Background: Radiotherapy as an adjuvant treatment modality is commonly used in the management of childhood extremity sarcomas. At therapeutic levels, radiation causes damage to the growth plates within the exposed fields. A variety of techniques are being investigated to preserve growth plate function in this setting. One of these techniques, a free radical scavenger radioprotector drug, amifostine (AMF) (S-2-[3-aminopropylamino]-ethylphosphorothioic acid) is known to protect normal cells at the expense of tumor cells. In order to better plan combination radioprotectant drug therapies, the mechanism of irradiation damage to the growth plate is being studied in detail. The effects on bone mineral density (BMD) of irradiation and the radioprotectant drug amifostine have not been previously reported.

Purpose. The purpose of this project was to examine the effects of irradiation on BMD of rat distal femurs with and without amifostine (AMF) pretreatment.

Methods. Seventy-two weanling Spague-Dawley rats were randomized into treatment groups. In all treated animals, the right knee (distal femur and proximal tibia) was irradiated with single fraction 17.5 Gy while the left leg was used as an internal control. Animals were sacrificed at 0.5, 1, 2, 3, 4, and 6 weeks following irradiation, 12 per time period. One half of the animals in each of the groups received 100 mg/kg amifostine twenty minutes prior to irradiation. After sacrifice, both lower extremities were harvested and skeletonized. Tibias were fixed in preparation for histological analysis (reported separately). For the bone density analysis, the skeletonized femurs were placed into a cylindrical holder and suspended in deionized water. Bone density (g/cm³) was determined using peripheral quantitative computed tomography (pQCT, Norland XCT 2000, Fort Atkinson, WI) at a resolution of 90 microns. Six 2.3 mm thick "slices" per specimen were measured with the pQCT. Slice 6 was located outside the irradiation field in the proximal proximal tibia. The remaining five slices were located within the field of irradiation in the distal femoral epiphysis and metaphysis. For analysis purposes, slice 1 (the most distal slice) was excluded due to its high variability. Slice 2 was also epiphyseal, slice 3 juxtaphyseal-metaphyseal, and slices 4 and 5 within the more proximal aspect of the distal femoral metaphysis. Area and density measurements were obtained for each of the slices from each specimen. Mean differences were analyzed using ANOVA, accepting a significance level of p<0.05.

Results. The control non-irradiated limb in slices 2 through 5 exhibited a relatively flat curve through 3 weeks followed by a significant (p<0.001) increase at 4 and 6 weeks. Slice 2 (epiphyseal) was nearly identical in the non-irradiated and irradiated limbs. However, in slices 3 through 5 weeks at slice 4, and through 3 weeks at slice 3. The slice closest to the metaphyseal side of the physis (slice 3) demonstrated a unique early peak BMD at 2 weeks which decreased dramatically by 3 weeks.(Fig. 1) This 2 week peak was also observed in the animals that received amifostine. However, the BMD for rats that received amifostine was significantly lower through 3 weeks than for rats that received radiation alone (p<0.02 for 0.5, 2, and 3 weeks).(Fig. 1) These BMD levels in the juxta-physeal region for the animals that received amifostine were close enough to the non-irradiated control limb BMD that there was no statistical difference. These positive effects of amifostine did not hold true for the slices farther away from the growth plate (slices 4 and 5). Slice 6, as expected, was unchanged by irradiation.

Discussion. Three conclusions are drawn from the current data. First, at early time periods following irradiation, BMD within the irradiation field is greater than control in this animal model. Second, a 2 week early peak in BMD occurs in the juxta-physseal metaphysis. Third, amifostine has a significant effect in holding BMD close to normal in the juxta-physseal region.

Concurrent histological examination of the irradiated proximal tibial physes of the treated and untreated rat limbs from these animals has suggested that there is at least a transient inhibition of osteoclast and chondroclast function in the first two weeks following radiation administration. Relatively increased matrix formation and provisional calcification is also seen during the same time period in the growth plate hypertrophic layer apparently unhindered by chondroclast resorption, resulting in an increased growth plate height (including the zone of provisional calcification) despite a diminished osteoclast function. Osteoclasts and chondroclasts then reappear histologically by the third week post-treatment concomitant with a reduction in overall growth plate height back toward normal.

The current densitometry data from the treated and untreated rats from these animals has suggested that within the juxta-physseal metaphysis increased provisional calcification is also seen during the same time period in the growth plate hypertrophic layer apparently unhindered by chondroclast resorption, resulting in an increased growth plate height (including the zone of provisional calcification) despite a diminished osteoclast function. Osteoclasts and chondroclasts then reappear histologically by the third week post-treatment concomitant with a reduction in overall growth plate height back toward normal.

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References.

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