Original Article

Effect of Melatonin on Bone Mineral Density of Irradiated Rats

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Abstract

Objective: Melatonin is a powerful endogenous antioxidant and it may play a role in prevention of radiation-induced damage. The aim of this study was to investigate the effect of melatonin on bone mineral density in rats receiving radiation.

Materials and Methods: Sprague Dawley rats were divided into four groups. Group 1 (control group) received neither melatonin nor radiation (control group). Group 2 (Mel group) was administered intraperitoneal injections of 5mg/kg melatonin daily for ten days. Group 3 (RT group) and Group 4 were exposed to total cranium radiation of 5 Gy in a single dose by using a cobalt-60 teletherapy unit. In addition to irradiation, group 4 (RT + Mel group) was administered 5mg/kg of melatonin intraperitoneally. At the end of the 10th day, the rats' cranium and vertebrae bone mineral densities (BMDs) were measured.

Results: When cranial BMDs were evaluated, statistically more significant BMD increases were seen in the Mel group and the RT + Mel groups than in the control group. No significant difference was seen in the Mel group versus the RT + Mel group; however, there was a significant difference between RT and RT + Mel groups. When vertebral BMDs were evaluated, the only significant difference was found between the control and Mel groups.

Conclusion: We think that melatonin is a radioprotective agent. However, we would like to emphasize that further studies are needed before clinical trials with melatonin are initiated.

Keywords: Melatonin, Bone Mineral Density, Rat, Irradiation

Introduction

The secretion of melatonin, a major pineal hormone, is regulated by neuronal inputs from the suprachiasmatic nucleus and displays a distinct daily rhythm that is linked with sleep. Melatonin also has regulatory actions on sexual activity and development (1), immunomodulation (2), and cardiovascular functions (3). In addition to these, it is a potent antioxidant (4) and has been suggested to possess antiaging (5) and oncostatic (6) properties. Recent data report that melatonin has an effect on bone remodeling (7). In 2002, Ladizesky et al. (7) found that melatonin augmented bone the bony area of spine and bone mineral content of the whole skeleton and tibia.

On the other hand, presence of radiation injury to the bone is known. Cranial irradiation has also been implicated in osteopenia, although the mechanism is unknown (8). Gait abnormalities and frequent fractures are well-documented consequences of osteopenia following treatments for acute lymphoblastic leukemia, brain tumors and various other solid tumors (9,10). This adverse effect contributes to the burden of morbidity in survivors of childhood cancer. Effective management and ultimate prevention of osteopenia would require the identification of etiologic factors and a clear understanding of the pathogenesis of osteopenia. Hence, the hypothesis tested in our animal model was to examine the effects of low-dose irradiation and melatonin on bone mineral density.

Materials and Methods

This study was performed in the Department of Pharmacology Experimental Animal Laboratory at Atatürk University's Medical School, and approved by the local animal committee for animal experiments. Thirty-two albino female Sprague

Dawley rats, 8-12 weeks old, weighing 185 ± 35g were used. The rats were quarantined for at least one day before irradiation, housed eight to a cage in a windowless laboratory room with automatic temperature (22 ± 1ºC) and lighting controls (12 hours light / 12 hours dark), and were fed standard solid rat chow and water ad *libitum*.

The rats were randomly divided into four equal groups of eight animals. Group 1 (control group) did not receive melatonin or irradiation but received both 0.1 ml saline intraperitoneally (IP) and sham-irradiation. Group 2 (Mel group) only received 5mg/kg/day melatonin IP plus sham irradiation. Group 3 (RT group) received a single dose of total cranial gamma radiation of 5 Gy plus 0.1 ml saline IP. Group 4 (RT+Mel group) received irradiation to the total cranium plus 5 mg/kg/day melatonin IP.

In order to evaluate the effect of low-dose irradiation (11), rats in the Mel and RT+Mel groups received 5 mg/kg/day of melatonin (Sigma-Aldrich Co, USA) thru daily intraperitoneal injections. First melatonin dose was given 30 minutes before the first radiation dose and continuing daily doses thereafter for a total of 10 days. The control and RT groups were both given daily IP injections of 0.1 ml saline starting before irradiation and continuing daily thereafter for a total of 10 days.

Prior to total cranium irradiation, the rats were anesthetized with 80 mg/kg ketamin HCl (Pfizer İlaçları Ltd, Turkey) and placed on a Plexiglas tray in prone position. While the rats in the control and Mel groups recieved sham irradiation, the rats in the RT and RT+Mel groups were irradiated using a Picker C/9 Cobalt-60 teletherapy unit (Picker Int, Cleveland, OH, USA). They received a total-cranium, 5 Gy, single-fraction dose of radiation with a source-skin distance of 80 cm, and a 5×5 cm anterior field. Five Gy was used for a low-dose cranial irradiation (11) and was targeted at the skull surface in the central axis so that the whole skull was irradiated. The dose was calculated for the central axis at a depth of 0.5 cm, and its rate was 0.59 Gy/minute.

Body bone mineral density (BMD) measurements were performed using dual photon X-ray absorptiometry (DEXA) with a Hologic QDR 4500 Elite bone densitometer (Hologic Inc, Bedford, MA, USA) set at small animal mode/high resolution. The rats were placed on the middle of the measurement table, and a series of transverse scans were obtained from the tip of the nose to the midpoint of their tails. BMD measurements were determined at the time of euthanasia. All images were processed by the same investigator and analyzed using the same method to minimize operational errors. Box-

es were drawn to limit the area of interest. Scan results for the skull and columnar vertebrae were expressed as BMD in grams per cm^2.

