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Parathyroid hormone reverses radiation induced hypovascularity in a murine model of distraction osteogenesis

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ABSTRACT

Background: Radiation treatment results in a severe diminution of osseous vascularity. Intermittent parathyroid hormone (PTH) has been shown to have an anabolic effect on osteogenesis, though its impact on angiogenesis remains unknown. In this murine model of distraction osteogenesis, we hypothesize that radiation treatment will result in a diminution of vascularity in the distracted regenerate and that delivery of intermittent systemic PTH will promote angiogenesis and reverse radiation induced hypovascularity.
Materials and methods: Nineteen Lewis rats were divided into three groups. All groups underwent distraction of the left mandible. Two groups received radiation treatment to the left mandible prior to distraction, and one of these groups was treated with intermittent subcutaneous PTH (60 μg/kg, once daily) beginning on the first day of distraction for a total duration of 21 days. One group underwent mandibular distraction alone, without radiation. After consolidation, the rats were perfused and imaged with micro-CT angiography and quantitative vascular analysis was performed.
Results: Radiation treatment resulted in a severe diminution of osseous vascularity in the distracted regenerate. In irradiated mandibles undergoing distraction osteogenesis, treatment with intermittent PTH resulted in significant increases in vessel volume fraction, vessel thickness, vessel number, degree of anisotropy, and a significant decrease in vessel separation ($p < 0.05$). No significant difference in quantitative vascularity existed between the group that was irradiated, distracted and treated with PTH and the group that underwent distraction osteogenesis without radiation treatment.
Conclusions: We quantitatively demonstrate that radiation treatment results in a significant depletion of osseous vascularity, and that intermittent administration of PTH reverses radiation induced hypovascularity in the murine mandible undergoing distraction osteogenesis. While the precise mechanism of PTH-induced angiogenesis remains to be elucidated, this report adds a key component to the pleotropic effect of intermittent PTH on bone formation and further supports the potential use of PTH to enhance osseous regeneration in the irradiated mandible.

Introduction

Patients with oral cancer that undergo resection and reconstruction of the mandible often require adjuvant radiation treatment to control their disease. While effective at treating the cancer, the radiation treatment severely impairs osseous healing and degrades existing bone. Radiation directly injures osteocytes, resulting in both immediate and delayed cellular death and a rise in empty lacunae [1–3]. Bone density is significantly decreased after radiation treatment [1]. Radiation treatment delivered to bone also induces progressive endarteritis, diminishing vascularity on a macroscopic and microscopic level [1,4,5]. Radiation induced depletion of vascularity is dose-dependent and the changes have been thought to be irreversible [1,3,4,6]. In a landmark article, Marx [2] described the "three H" principle, stating that irradiated tissue is hypoxic, hypocellular, and hypovascular compared to nonirradiated tissue. While radiation treatment impairs many factors required for successful bone healing, the impairment of vascularity is believed to play a key role in the majority of radiation-related osseous complications [1]. Adjunctive agents that can potentially restore vascularity to irradiated bone and/or mitigate

Abbreviations: PTH, parathyroid hormone; DO, distraction osteogenesis; XRT, radiation treatment; Gy, gray; POD, postoperative day; CT, computed tomography; ROI, region of interest; VVF, vessel volume fraction; VN, vessel number; VT, vessel thickness; DA, degree of anisotropy; VS, vessel separation; VEGF, vascular endothelial growth factor; HIFα, hypoxia-inducible factor-1 alpha.

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radiation induced vascular depletion could have a profound effect on assuaging radiation related injury.

Parathyroid hormone (PTH) has the potential for anabolic and catabolic effects on bone metabolism, depending on the dosage regimen. Intermittent dosing of PTH results in a net increase in bone mass while continuous infusion of PTH results in a net loss of bone mass [7] as the anabolic and catabolic effects of PTH are determined primarily by the duration of time that serum concentrations of PTH remain elevated [8]. The anabolic effect of intermittent PTH on bone formation is largely attributed to an increase in the number of matrix-synthesizing osteoblasts [9–15]. Intermittent PTH has been shown to have a pleiotropic effect, increasing osteoblast number by promoting osteoblastogenesis, inhibiting osteoblast apoptosis, and reactivating lining cells to resume matrix synthesizing function [16,17].

In animal fracture models, intermittent PTH improves and accelerates union [18,19] and increases callus formation and mechanical strength [20]. The osteogenic potential of PTH is also demonstrated by its ability to enhance the healing of bone grafts [21]. Teriparatide [rhPTH(1-34)] is currently FDA-approved for the treatment of osteoporosis, and has shown the ability to increase bone density in postmenopausal women [22]. In patients undergoing posterolateral lumbar fusion with local bone grafts, treatment with PTH significantly improved the rate and duration of bone union [23]. PTH also accelerates fracture healing in patients with pelvic [24] and distal radius fractures [25]. Case reports suggest that PTH treatment may have a role in ameliorating bisphosphonate-related osteonecrosis of the jaw [26,27].

Previous work in our laboratory has shown that PTH has the ability to reverse the deleterious effects of radiation on osseous regeneration in a murine model of distraction osteogenesis (DO) [28]. DO is a form of endogenous tissue engineering whereby new bone formation is stimulated by the gradual separation of two osteogenic fronts [29]. The intense osteogenic and angiogenic response that occurs in close temporal and spatial proximity also makes DO a valuable experimental model to analyze the potential effects of therapeutic agents on osteogenesis and angiogenesis. Gallagher et al. [28] observed that treatment with intermittent PTH enhanced bone regeneration in irradiated bone undergoing DO, demonstrated by significant improvements in bone volume fraction, bone mineral density, and union quality.

