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BONE MARROW STEM CELLS

Bone marrow stem cells assuage radiation-induced damage in a murine model of distraction osteogenesis: A histomorphometric evaluation

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Abstract

The purpose of this study is to determine if intraoperatively placed bone marrow stem cells (BMSCs) will permit successful osteocyte and mature bone regeneration in an isogenic murine model of distraction osteogenesis (DO) following radiation therapy (XRT). Lewis rats were split into three groups, DO only (Control), XRT followed by DO (xDO) and XRT followed by DO with intraoperatively placed BMSCs (xDO-BMSC). Coronal sections from the distraction site were obtained, stained and analyzed via statistical analysis with analysis of variance (ANOVA) and subsequent Tukey or Games-Howell post-hoc tests. Comparison of the xDO-BMSC and xDO groups demonstrated significantly improved osteocyte count (87.15 ± 10.19 vs. 67.88 ± 15.38, $P = 0.00$), and empty lacunae number (2.18 ± 0.79 vs 12.34 ± 6.61, $P = 0.00$). Quantitative analysis revealed a significant decrease in immature osteoid volume relative to total volume ($P = 0.00$) and improved the ratio of mature woven bone to immature osteoid ($P = 0.02$) in the xDO-BMSC compared with the xDO group. No significant differences were found between the Control and xDO-BMSC groups. In an isogenic murine model of DO, BMSC therapy assuaged XRT-induced cellular depletion, resulting in a significant improvement in histological and histomorphometric outcomes.

Key Words: *histology, mandible, osteocyte, radiotherapy*

Introduction

Nearly 60,000 new cases of head and neck cancer (HNC) are diagnosed annually in the United States [1]. Many patients afflicted with HNC require a multimodal treatment regimen to address the cancer. The therapeutic strategy to address the HNC often includes tumor extirpation with adjuvant radiation (XRT) and subsequent reconstruction. Although XRT is necessary and has led to greater overall and relapse-free survival, the negative sequelae on the surrounding tissue make reconstruction difficult [2,3]. Vascularized bone flaps are the most common surgical method used during mandibular reconstruction. Free flap reconstruction makes use of a vascular pedicle from a donor site in an unaffected region that can be grafted into the desired mandibular defect [4]. However, free tissue transfers can be a highly demanding surgical technique on both the surgeon and the patient. These operations often necessitate many hours to perform, and are associated with donor site morbidities [4]. Although these flaps provide a substantial amount of bone, this can come at a cost to the soft tissue coverage. A less-invasive reconstructive method that reliably restores mandibular structure and function, while providing a viable option for a greater number of patients, would certainly be an improvement to the current status quo.

Distraction osteogenesis (DO) offers a powerful surgical option that stimulates new bone formation

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through the controlled separation of two osteogenic fronts. This technique has proven to be an effective tool in reconstructing congenital mandibular defects. In addition to promoting the growth of bone using endogenous tissue, DO promotes the expansion of the surrounding soft tissue [5]. DO offers unique benefits over alternative reconstructive methods through the avoidance of donor site morbidity and concurrent growth of both bone and soft tissue, in addition to shorter operative and recovery periods [6]. The use of DO for tissue regeneration following surgical resection of oncogenic tissue and XRT could offer an additional reconstructive strategy with immense therapeutic benefit. However, DO as an option for mandibular reconstruction in HNC is restricted due to the destructive effect of XRT on local substrate.

Marx first described the damage wrought by radiation in his well-known observation of the pathogenesis of osteoradionecrosis [7]. Hypocellularity, hypovascularity and hypoxia inhibit viable bone formation, and the affected bone demonstrates significantly fewer osteoblast progenitor cells and mature osteocytes [8]. In addition to diminished cellularity, XRT reduces vessel size in non-extirpated bone [9]. The reduced perfusion of oxygen-filled blood to the area of damaged bone compounds the effects of decreased vasculature via XRT and tissue removal, and results in locally incapacitated vasculature [10–12]. While therapeutic XRT destroys cancer cells, these effects are not isolated to the malignant tissue and result in severe capillary degradation, suppression of osteoblast proliferation and diminution in osteocyte survival in the surrounding healthy tissue [13–15]. Our laboratory has previously used histomorphometric analysis to quantitatively assess the XRT-induced increase in empty lacunae and decrease in osteocyte formation in regenerated bone [16]. Insufficient osteocytes in irradiated fields can be explained in part through the inhibition of the proliferation and differentiation of osteoblasts. Additionally, reduced osteoblast proliferation results in insufficient expression of necessary angiogenic factors, such as vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β) [17].

Bone marrow stem cells (BMSCs) possess the capability to directly differentiate into osteoblasts. Osteoblasts are active early in osteogenesis and are critical for bone mineralization and eventual differentiation into osteocytes [18]. Additionally, BMSCs offer an advantageous therapy through the expression of growth factors such as bone morphometric proteins (BMPs), VEGF and TGF-β [18]. Prior clinical studies have demonstrated the capability of osteogenic progenitor cells from BMSCs to promote new ossification. Quarto et al found that BMSCs aid in bone regeneration in a mandibular defect [19]. These results, in corroboration with a growing body of research, imply that BMSCs offer a strong possibility for improving bony healing [20,21]. Our current study used the restorative properties of BMSCs in an isogenic immunocompetent murine mandibular model. The use of BMSCs in this model offers substantial new insight into improving the healing of bone with impaired osteogenic capabilities, and builds upon a model our laboratory has recently established. Our laboratory has previously created a reliable isogenic model establishing the cellular destruction that occurs subsequent to XRT. The reduced healing capability in our control model further exemplifies the need for a cellular therapy that can restore the osteogenic potential of bone exposed to XRT [20]. Given the immunocompentency of this model, it offers the additional opportunity to study stem cell remediation without the risk of generating an immune response.

The potential for BMSCs to replenish cells depleted by XRT, as well as incite proliferation of osteoblasts and differentiation of new osteocytes puts forth an exciting option to ameliorate XRT-induced cellular devastation. However, there is a paucity of research explicitly delineating the ability of BMSCs to induce osteogenesis in irradiated bone. Our global goal is to use the reparative properties of BMSC therapy to create a viable model of mandibular DO in the face of XRT. Our laboratory has previously demonstrated the effectiveness of BMSCs in remediating the hypovascular impact of XRT, as well as restoring bone mineral density and biomechanical strength [22,23]. However, we have yet to examine the specific histological and histomorphometric outcomes of BMSC therapy. We recently completed a study examining the effects of radiation on DO in an isogenic model [20]. As such, we used this data for the control and non-treatment group in this study. In the current study, we used BMSCs in an isogenic murine model to determine their ability to restore osteocyte number as well as increase the volume of mature woven bone. Due to their inherent osteogenic potential and ability to recruit endogenous factors critical for mature bone development, we hypothesize that BMSCs will replenish cell number and remediate cell function in a model of irradiated mandibular DO. We further hypothesize that the added mesenchymal stromal cells will increase the formation of new, mature regenerate bone, such that it resembles the composition of bone formed by non-irradiated DO.

Methods

Animal

Male Lewis rats, 325 g, were obtained through the University of Michigan's Unit for Laboratory Animal