesia. The right leg was preserved and evaluated as a control.Source-skin distance 100 cm using a centimeter bolus has been set. A total of 24 Gy fractionated RT was applied for 3 consecutive days using 6 MeV electrons (Linac DHX-3323, Varian Medical Systems, Palo Alto, CA, USA). The rats in the first and third groups were anesthetized with the same dose of anesthetics and sham RT was applied.

Measuring Leg Lengths

Before RT (day 0), each rat under anesthesia was fixed to lie on its back with adhesive tapes by stretching its front and hind legs on 20x30 cm prepared wooden boards. Direct radiographs of X-rays (40 mA, 80 kVp, 2.7 mAs) were visualized using a simulator device (Acuity, Varian Medical Systems, USA). Six weeks later, control radiographs were taken using the same technique. On the digital images, using the measuring ruler, each rat was measured from the most proximal points of the right and left femoral head to the most distal points of the femoral condyles at day 0 and week 6. Total leg length was recorded by calculating the sum of the measured femur and tibia lengths.

Evaluation of Bone Growth

The growth difference between irradiated and non-irradiated legs was used as the primary indicator of growth inhibition due to radiation exposure. Endpoints were calculated including actual growth (AG), percent growth loss (PGL), percent improved growth loss (PIGL), and percent leg length difference (PLLD).

Statistical analysis

Analyzes were performed using SPSS 20.0 statistical software (IBM SPSS Inc,

Chicago, IL, USA). All data were expressed as mean ± standard deviation. Statistical analysis was performed using factorial analysis of variance (ANOVA) method with bone length measurements as a variable to determine the main effect of each treatment. A p value of <0.05 was considered significant for all statistical evaluations.

RESULTS

No deaths due to radiation or drug administration were observed in the study. The right and left total leg (B) length measurements and weight gain values of the rats in each group, measured before RT (0) and 6 weeks after RT (6), are as in Table 1. As seen in the table, weight gain, vitamin D and RT groups were similar and there was no significant difference associated with prophylactic drug use (p=0.51). Bone lengths of the non-irradiated right extremities were similar in both groups after 6 weeks (p > 0.05). However, the right-left difference in final leg lengths was significantly different between the RT and VIT D + RT groups (p < 0.005), favoring the VIT D + RT group.

Table 1: Weight gain and femur, tibia and total leg growth data for each group

Parameters	GROUP 2	GROUP 4	P value
6 weeks weight gain (g)			
Mean±SD	47,7 ± 22,6	51,5 ± 14,5	0.51
Range	19,0-73,0	28,0-69,0	
Right and Left L_0 (mm)			
Mean±SD	47,1 ± 2,1	47,6 ± 1,7	0,22
Range	45,2-50,8	44,2-51,8	
Left L_6 (mm)			
Mean±SD	53,0 ± 1,1	57,1 ± 1,0	0,11
Range	50,2-54,2	56,6-59,4	
Right L_6 (mm)			
Mean±SD	61,3 ± 1,8	62,2 ± 0,5	0,19
Range	58,2-63,9	59,3-64,3	
Real Growth, Right L_6 (mm)			
Mean±SD	12,4 ± 2,5	13,7 ± 1,3	0,39
Range	10,6-13,7	10,7-15,8	
Real Growth, Left L_6 (mm)			
Mean±SD	4,2 ± 1,5	8,2 ± 1,2	<0,001
Range	2,0-6,6	4,9-11,2	
Real Growth, Left T_6 (mm)			
Mean±SD	1,8 ± 0,6	4,5 ± 0,9	<0,001
Range	1,0-2,6	2,6-6,1	
Real Growth, Left F_6 (mm)			
Mean±SD	2,4 ± 1,0	3,7 ± 0,7	<0,001
Range	1,2-4,0	2,8-4,8	
Right and Left L6 difference (mm)			
Mean±SD	8,3 ± 1,2	4,4 ± 1,8	<0,001
Range	6,2-10,2	1,1-6,4	

Abbreviations: L_0: Leg length before radiotherapy, L_6: 6 weeks leg length after radiotherapy, F6: Femur length 6 weeks after radiotherapy, T6: Tibia length for 6 weeks after radiotherapy, Group 2: RT, Group 4: RT + vitamin D3

In the left leg irradiated with fractionated RT, it caused a mean percentage of total leg growth loss of 56.2% ± 6.7%. Prophylactic use of VIT D brought the mean percentage of growth loss in the total leg to 28.5% ± 5.6%, and this value was found to be statistically significant (p = 0.001) (Figure-1**).** The mean percentages of growth loss for the femur and tibia in the RT alone group were 49.7% ± 7.6% and 62.3% ± 10.8%, respectively.

