

Effect of Palliative Dose Radiotherapy on Rat Vertebral Bone Architecture, Composition, and Mechanical Properties

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INTRODUCTION: Vertebral bone metastases, highly prevalent in patients with cancer, result in pathologic vertebral fracture (PVF) in up to 30% of affected vertebrae. Palliative Radiotherapy (RTx), applying ionizing radiation as high as 50 Grays (Gys), is commonly employed to help relieve pain and to offer local control of the metastases [1]. However, previous studies demonstrated that RTx deleteriously affects human bone fragility and is a significant risk factor for PVF [2, 3]. In murine hindlimb, the application of fractionated RTx degraded bone spatial architectural parameters, altered bone mass, collagen, and mineral chemistry, and increased apoptotic osteocytes [4-6]. By contrast, the temporal effect of a high irradiation dose, simulating palliative treatment on rat vertebral bone quality, remains unclear. We hypothesize that a clinically relevant radiation dose, simulating single fraction RTx, causes a progressive temporal decline in vertebral bone architecture, composition, and mechanical properties.

METHODS: Thirty-six male 15-weeks-old Sprague Dawley rats received a radiation dose (RTx group) of 15.5 Gy to the lumbar spine using a small animal X-ray irradiator (2 Gy/min, X-Rad320, Precision, CT). A separate group of control rats at 15-weeks (0-day) and 19-weeks-old (28-day) received sham treatment to the same region to quantify age-related changes during the study. Animals were euthanized at 7, 14, and 28 days post-RTx for the RTx group and at 0- and 28- days for the control group. The spines were removed un-block (L1-L6), and the L4 vertebra dissected. Each vertebra was imaged with micro-computed tomography (μ CT) at 10.2 μ m (Bruker Skyscan 1276), reconstructed, aligned, and analyzed to obtain vertebral bone mineral density (BMD), bone volume fraction (BV/TV), and trabecular bone architectural parameters [thickness: Tb.Th; spacing: Tb.Sp; and number: Tb.N]. The vertebral endplates were removed to create a plano-parallel segment, and the vertebral segment was tested in unconfined compression to measure strength and stiffness at a rate of 0.033 mm/s (ElectroForce 3200, TA Instruments, DE). Accumulation of advanced glycation endproducts (AGEs) and collagen content were measured using a colorimetric assay [7]. The following analyses (JMP Pro 14, SAS, NC) were performed based on normality and homogeneity of variances tests: 1) Welch's ANOVA was used to test for differences among the groups in bone architecture, mechanical properties and AGEs, 2) Steel-Dwass non-parametric post-hoc comparisons to test for differences between specific groups (i.e. 0-day control, 7, 14, and 28 days post-RTx group and 28-day control) on bone architecture, mechanical properties, and AGEs. Statistical significance was set at 5%.

RESULTS: Across the experimental time points (Welch's ANOVA), RTx resulted in a continual decrease in bone mineral density ($p=0.0015$), BV/TV ($p=0.008$), and trabecular number ($p=0.0026$), while trabecular separation increased ($p=0.0065$), Fig 1. Comparison of the 28-d control and irradiated animals showed RTx resulted in lower BMD ($p<0.0012$), BV/TV ($p<0.0001$), and Tb.N ($p<0.0013$) but higher Tb.Sp ($p<0.0051$). Similarly, collagen content declined with RTx ($p<0.0389$), while AGEs ($p<0.0009$) increased, Fig 2. We found no statistically significant differences for Tb.Th ($p=0.5946$). Compared to the 28-d control, the irradiated vertebrae showed lower stiffness ($p<0.0012$) and strength ($p<0.0003$), Fig 3. Although the analysis indicated overall significant differences for trabecular thickness ($p=0.0150$) and AGEs ($p=0.0165$), Fig 2., the post hoc analysis was not significant comparing the changes at 7d and 14 (an increase) and 28d (a marked decrease) post RTx with 0d group. For the mechanical properties, Fig 3, we found irradiation to yield a continual decrease in stiffness ($p<0.0001$) and strength ($p<0.0001$) with time. Post hoc analysis found these differences predominantly manifested in the longest-time group (28d), Figs 1- 3.

DISCUSSION: Using a rat model for palliative irradiation, this study demonstrates that high-dose RTx temporally yields rapid deterioration in bone stiffness and strength while highlighting a concomitant compromised in both bone architecture and composition. The initial early increase in trabecular thickness and increased variation in the collagen crosslinking post-RTx suggest a compensatory remodeling mechanism in response to the decline in trabecular number and bone volume fraction. Future work will include quantifying histological and serum markers of bone remodeling, bone mineral crystallinity, osteocyte apoptosis, and assessment of RTx effect at other skeletal sites.

SIGNIFICANCE/CLINICAL RELEVANCE: Palliative radiotherapy, RTx, routinely used in advanced cancer patients, has been linked with increased vertebral fracture risk. Here, we show for the first time that a clinically relevant RTx dose in a healthy rodent model decreases bone mass, deteriorates bone architecture and composition, and consequently decreases resistance to fracture, underlying the increased risk observed in patients.

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