Bone 57 (2013) 56–61

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

The effect of Amifostine prophylaxis on bone densitometry, biomechanical strength and union in mandibular pathologic fracture repair☆

Catherine N. Tchanque-Fossuo [a,1], Alexis Donneys [a,1], Deniz Sarhaddi [a,1], Behdod Poushanchi [a,1], Sagar S. Deshpande [a,1], Daniela M. Weiss [a,1], Steven R. Buchman [b,*]

[a] University of Michigan Hospital and Health Systems, Pediatric Plastic Surgery Section, 1540 E. Hospital Drive, Ann Arbor, MI 48109, USA
[b] 4-730 C.S. Mott Children's Hospital, 1540 E. Hospital Drive, Ann Arbor, MI 48109-4215, USA

ARTICLE INFO

Article history:
Received 15 May 2013
Revised 2 July 2013
Accepted 3 July 2013
Available online 13 July 2013

Edited by: Thomas Einhorn

Keywords:
Pathologic fracture
Osteoradionecrosis
Amifostine
Bone density
Biomechanical strength

ABSTRACT

Background: Pathologic fractures (Fx) of the mandibles are severely debilitating consequences of radiation (XRT) in the treatment of craniofacial malignancy. We have previously demonstrated Amifostine's effect (AMF) in the remediation of radiation-induced cellular damage. We posit that AMF prophylaxis will preserve bone strength and drastically reverse radiotherapy-induced non-union in a murine mandibular model of pathologic fracture repair.
Materials and methods: Twenty-nine rats were randomized into 3 groups: Fx, XRT/Fx, and AMF/XRT/Fx. A fractionated human equivalent dose of radiation was delivered to the left hemimandibles of XRT/Fx and AMF/XRT/Fx. AMF/XRT/Fx was pre-treated with AMF. All groups underwent left mandibular osteotomy with external fixation and setting of a 2.1 mm fracture gap post-operatively. Utilizing micro-computed tomography and biomechanical testing, the healed fracture was evaluated for strength.
Results: All radiomorphometrics and biomechanical properties were significantly diminished in XRT/Fx compared to both Fx and AMF/XRT/Fx. No difference was demonstrated between Fx and AMF/XRT/Fx in both outcomes.
Conclusion: Our investigation establishes the significant and substantial capability of AMF prophylaxis to preserve and enhance bone union, quality and strength in the setting of human equivalent radiotherapy. Such novel discoveries establish the true potential to utilize pharmacotherapy to prevent and improve the treatment outcomes of radiation-induced late pathologic fractures.

Introduction

Late pathologic fractures are one of the most debilitating and devastating sequelae related to adjuvant radiotherapy in the treatment of craniofacial malignancies [1–9]. Pathologic fractures are also a part of a corrosive spectrum of clinical manifestations of osteoradionecrosis that include bone necrosis and fistula formation [1–9]. Osteoradionecrosis takes place after irradiated bone undergoes devascularization and exposure through its overlying skin or mucosa and fails to heal over a three-month period in the absence of a local tumor [2,9]. The injury occurs secondary to a cascade phenomenon of radiation induced-hypoxic milieu, cellular depletion or hypocellularity and blood vessels obliteration or hypovascularity [2,3,9,10].

In the craniofacial skeleton, the mandible is the most commonly affected bone with the incidence of mandibular osteoradionecrosis and late pathologic fractures reportedly as high as 8.2% and 6% respectively [4–6,8,11,12]. The discrepancy in the incidence rates reported is based on the various doses of radiation delivered in those reports. The mandible is more susceptible to pathological fracture because of its particularly vulnerable set of anatomical features. It is a cortical and compact bone with a tenuous blood supply from the inferior alveolar artery and limited collateral circulation. Pathologic fractures are commonly reported in relation to osteoradionecrosis, which may occur as early as few months after the completion of radiotherapy to several years post-radiation or the so-called later onset osteoradionecrosis [4–6,8,11,12]. The basis of the timeframe of pathologic fracture occurrence resides in many factors such as those inherent to the patient (oral hygiene, periodontal disease, infections, abscesses), tumor site, surgical extirpation during irradiation and radiotherapy delivery and dosage [9,10]. Radiation inflicts multiple insults on both the cellular and the vascular constituents of bone; significantly escalating the risk for delayed fracture unions and severely undermines the chances to heal and achieve union [2,3,9–12]. Osteosynthesis and efforts at reconstruction of mandibular

☆ Funding: This work was supported by the National Institutes of Health (NIH-R01# CA 125187-01 to S.R.B) "Optimization of Bone Regeneration in the Irradiated Mandible".
* Corresponding author. Fax: +1 734 936 7815.
E-mail address: sbuchman@umich.edu (S.R. Buchman).
[1] Fax: +1 734 936 7815.

8756-3282/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bone.2013.07.005

fractures secondary to radiation can be quite challenging; requiring both structural and functional considerations as well as strategies that can address the devastating effects upon the patient's quality of life. Surgeons have attempted a large variety of treatment options to address the noxious sequelae of radiation on fracture healing. These options have ranged from conservative approaches with local wound care, ultrasound treatment, and adjuvant hyperbaric oxygen therapy, to the insertion of large metal plates, the use of non-vascularized bone grafts and the use of vascularized osseous free flaps [6–8,11,12]. Unfortunately, each of those options have drawbacks such as plate extrusion, infections, donor site morbidity, high cost and modest to poor outcomes [8]. Consequently, the development of late pathologic fracture presents the clinician with an exceptionally difficult infirmity to successfully treat necessitating the discovery of effective therapies that would thwart the corrosive and detrimental effects of radiation on bone healing and repair.

