RADIATION INDUCES BRITTLE BONE FAILURE

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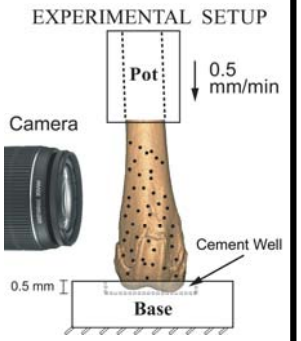
Clinical Issue and Approach

Radiation therapy is an effective treatment for the control of tumor growth. However, long-term complications from radiation therapy such as insufficiency fracture, osteonecrosis, and osteosarcoma are clinical challenges. The mechanism leading to these complications is unclear and research has been limited without rigorous animal model validation. Recent research in our lab led to the development of a mouse model which allows for an assessment of bony changes following radiation therapy. Here we developed an approach to quantify the new fracture surfaces created following mechanical loading to failure, with more fracture surfaces representing a more brittle bone material. The specific aims of this study are to: 1) measure fracture force of irradiated and control bones at different time points, 2) determine accurate energy to failure measures and 3) utilize computed tomography imaging techniques to quantify the bone crack surface generated after bone fracture.

Experimental Model

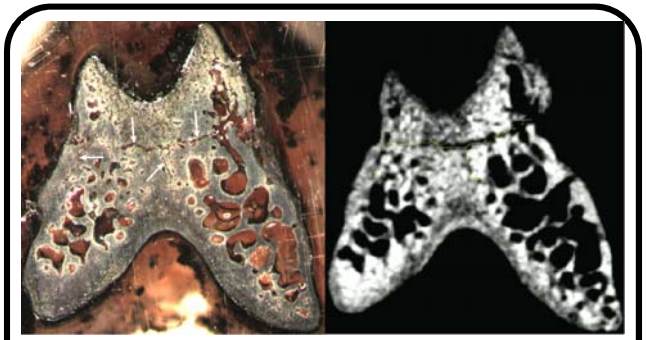
13 week old Balb/c mice were irradiated on the left hind limb with either 0 Gy (sham control- n=4) 5 Gy (n=16), 20 Gy (n=12) or fractionated 20 Gy (5 Gy over 4 days- n=6). Left limbs served as the non-irradiated control. At 2 and 6 weeks post-irradiation, 4-8 animals per radiation dose were euthanized, femora were explanted and the proximal ends potted in PMMA.

Axial compression tests to fracture (utilizing a failure criteria of a 30% reduction from peak load), using a mechanical test frame, were combined with digital image correlation to determine accurate energy to peak (E peak), energy to fail (E Fail) and post-peak energy (E Post Peak). The distal condyles were set into pre-polymerized PMMA, allowed to cure, and then loaded at 0.5 mm/min with 0.5 Hz image capture.



Crack Mapping With Validation

Fractured bones were CT scanned at 12 µm to assess the bone fracture surface over the distal 5 mm of the femur. Image sets were traced in Mimics (Materialise) with a one pixel wide tool in each image slice. Crack area was determined by multiplying total crack length by the inter-slice distance (12 µm). The technique was verified by two methods. First, 8 control bones were loaded to varying failure levels. The bones were then infiltrated and embedded using PMMA, and CT scanned for crack analysis. Each bone was sequentially ground and polished (0.05 µm alumina) to five separate locations, varying in distance from 0.5 to 3.5 mm from the condyles.

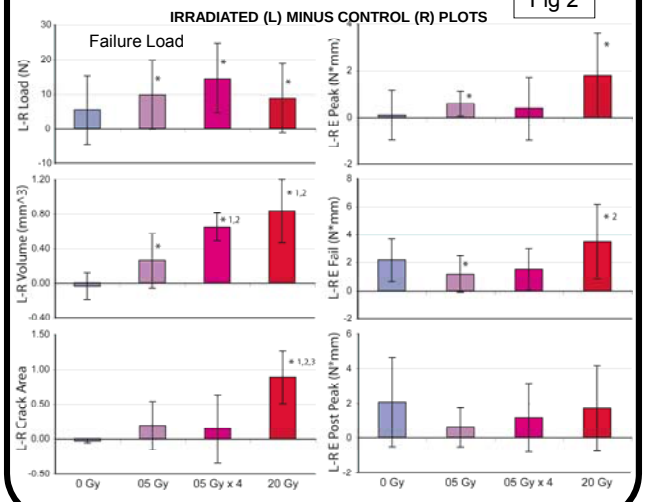


At each location the exposed bone was stained with silver nitrate, and high resolution images (2.8 µm resolution) were captured. Cracks were mapped optically for each slice and compared to measurements made by CT (**above**). Average error between the two techniques was 2.3%. Second, a repeatability study was conducted on the evaluation of cracks within a single CT image set. Over the course of 10 trials, the standard error of the mean was 0.0147 mm^2, or roughly 1%.

Results

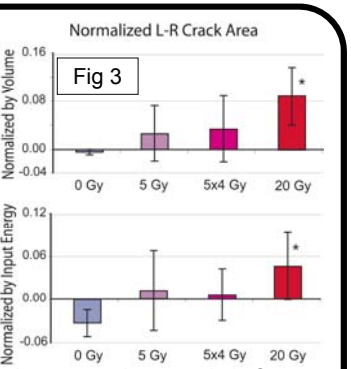
Results are presented as irradiated minus control (L -R). Radiation leads to increased femur strength (load capacity), volume, and crack area (**Figure 1**), when compared to non-irradiated, contralateral controls (**Figure 2**). There appears to be a dose dependency effect of radiation on volume, and a minimum threshold of 20 Gy to significantly result in more crack area. Further, irradiated bones require more energy to peak and energy to fail ($p < 0.05$).

Fig 2



There is no relationship between load level and crack area. ($R^2=0.057$, $p=0.174$). Even after normalizing for bone volume and energy to failure, the 20 Gy irradiated bones exhibited the most crack area (**Figure 3**).

Fig 3



Time course effects show that Irradiated femora have more bone volume at both 2 and 6 weeks compared to contralateral controls ($p<0.05$). They are also stronger at two weeks compared to controls (5 Gy- $p=0.011$, 5 Gy x 4- $p=0.0035$, 20 Gy- $p=0.013$). At six weeks, irradiated bones are no stronger than controls.

Fig 1

Discussion

Increased strength in irradiated bones at early time points is associated with an increase in bone volume and is likely due to early reduction in osteoclast activity, with the increased strength of bone resulting from increased density of bone (more, older bone) and new bone formation. However, bones are more brittle following radiation, with brittleness (crack area) dependent on radiation level. The amount of cracking is not an artifact of increased volume or energy necessary to fail the bones, as shown by normalization plots.

These results suggest that irradiated bones, although stronger and larger initially, have a material change that makes the bone more brittle at high radiation dosages. This material-change induced brittleness likely contributes to the observed increase in fracture incidence after clinical radiation therapy treatments.

Acknowledgements

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