The capability of PTH to enhance an irradiated bone's ability to form a substantial and bountiful regenerate is promising. However, the effect of PTH on angiogenesis in irradiated bone has yet to be elucidated. More work is needed to determine the mechanism by which it can function to mitigate the deleterious effects of radiation induced injury. In this murine model of DO, we hypothesize that radiation treatment results in a diminution of vascularity in the distracted regenerate and that delivery of intermittent systemic PTH will promote angiogenesis and thereby reverse radiation induced hypovascularity. To our knowledge, a key link between the anabolic effect of intermittent PTH administration and angiogenesis has yet to be established.

Materials and methods

Experimental groups

Nineteen twelve-week-old isogenic male Lewis rats were randomly assigned to one of the three experimental groups: DO, xDO, and xDO-PTH (Fig. 1). All groups underwent surgical placement of mandibular distractors and underwent mandibular distraction osteogenesis. The DO (n = 5) group was the normal control group and underwent mandibular distraction osteogenesis. The DO group did not receive radiation treatment. The xDO (n = 7) group was the radiation control group and received preoperative radiation treatment followed by mandibular distraction osteogenesis. The xDO-PTH (n = 7) group received preoperative radiation treatment followed by mandibular distraction osteogenesis and intermittent subcutaneous PTH (60 μg/kg, once daily) beginning on the first day of distraction for a total duration

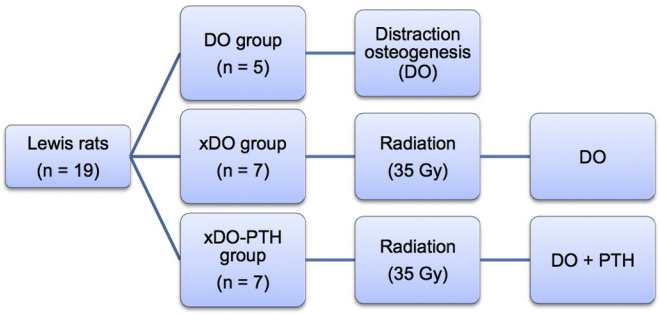

Fig. 1. Experimental groups. The DO group was the normal control group and underwent mandibular distraction alone without radiation treatment. The xDO group underwent radiation treatment followed by mandibular distraction. The xDO-PTH group underwent radiation treatment, mandibular distraction, and intermittent PTH treatment (60 μg/kg/day) for 21 days. DO, distraction osteogenesis; Gy, gray; PTH, parathyroid hormone.

of 21 days. Our animal protocol has been reviewed and approved by the University Committee on the Use and Care of Animals. The experimental timeline is shown in Fig. 2.

Radiation treatment

Rats were acclimated for 7 days in light and temperature controlled facilities and given hard chow and water without restriction. Radiation was delivered to the xDO and xDO-PTH groups; the DO group did not receive radiation. Prior to radiation, rats were anesthetized with inhaled isoflurane. Induction was begun at 4%, after which anesthesia was maintained at 1.5%.

All radiation treatments were performed in the Irradiation Core at the University of Michigan Cancer Center using a Philips R250 orthovoltage unit (250 kV, 15 mA; Kimtron Medical, Woodbury, CT). Radiation was delivered to the left hemimandible, 2-mm posterior to the third molar. Lead shielding was used to ensure localized delivery and to protect surrounding tissue. 7 Gy was delivered daily for 5 days for a total fractionated treatment dose of 35 Gy, which is the bioequivalent dose of 70 Gy in humans [30]. Dosimetry was carried out using an ionization chamber connected to an electrometer system, which was directly traceable to a National Institute of Standards and Technology calibration.

After completion of radiation, the xDO-PTH and xDO groups were allowed to recover for 14 days. During this period, all three groups were acclimated to a soft chow high-calorie diet (Hills-Columbus Serum; Columbus, Ohio). The percent of ration of calcium and phosphorus were 0.95% and 1.05% respectively, and the content of vitamin D was 4.5 IU/g.

Surgery and postoperative care

All three groups underwent surgical placement of a mandibular distraction device with unilateral left mandibular osteotomy. The surgery was performed 14 days after completion of radiation in the xDO-PTH and xDO groups. Anesthesia was achieved with inhalational isoflurane (4% induction, 1.5% maintenance) and subcutaneous buprenorphine

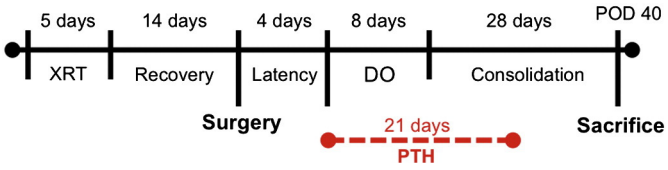

Fig. 2. Experimental timeline. In the xDO-PTH group, parathyroid hormone (PTH) treatment was administered in one subcutaneous injection daily (60 μg/kg/day) for 21 days, beginning on the first day of mandibular distraction. XRT, radiation treatment; DO, distraction osteogenesis; PTH, parathyroid hormone.