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Effects of Amifostine and Zoledronic Acid on Radiation-Associated Bone Damage in a Pediatric Mouse Model

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Background: While radiation therapy is an effective treatment option for management of metastatic disease and surgical adjunct for soft tissue sarcoma, many patients will experience radiation-associated damage to normal tissues surrounding the targeted area. Bone damage is characterized by osteocyte death and loss of mechanical strength. Pediatric patients may suffer additional complications if epiphyseal tissue is included within the radiation field, including limb length discrepancies and angular deformities. Inclusion of underlying bone within the irradiation field is often unavoidable, and clinical interventions to prevent bone damage are largely limited to fractionation and beam collimation.

Purpose: The goal of this study was to explore the potential for two pharmacologic agents to prevent radiation-induced bone damage in a pediatric mouse model. Specifically, we evaluated the ability of amifostine and zoledronic acid (individually and in combination) to maintain bone strength, quantity, and quality following limited field irradiation in a pediatric mouse model.

Methods: Female BALB/CJ mice aged four weeks (Jackson Labs, Bar Harbor, ME) were anesthetized and exposed to unilateral hindlimb irradiation (RTx), delivered as four consecutive daily fractions of 5 Gy each (4x5 Gy at 300 kV and 10 mA). The non-irradiated hindlimb (0 Gy) served as a control. Mice were assigned to one of four treatment groups: vehicle, amifostine (AMF), zoledronate (ZA), or AMF + ZA. AMF or vehicle was delivered 20-30 minutes prior to RTx (100 mg/kg, IP). ZA (or vehicle) was given 4 days prior to the first RTx, and once weekly for 3 weeks thereafter (100 ug/kg, SC). Mice were euthanized at 0, 1, 2, 4, 8, 12, and 26 weeks post-RTx (n=7 mice/group/time point). The femurs were imaged by micro-computed tomography (micro-CT) for 3-D quantification of bone volume, bone mineral density, and cross-sectional morphology over a 1 mm (axial height) section of the mid-diaphysis. Following this, the mechanical strength of the femurs was assessed by three-point bending. Femurs were placed in the testing fixture (8 mm support span), and loaded in the posterior-anterior at a rate of 1 mm per minute with simultaneous image capture. Outcome measures included bending strength, stiffness, and flexural strength. For each sample, cross-sectional geometric parameters at the point of loading were determined from the reconstructed micro-CT images using the BoneJ plugin to ImageJ, and included cross-sectional area, mean cortical thickness, second areal moments of inertia, and section moduli).

Results: Radiation did not significantly alter mid-diaphyseal bone volume (BV) or cross-sectional area (CSA), but did induce a decrease in bone mineral density (BMD) over weeks 4-26 and an increase in mean cortex thickness. Delivery of ZA did not consistently alter BV, but further decreased BMD between 8 and 26 weeks post-RTx. CSA in irradiated femurs was increased by ZA treatment over weeks 4-12 and restored cortex thickness comparable to non-RTx controls. There was no persistent effect on post-RTx BV, BMD, CSA, or cortical thickness resulting from AMF delivery. Bending strength was decreased at 26 weeks post-RTx, and ZA treatment increased post-RTx bending strength over weeks 4-26 to near control (0 Gy) levels (Figure 1). Bending stiffness followed a similar pattern, demonstrating an RTx-associated decrease at 12 and 26 weeks that could be rescued with ZA treatment. AMF did not alter mechanical properties long-term in irradiated femurs. Flexural strength was decreased following irradiation, but was not restored by ZA or AMF treatment (Figure 2).