There was significantly less growth loss for the femur (31.5% ± 3.3%) and tibia (35.5 % ± 4.3%) in the VIT D + RT group (p= 0.001, for each).

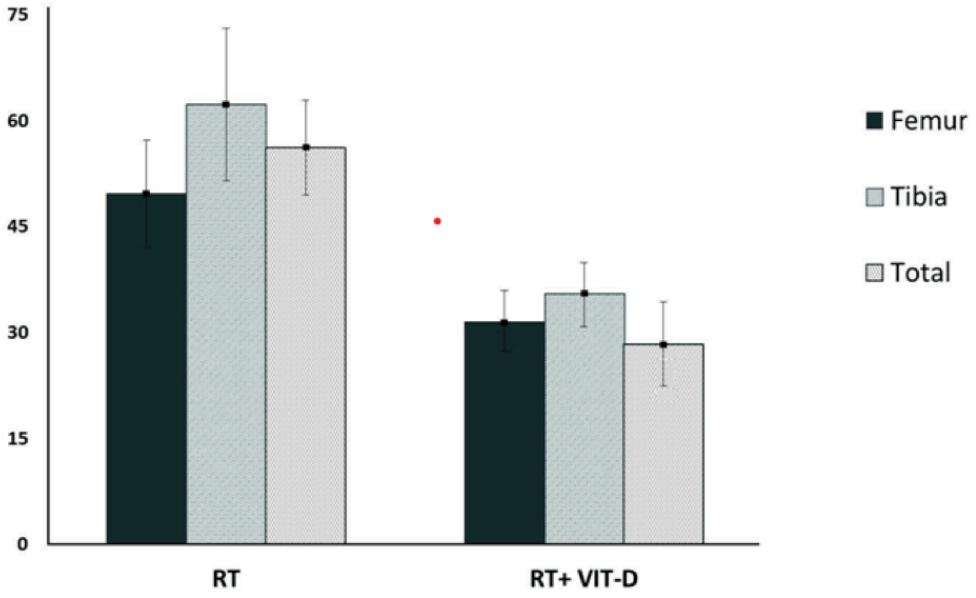

Figure 1: Radiation-induced growth loss as percent reduction in growth in the normal femur, tibia, and total leg relative to the treatment group

When the mean leg length difference values were examined, it was found to be 12.7% ± 1.3% in the RT group alone and 4.4% ± 3.03% in the VIT D + RT group (Figure2).

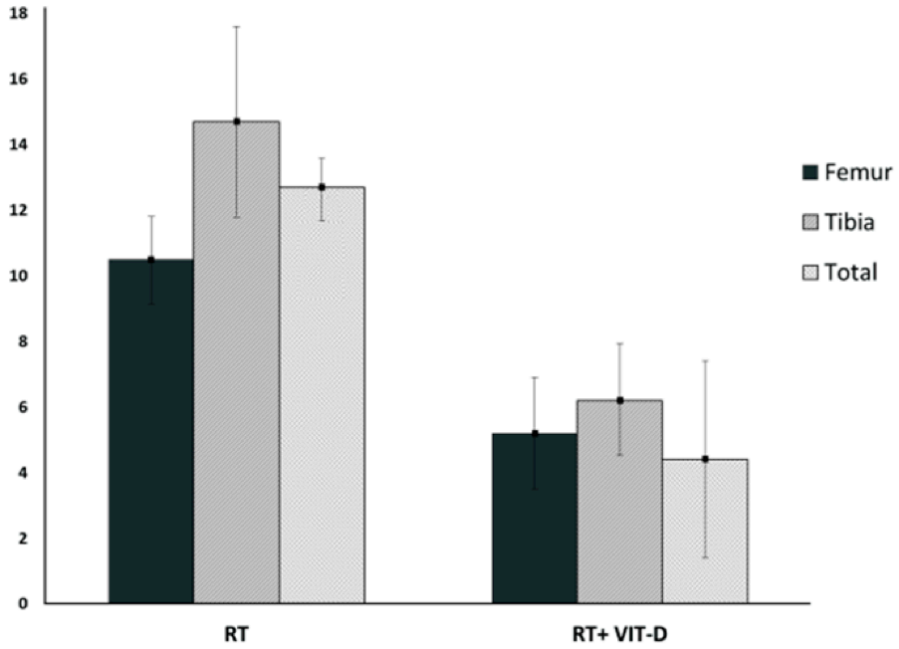

Figure 2. Radiation-induced leg length difference as percent reduction in growth in normal femur, tibia, and total leg compared to treatment group

