ALENDRONATE INCREASES TRABECULAR DENSITY BUT DOES NOT AFFECT BONE GROWTH FOLLOWING RADIOTHERAPY IN YOUNG RATS


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**BACKGROUND:**
Bisphosphonates are generally known to suppress bone resorption in adult bone leading to increases in macroscopic bone mineral density (BMD). Their effects in growing bone are less widely studied. In contrast, the effects of radiotherapy on growing bone has recently been under increasing study with an eye to preventing growth loss and deformity that can occur in children undergoing such therapy. The possibility of using bisphosphonates to prevent early osteoporosis in survivors of pediatric cancers, raises questions about the combined effects of radiotherapy and bisphosphonates in growing bone. Our previous study with the bisphosphonate, alendronate (ALN) (without radiation) suggested negligible effects on bone length, but large increases in BMD in normal growing bone in rats. This follow-on study looked at the influence of alendronate on bone growth and density following radiation treatment. We asked whether ALN would exaggerate or reduce the growth loss due to radiation. We also wanted to describe micro-radiographically the effects of ALN on the metaphyseal trabecular bone.

**METHODS:**
Eight male Sprague-Dawley rats, 5 weeks of age, were exposed to a single dose of 15.7 Gy external beam x-irradiation (300 kV, 10 mA, 285 RADs/min.) to the distal femur and proximal tibia of the right hind limb. In this model, used in a number of prior studies, the left hind limbs serve as non-irradiated controls. Half the animals received in addition, weekly injections of alendronate (0.3 mg/kg, subcutaneous, Merck) starting 1 week after irradiation. The animals were euthanized 6 weeks after radiation (2 days after the final ALN dose), and the hind limbs were disarticulated and bone lengths measured from contact radiographs. The proximal tibiae were removed and split sagittally and fixed in neutral formalin. The hemi-sections were then scanned by micro-CT (Scanco µCT40) using 20 µm serial axial cuts and sagittal reconstructions.

**RESULTS:**
The final tibial and femoral bone lengths (fig. 1) were reduced by the radiation by 14% and 17% respectively, consistent with previous observations (p<0.01). These radiation-induced reductions were unchanged by the addition of alendronate. Bone lengths were slightly shorter on average in alendronate treated animals but only in the non-irradiated limbs (femur 2.2% p<0.05; tibia 4.4% p<0.02).

The most interesting and dramatic response was seen in the metaphyseal trabecular bone where most of the longitudinal growth occurs (fig 2). In the ALN group, the trabecular bone below the growth plate was remarkably dense with many fine, longitudinal spicules. In the non-irradiated leg the density banding corresponding to the weekly ALN dosing was clearly evident, while in the irradiated limbs, the trabeculae were poorly organized, especially in the early post-radiation growth period (more distal). This is when longitudinal growth slows considerably, resulting in about half of the normal growth before corresponding control without radiation or ALN. The growth plate and subchondral bone appears thinner and less uniform, even at 6 weeks, but not very different from control at this level.

**DISCUSSION:**
Therapeutic levels of irradiation tends to reduce chondrocyte proliferation, hasten hypertrophic terminal differentiation and increase transient extracellular matrix production. The findings in this experiment suggests that bisphosphonate treatment does not significantly reduce, or enhance these sequela, but instead markedly affects the formation and remodeling of the subchondral metaphyseal bone as growth recovers. This is somewhat surprising given the complexity of the paracrine controls of growth plate function and recovery and the potential contribution of factors from metaphyseal bone remodeling and vascular ingrowth.

**CONCLUSION:**
Alendronate administration during growth dramatically altered trabecular morphology and led to remarkable increases in trabecular density in both irradiated and non-irradiated growing rat tibiae. It did not, however, appear to alter the growth loss induced by the radiation in a substantial way. This suggests that bisphosphonates may have a role in preserving bone density (and strength) after radiotherapy for childhood cancers.

![Fig. 1: Bone length 6 weeks after radiotherapy to the right hind limbs with and without weekly ALN. Left limbs were non irradiated in both groups. (mean ± SD)](image)

![Fig. 2: Micro-CT sagittal views of proximal rat tibiae; A. Radiation + ALN; B. ALN only; C. Radiation only; D. Non-irradiated control.](image)


**Acknowledgements:** NIH/NCI R01CA083892-04(TAD); M.J. Allen, VetMB, Ph.D, Rebecca. Hickman, & John. Lee for µCT assistance, & Gideon Rodan MD (Merck) for the alendronate.