SPSS version 10.0 statistical software package for PC was used for the our statistical analysis. Data were expressed as mean ± SD. Mean values of different parameters were compared using the Mann-Whitney U test. A p-value less than 0.05 was considered significant.

Results

The mean BMD measurements and statistical comparisons of the groups are shown in table 1. On day 10, control and gamma radiation groups respectively presented the lowest and the highest cranial and vertebral BMD levels. Although cranial BMD was significantly increased following cranial irradiation and melatonin administration, vertebral BMD was significantly raised after melatonin administration only (Table 1). Melatonin affected both cranial and vertebral BMDs.

Table 1: Descriptive values and statistical comparisons of groups

	Cranial BMD Mean ± SD	Vertebral BMD Mean ± SD
Control	193.3 ± 11.7	168.8 ± 12.2
RT	247.6 ± 26.7	186.9 ± 17.3
Mel	222.4 ± 7.7	183.7 ± 12.7
Mel+RT	226.9 ± 11.5	175.4 ± 16.9
P-Value		
Control-RT	0.01	NS
Control-Mel	0.01	0.01
Control-Mel+RT	0.01	NS
RT-Mel	0.03	NS
RT-Mel+RT	0.02	NS
Mel-Mel+RT	NS	NS

NS: not significant

Discussion

The sole purpose of this study was to examine the effects of low-dose irradiation on bone mineral density in a Sprague Dawley rat model. We expected that irradiation caused bone mineral loss. However, we found that a single-dose irradiation of 5 Gy alone increased cranial BMD but not vertebral BMD in the RT group versus the control group. Inhibitory effects of irradiation on osteoclasts were also well described in previous studies (12, 13). According to animal study observations made by Fukuka et al. (14), the convex curves of total BMD, trabecular BMD, bone volume and trabecular thickness in the different radiation dose

groups show that these values increased after low dose and decreased after high dose irradiation when compared to those of the control group. Irradiation in this animal model did not produce the more commonly observed problem of decreased BMD associated with postirradiation fractures (14). Margulies et al. (15) found that statistically significant increases in overall radiation-field BMDs were seen in their irradiation (single-dose 17.5 Gy radiation) and irradiation plus amifostine treatment groups; these increases were observed in the right irradiated legs and compared to the control unirradiated left legs of the animals at all time points from 0.5 through 6 weeks. In the same study, a statistically significant difference between the 2 treatment groups, Rad and Rad + amifostine, was observed only at 3 weeks post treatment (15), and concurrent histologic examinations suggested that there was a transient inhibition of osteoclast and chondroclast function during the first 2 weeks after radiation administration. In our last referenced study (16), in agreement with our results, the radioprotective effect of sodium selenite on the bone-repair process in the irradiated tibiae was evaluated and it was found that the selenium treated group and the 8 Gy gamma irradiated group presented significantly higher bone volumetric density than the control group at 14 days after treatment. However, while no significant differences were observed among the post treatment groups at 21 days, there were significant differences between the gamma radiation and control groups at 28 days in that report (16). Our results are possibly caused by several reasons. First, a relatively low-dose irradiation was used in this study. Second, the irradiation induced higher BMD was a transient and an early effect. Therefore, we can say that a 5 Gy single-dose of irradiation in this rat experiment does not provide an adequate model of radiation-induced osteopenia.

Various methods have been proposed for increasing bone mineral density and/or preventing bone mineral loss. One of investigated methods during the past few years is melatonin supplementation. Roth et al. (17) reported that melatonin was capable of promoting differentiation and mineralization of osteoblast cells grown in rat cultures.

When cranial BMDs were evaluated in our study, statistically more significant BMD increases were seen in the Mel and RT + Mel groups than in the control group. No significant difference was seen in the Mel group versus the RT + Mel group, although there was a significant difference between RT and RT + Mel groups. When vertebral BMDs were evaluated, the only significant difference was found between the control and Mel groups. As ex-

pected, cranial irradiation only affected the irradiated regions. Because it increased both cranial and vertebral BMDs, we think the effect of melatonin on BMD is a systemic effect which may also be permanent. Our findings provide the conclusive evidence that melatonin treatment alone could lead to BMD increase in rats. The physiological basis of melatonin effect on bone metabolism remains to be determined. *In vitro* studies give credit to the existence of significant direct effects of melation on bone (18). Melatonin stimulateds in vitro human osteoblast proliferation and differenciation (19). It dose-dependently augments procollagen type l C-peptide production in human bone cells and human osteoblastic cell lines (19). Another possible target cell for melatonin is the osteoclast. Osteoclasts use a variety of chemical agents to degrade bone. One important component of this process is the generation of free radicals (20). Osteoclasts generate high levels of superoxide anions during bone resorption which contributes to the degradative process (21). Melatonin is a significant free-radical scavenger and antioxidant at both physiological and pharmacological concentrations. Besides its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, melatonin stimulates several antioxidative enzymes which increase its efficiency as an antioxidant (18, 22). Umegaki et al. (23) reported that radiation's oxidative damage significantly increased at 3 to 5 hours after a total body irradiation with 3 Gy, with maximum levels occurring at 24 hours and gradually decreasing up to 144 hours post irradiation of the bone marrow. Also, they suggested that the onset of oxidative damage due to total body irradiation did not occur immediately after total body irradiation, and that the damage appeared at different times in different tissues (23).

Conclusion

In our study, Mel and RT + Mel groups had significantly lower BMD levels than the control group. Thus, we think that melatonin is a radioprotective agent. However, we emphasize that further studies are needed before clinical trials of melatonin supplementation. Whether melatonin can be used as a novel mode of therapy to achieve bone mass augmentation in diseases characterized by low bone density or for preventing bone mineral loss remain to be established.

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any product, service and/or company that could be construed as influencing the position presented in our study.

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