Thus, the leg length difference was observed to be significantly less in the treatment group than in the RT group (p = 0.001). The leg length difference at the femur was similarly greater in the RT group than in the VIT D + RT group (10.6% ± 1.3%, 5.3% ± 1.7%, respectively). This difference is statistically significant (p = 0.001). The difference in leg length in the tibia was significantly greater in the RT group (14.7% ± 2.9%) than in the VIT D + RT group (6.1% ± 1.7%, p=0.001).

Compared to the group receiving RT alone, the percentage of growth loss improved with VIT D was 26.4% for the tibia, 33.8% for the femur, and 31.6% for the total leg. These values indicate that vitamin D3 treatment is significantly effective in improving growth loss caused by fractionated RT.

DISCUSSION

In this study, it was investigated whether pre-RT vitamin D3 application had a reducing effect on epiphyseal damage due to RT in rats that underwent 24 Gy (8 Gy/day) knee irradiation. While RT alone has been shown to shorten the femur, tibia and total leg lengths statistically significantly, pre-RT vitamin D3 application has been found to statistically protect the growth plate from the effects of radiation and increase bone growth.

Radiotherapy has an important place in the multimodal treatment of pediatric soft tissue and bone tumors. However, in the growing child, decreased bone growth when the epiphyseal plate was included in the RT field; Permanent shortness, shape deformity and late pathological fractures are the most important dose-limiting factors. To the best of our knowledge, there is no literature available on the possible efficacy of vitamin D3, a growth factor and bone formation regulator, in the prevention of RT-induced epiphyseal damage. However, Graham et al. (27) showed that vitamin D3 effectively reduced RT-induced lung injury. Based on this finding, we aimed to test whether this activity of vitamin D3, a bone growth

factor, would also be effective on the epiphyseal growth plate.

In this study, we showed that fractionated RT caused 56.2% ± 6.7% growth loss in the total leg, 49.7% ± 7.6% in the femur, and 62.3% ± 10.8% in the tibia. We found that the mean growth loss for the total leg (28.5% ± 5.6%), femur (31.5% ± 3.3%), and tibia (35.5 ± 4.3%) was significantly less with prophylactic vitamin D3 administration (p=0.001, each for). There is no similar study in the literature regarding the combination of vitamin D3 and RT. Therefore, it is not possible to make a meaningful comparison. However, these findings can be compared with the results of studies with other potent radioprotectants. In the literature, there are studies on amifostine and melatonin, which have shown protective properties against the damage caused by RT on the epiphyseal plate (6,12,17,28).

It is not possible to clearly interpret the radioprotectant effect that we have shown in this study, since there is no morphological and biochemical study. However, we think that one of the possible mechanisms may be the role of vitamin D in the regulation of growth, differentiation and functions in growth plate chondrocytes. Vitamin D3 inhibits metabolite proliferation in the growth zone, while stimulating alkaline phosphatase activity, collagen synthesis and proteoglycan synthesis (29,30). Another possible mechanism is the effect of vitamin D3 on osteoblast gene transcription, proliferation, differentiation and mineralization (31,32).

Li et al. (33) showed that VEGF mRNA expression, which causes VEGF secretion in human osteoblast-like cells, increased with vitamin D3 in their in vivo studies. These results suggest that vitamin D3 can prevent vascular changes in epiphyseal damage due to RT by forming a large amount of new capillaries, giving the image that it may be the underlying cause of our findings.

No toxicity was observed with the depot 50000 IU/kg dose of vitamin D3 used in this study. In studies investigating the effects of vitamin D3 on bone healing in rats, doses of 60 ng/kg/day and 2.5 *μ*/kg/day, 1.25 *μ*/kg/day, 0.125 *μ*/kg/day and 50000 IU/kg were used as in our study. No toxicity was found at doses (34,35).

In conclusion, this study shows that vitamin D3 can significantly reduce epiphyseal plate damage due to RT. However, it will be possible to reach definite results with clinical studies. We believe that it is worth investigating in the future whether vitamin D3 is increased to higher doses with standard fractionated RT schemes and/or the addition of radioprotectants such as amifostine and melatonin will increase the protective efficacy.

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