Our laboratory has previously investigated a radio-protective drug Amifostine (AMF, WR-2721, Ethiol MedImmune, Gaithersburg, MD) on irradiated murine mandibles. AMF is a pro-drug that gets dephosphorylated by the cell membrane alkaline phosphatase into an active metabolite that scavenges radiation-induced free radicals; thereby protecting cellular DNA from an avalanche of significant deleterious effects [13]. AMF selectively guards normal cells because they have a significantly higher content of the key activating enzyme alkaline phosphatase in contrast to the tumor cells [13,14]. AMF has been FDA-approved for the prevention of radiotherapy-induced xerostomia and mucositis in head and neck cancer patients [13,14]. It has also shown successful remediation of radiation damage on osteocytes [15], microvasculature [13,14] and bone mineralization [17–19] in our in vivo experimental model of murine mandibular distraction osteogenesis [20].

We posit that AMF prophylactic therapy will significantly prevent radiation-induced non-union and maintain bone quality and strength in our murine mandible model of pathologic fracture repair. Our goal is to establish a successful pharmacologically based treatment strategy aimed at the prevention of late pathologic fracture in any patient where bone is in the target field. Achieving such objective holds the promise to significantly impact the scourge of radiation associated morbidity for the tens of thousands of patients diagnosed with cancer every year.

Materials and methods

Sprague Dawley male rats (~400 g) were randomized into three experimental groups: Fx (n = 5), XRT/Fx (n = 14), and AMF/XRT/Fx (n = 10). The University of Michigan's Committee for the Utilization and Care of Animals approved all animal experiments as performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Radiotherapy

A human equivalent dose of radiation (or bioequivalent radiation dose of 70 Gy in human) was delivered in a 7 Gy daily fraction over five days to the left hemimandible of both XRT/Fx and AMF/XRT/Fx animals, utilizing the Philips RT250 orthovoltage unit (250 kV, 15 mA-Kimtron Medical, Woodbury, CT), in accordance to a protocol performed and previously described in our laboratory [16–19,21–23]. A subcutaneous injection of 100 mg/kg of AMF was prophylactically administered to AMF/XRT/Fx 45 m prior to irradiation. Fx did not undergo any radiation or AMF pre-treatment.

Surgical and postoperative procedures

Following preoperative analgesia and antibiotic administration, all animals underwent a unilateral left mandibular osteotomy with bilateral external fixator placement as previously described [15,16,19–24]. To prevent peri-operative blood loss, a 4 to 8-hour interval was observed prior to setting a 2.1 mm fracture gap. On post-operative day forty, animals were euthanized and left hemimandibles were harvested devoid of soft tissue and evaluated for bony bridging or complete union versus non-union.

Micro-computer tomography (μCT)

Hemimandibles were scanned with eXplore Locus SP μCT (GE Healthcare Bioscience, Fairfield, CT) utilizing 80 kVp, 80 mA and 1100 ms exposure, where 392 projections were taken at a resolution of 45 μm voxel size. The μCT machine was calibrated prior to scanning each specimen into a chilled dH_2O solution. Each individual scan was reconstructed and reoriented in a 3-dimensional (x, y, z) plane until all axial, coronal and sagittal sections were aligned with a +X representing the mandibular length from posterior to anterior, +Z representing the mandibular height from inferior to superior, and +Y representing the depth (or lingual to buccal thickness) of the mandible. The region of interest (ROI) is delineated posteriorly by the third molar, extends 2.1 mm and corresponds to the fracture gap. Utilizing the spline tool of the MicroView 2.2 software (GE Healthcare, Milwaukee, WI), several contours were generated on serial sagittal sections that highlighted the ROI, with selective inclusion of bone. For uniform data analysis of the ROI, rotations, cropping of non-bone space and exclusion of the incisor, canal, tooth and periodontal tissue were performed. Microview assigns a grayscale value to each voxel and uses an algorithm that converts the grayscale to mineral content. Microdensitometry parameters including bone volume fraction (BVF), bone mineral density (BMD) and tissue mineral density (TMD) were quantitatively measured.

BVF is defined as the volume of newly formed bone divided by the total volume of the gap or region of interest (ROI) (which includes air, soft tissue, water and marrow). BMD is defined as the ratio of bone mineral content (mass) of newly remodeled bone over the total volume of the ROI. BMD represents the amount of bone within the entire ROI (which includes air, soft tissue, water and marrow).

Tissue mineral density (TMD) is defined as the ratio of tissue mineral content over the bone volume of the ROI. TMD represents the average mineral density only for the tissue identified as bone within the ROI (which excludes air, soft tissue, water and marrow).

Maximum intensity projections (MIPs) of each hemimandible were obtained for qualitative illustration. MIPs allow for instant volume representation of a volumetric data set into a three-dimensional visualization of the specimen scanned.

Biomechanical testing

Hemimandibles were mounted on cylindrical fixtures with initial potting of the posterior hemimandible in bismuth alloy medium (Cerrobend, Cerro Products, Bellefont, PA) to a depth that was excluding the ROI, followed by the potting of the anterior hemimandible. Once the potted medium and specimens have solidified and sufficiently cooled down, they were loaded to failure for uni-axial tension testing at a displacement rate of 0.5 mm/s. Grip to grip displacement was recorded and data were acquired at a 2000 Hz sampling frequency. Load–displacement curves were generated and analyzed for breaking load (BL) and yield strength (Y).

Statistics

One-way ANOVA with post-Tukey (SPSS 19.0; SPSS, Inc., Chicago, IL) was performed and results were considered statistically significant at $p < 0.05$.