Effectiveness of OP-1 in the Treatment of Radiation-Induced Atrophic Non-Union in a Rat Femoral Fracture Model

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INTRODUCTION:

The current management paradigm for soft tissue sarcoma via limb preservation often necessitates the use of multimodal treatment, including both surgery and external beam radiotherapy. Pathologic fracture is a serious, late complication of radiotherapy. In patients who have also undergone wide excision of soft tissue sarcoma, nonunion rates in the femur of 80–90% persist despite optimal internal fixation. No reliable biologic solution currently exists for this problem.

Many sequelae of the management of soft tissue sarcoma exhibit the potential to perpetuate failure of bony union. Limb salvage surgery may be associated with extensive periosteal excision and disruption of vascular supply, which can lead to eradication of local osteoprogenitor cells. External beam radiotherapy leads to obliterator derangements, decreased osteoblast proliferation and reduction in bone matrix production. Our previous work has demonstrated that combining a single 18 Gy dose of directed radiation and complete diaphyseal periosteal excision reliably generates atrophic non-union of femoral fractures in a rat model.

Osteogenic protein 1 (OP-1, or bone morphogenetic protein 7 (BMP-7)) is a member of the Transforming Growth Factor β (TGFβ) superfamily of proteins. It has been demonstrated to be effective at inducing alkaline phosphatase in osteogenic cells and in generating bone formation. OP-1 has also been shown to improve the rate of fracture union in patients with persistent non-union of long bone fractures. We hypothesized that augmentation of fracture fixation with OP-1 would lead to an increased rate of union in rat femoral fractures subjected to periosteal excision and external beam radiotherapy.

METHODS:

We previously validated an animal model of femoral non-union in patients undergoing combined modality treatment for soft tissue sarcoma. This model was applied to 144 female Sprague-Dawley, retired breeder rats, separated into four treatment groups of 36 animals each: control, combined therapy, control + OP-1 and combined therapy + OP-1. Animals were then further randomized to temporal end-points of 28, 35 and 42 days post-fracture, leaving 12 animals per sub-group. Animals were allowed a four-week adjustment period upon arrival to the housing facility prior to being entered into the study.

Animals in the combined therapy group first underwent external beam irradiation to a total of 18 Gy administered as a single fraction and focused on the left femur. Three weeks following irradiation, animals assigned to combined therapy groups underwent surgical excision of the periosteum along the entire left femoral diaphysis. Three weeks later, the left femurs of animals from all groups were prophylactically fixed with an intramedullary 1.25 mm K-wire and subjected to controlled fracture using a manually powered device patterned after those described in the literature. 80 μg of OP-1 was introduced directly into the femoral canal using a 20 gauge spinal needle immediately preceding prophylactic femoral fixation in animals so designated. Animals were sacrificed at their randomly assigned end-points. Samples were then analyzed using MicroCT, Back Scattered Electron Microscopy (BSE) and Histomorphometric modalities.

RESULTS:

Radiographic and gross examination of left femurs revealed a 0% union rate in rats from combined therapy groups, despite treatment with OP-1 in half of these animals. The overall union rate in animals not undergoing radiotherapy and periosteal excision was 98.6%. Heterotopic ossification was only observed in animals receiving OP-1.

MicroCT analysis revealed a significant decrease in Bone, Callus and Mineralized tissue volumes in irradiated femurs at all time points (p < 0.0001). At 42 days, a significant increase in callus formation was observed in irradiated animals receiving OP-1 (Figure 1). This callus was found to have significantly lower density than that formed by non-irradiated animals. Examination of 3D reconstructions of these samples revealed persistent non-union and the presence of heterotopic ossification, discontinuous with underlying bone.

Figure 1: Comparison of Mineralized Callus Volume (mm³) at 42 days between irradiated (green) and non-irradiated (blue) samples, with or without supplementary OP-1 (A). A baseline presence of callus is observed due to the edge effect present in digitally acquired images. Axial cuts through 3D reconstructed MicroCT scans of Control (B) and Irradiated (C) femurs at 42 days are pictured on the right. Sample C was treated with OP-1. Both atrophic non-union and heterotopic ossification can clearly be observed in sample C. Color scale represents density from low to high.

DISCUSSION/SIGNIFICANCE:

The lack of effectiveness of OP-1 at stimulating fracture union in this model suggests that the endogenous repair mechanism has been compromised beyond what can be compensated for with osteoinductive biologics. Further research into the underlying biology of osteoradionecrosis is still needed. The solution to this challenging clinical problem may lie instead in exogenous supplementation of that repair mechanism with administration of mesenchymal progenitor cells.

REFERENCES:


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