A MOUSE MODEL OF BONE LOSS AND MECHANICAL COMPROMISE SECONDARY TO RADIATION THERAPY

+1Allen, MJ; 2Fisher, ER; 2Ku, CW; 3Hsie, M; 3Bogart JA; 3Damron TA; 3Mann, KA
+4College of Veterinary Medicine, The Ohio State University, Columbus, OH, 2SUNY Upstate Medical University, Syracuse, NY
Corresponding author: matthew.allen@cvm.osu.edu

Introduction: Radiation therapy (RTX) is effective in controlling the growth of many tumors but clinical studies have shown that irradiation of the adult skeleton may be associated with long-term skeletal complications such as osteoradionecrosis, pathological fracture and, in rare cases, radiation-associated osteosarcoma. There is often a significant delay (months to years) between the time of therapy and clinically evident bone disease. The mechanisms through which RTX causes long-term bone damage have not been determined and research in this field has been limited by the lack of a validated animal model. The specific aim of this study was therefore to develop a mouse model of radiation damage to bone and to define the temporal pattern of bone disease following RTX.

Methods: Under an IACUC-approved protocol, skeletally mature female Balb/c mice were anesthetized and the right hind limb irradiated with 5 Gy (n=21 mice) or 20 Gy (n=22 mice) using an orthovoltage X-ray unit (MGC-30; Philips Medical Systems). The left hind limb served as the non-irradiated control. Distal femoral bone mineral density (BMD) was determined by dual-energy X-ray absorptiometry (DEXA) immediately after radiation and at 2-week intervals throughout the course of the experiment. At the conclusion of the study period (2, 6, 12 or 26 weeks), groups of 4 to 6 animals per RTX dose were euthanized and the hind limbs examined using Faxitron high-resolution X-rays. The femur and tibia were then stripped of soft tissues, scanned in a µ-CT unit and submitted for either mechanical testing or histology. Axial compression tests to failure were conducted on a subset of all distal femurs to determine if there was a decrease in structural integrity with time for the irradiated (right) limbs. The proximal femur was potted in PMMA cement and secured to the cross-head of a mechanical test frame (Q-Test, MTS Corporation), while the distal femur was placed against a flat platen using a thin layer of PMMA cement to distribute load to both condyles. Specimens were loaded to failure and peak load and energy to failure were measured. Data was paired (radiation limb minus control limb, R-L) to allow for internal control. A total of 17 pairs of femora were tested (6@2wk, 5@6wk, 6@12wk).

Results: Radiographs of the femora exposed to 20 Gy were characterized by a consistent pattern of bone loss in the metaphyseal region, immediately proximal to the growth plate. BMD values in irradiated femora were significantly lower than those from non-irradiated femora at 26 weeks and 12 weeks (both p<0.05), but not at earlier time points. Micro-CT analysis (Figure 1) revealed significant decreases in trabecular bone volume fraction (BV/TV) at 6 weeks in the irradiated (right) limbs. The changes in bone microstructure were associated with statistically significant decreases in the mechanical properties of irradiated bones. In this mouse model, RTX produced significant decreases in trabecular bone volume and an associated increase in cortical bone volume within 6 weeks of treatment. The increase in bone density at the growth plate appears to be due to a relative increase in the fraction of cortical bone within the volume of interest. The loss of trabecular bone was reversible for low dose RTX (5 Gy), with BMD and µ-CT parameters normalizing by 12 weeks post-treatment. However, the higher radiation dose (20 Gy) was associated with reductions in trabecular bone that persisted for at least 6 months. Most importantly, the changes in bone microstructure were associated with statistically significant decreases in the mechanical properties of irradiated bones. Radiation has been shown to have negative effects on growing bone [1, 2] and fracture healing [3], but its effects on adult bone have been less well studied [4] and, to our knowledge, this is the first documented report of mechanical compromise following radiation therapy in an adult animal. Although further experiments will be needed to determine the mechanistic basis for these changes, the mouse model appears to provide a clinically relevant model for assessing radiation injury to adult bone, as well as for evaluating the efficacy of candidate therapies for protecting and/or repairing bone damage after RTX.

Discussion: In this mouse model, RTX produced significant decreases in trabecular bone volume and an associated increase in cortical bone volume within 6 weeks of treatment. The increase in bone density at the growth plate appears to be due to a relative increase in the fraction of cortical bone within the volume of interest. The loss of trabecular bone was reversible for low dose RTX (5 Gy), with BMD and µ-CT parameters normalizing by 12 weeks post-treatment. However, the higher radiation dose (20 Gy) was associated with reductions in trabecular bone that persisted for at least 6 months. Most importantly, the changes in bone microstructure were associated with statistically significant decreases in the mechanical properties of irradiated bones. Radiation has been shown to have negative effects on growing bone [1, 2] and fracture healing [3], but its effects on adult bone have been less well studied [4] and, to our knowledge, this is the first documented report of mechanical compromise following radiation therapy in an adult animal. Although further experiments will be needed to determine the mechanistic basis for these changes, the mouse model appears to provide a clinically relevant model for assessing radiation injury to adult bone, as well as for evaluating the efficacy of candidate therapies for protecting and/or repairing bone damage after RTX.

Table 1. BV/TV for metaphyseal trabecular bone illustrating marked bone loss with time. Mean and (standard deviation) values shown.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 wk</th>
<th>6 wk</th>
<th>12 wk</th>
<th>26 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Gy (control)</td>
<td>0.16 (0.03)</td>
<td>0.10 (0.01)</td>
<td>0.13 (0.03)</td>
<td>0.09 (0.05)</td>
</tr>
<tr>
<td>5 Gy (control)</td>
<td>0.18 (0.03)</td>
<td>0.16 (0.02)</td>
<td>0.18 (0.04)</td>
<td>0.12 (0.04)</td>
</tr>
</tbody>
</table>

Figure 2. Radiation leads to a reduction in structural properties of adult bone. Data represents mean ± SD for difference in energy to failure between irradiated (right) and control (left) femora.


Acknowledgement: Carol M. Baldwin Breast Cancer Research Foundation.