Conclusions: In this juvenile animal model, delivery of AMF prior to each radiation dose did not significantly affect femoral cortical bone morphology or mechanical strength. Treatment with ZA resulted improved bending strength of irradiated femurs by increasing the quantity of bone present. Delivery of ZA did not improve flexural strength of irradiated femurs, indicating that the material properties remain diminished by irradiation. Increasing bone quantity may be an effective early intervention to preventing radiation-associated fragility fractures. Long-term benefits of this approach may, however, be diminished as the bone remodels and resorbs. Furthermore, some bone matrix modifications, such as non-enzymatic collagen cross-links, are known to alter both mechanical

properties and rates of osteoclast-mediated bone turnover. Identifying specific radiation-associated matrix alterations could provide an additional area for clinical interventions to prevent fragility fractures.

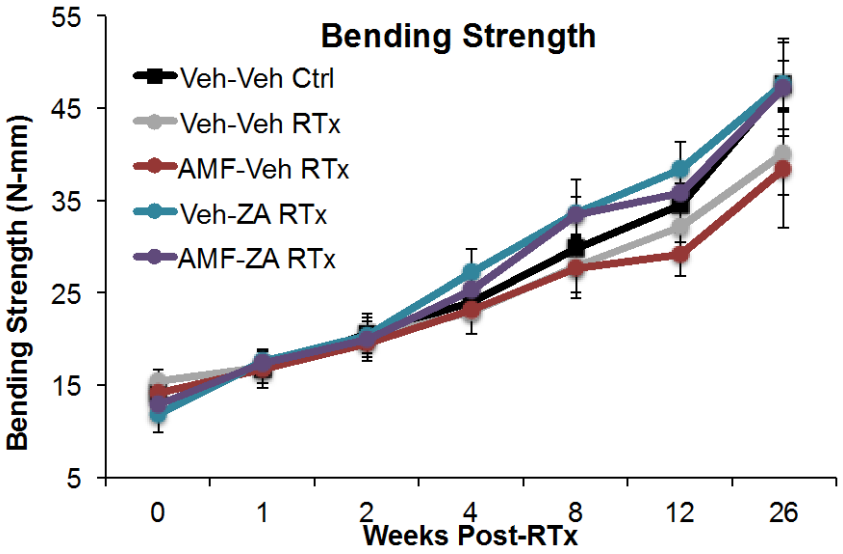

Figure 1: Bending strength of femurs following radiation. Delivery of zoledronate facilitates long-term recovery of bending strength to near control (0 Gy) levels through addition of bone matrix.

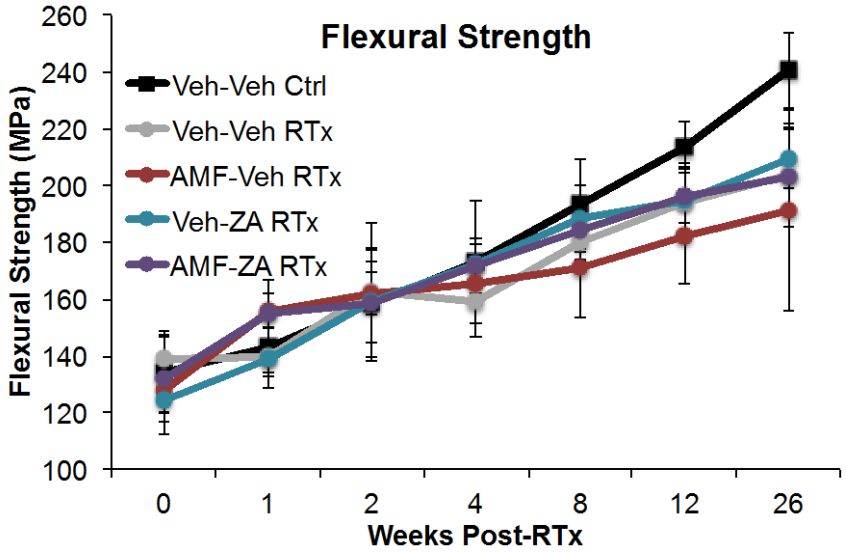

Figure 2: Flexural strength of femurs following radiation. Zoledronate delivery does not recover flexural strength of the bone post-RTx, suggesting that ZA treatment does not attenuate radiation-induced alteration of the bone matrix material properties.