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Dose-Response Effect of Human Equivalent Radiation in the Murine Mandible: Part II. A Biomechanical Assessment

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Abstract

Background—Despite the widespread use of adjuvant irradiation for head and neck cancer, the extent of damage to the underlying bone is not well understood. However, patients can suffer serious clinical consequences, including pathologic fractures, nonunion, and osteoradionecrosis. The authors' specific aim was to objectively quantify the human equivalent radiation dose-response effect of radiation on the biomechanical properties of the murine mandible.

Methods—Twelve Sprague-Dawley rats were randomized into three radiation dosage groups—low (5.91 Gy), middle (7 Gy), and high (8.89 Gy)—delivered in five daily fractions. The fractionation regimen was used to approximate 75, 100, and 150 percent, respectively, of the bioequivalent dose humans receive in conventional head and neck cancer treatment. Fifty-six days after irradiation, hemimandibles were loaded to failure in a uniaxial tension at 0.5 mm/second. Load displacement curves were analyzed for yield and breaking load, and values were considered statistically significant at $p < 0.05$.

Results—The authors' data demonstrated a statistically significant decrease in the yield and breaking load metrics. The authors' reported averages for low, middle, and high radiation dosages were 162, 136, and 69 N, respectively, for yield; and 215, 211, and 141 N, respectively, for breaking load. Both of these quantitative biomechanical properties were diminished in a dose-response pattern.

Conclusions—In this article, the authors report a dose-response effect in the degradation of the biomechanical properties of the mandible after fractionated human equivalent radiation. The authors' findings and model can now be used to formulate therapies aimed at remediating those effects and augmenting bone regeneration and healing after adjuvant radiotherapy in head and neck cancer patients.

Management of head and neck cancer has evolved significantly with the advent of new therapeutic interventions, including advances in radiotherapy. The deleterious effects of radiation on bone, however, are numerous and range from increasing morbidity to the

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unforgiving sequelae of osteoradionecrosis and associated pathologic fractures. The incidence of osteoradionecrosis described in the literature, although variable, has been reported to be as high as 56 percent.¹ Radiation-induced tissue damage is proportional to the dose, and the overall dose is an important factor in patient outcome. Therefore, fractionated dosing has become an important strategy that is used to maximize patient outcomes and minimize the soft-tissue side effects. In the United States, conventional radiotherapy for head and neck cancer, such as squamous cell cancer, involves a fractionated regimen of 1.8 to 2 Gy per fraction, five daily fractions per week, to a total dose of 65 to 70 Gy.² However, the secondary target, such as the underlying exposed mandible, may endure much greater consequences from the same dose of radiation. The concept of differential effect of radiation on different types of tissue is known as the biologically effective dose or biologically equivalent dose.

The biologically equivalent dose is defined as an approximate quantity by which radiotherapy fractionation schedules may be compared. In external beam radiotherapy using equal fractions of conventional size, the biologically equivalent dose will be as follows:

$$\text{Biologically equivalent dose} = E/\alpha = n \cdot d \cdot [1 + d/(\alpha/\beta)]$$

expressed in units of $\text{Gy}_{\alpha/\beta}$, where n is the number of fractions, d is the dose per fraction, nd is the total dose, and α and β are survival parameters of the target cells in the tissue that quantify fractionation sensitivity for normal tissue after different radiation fractionation regimens.^{3,4} This formulation assumes that full repair occurs between fractions so that the biological effect of each fraction is the same. Unfortunately, there are insufficient data on the dose-response effect of radiation on the structural integrity of the mandible. In this study, we evaluated the dynamics and pathologic effects of radiation dose on the murine mandible using human equivalent radiation.⁵ Our specific aim was to assess the effect of radiation on the biomechanical properties of the murine mandible in a dose-response manner at 75, 100, and 150 percent of the dose that a human would receive in standard treatment for head and neck cancer. In conventional squamous cell cancer treatment, the human mandible receives a biologically equivalent dose of 116.66 Gy_3 or an isodose of 100 percent, which approximates to our experimental fractionation scheme of 7Gy delivered in five daily fractions. The dosing at 75 and 150 percent approximates to a biologically equivalent dose of 87.76 Gy_3 (dose per fraction, 5.91 Gy) and a biologically equivalent dose of 176.17 Gy_3 (dose per fraction, 8.89 Gy), respectively, with a similar fractionation regimen. Our hypothesis is that human equivalent radiation dosing will demonstrate a significant degradation in the biomechanical properties of the mandible and do so in a dose-response manner. Our overall goal is to determine the adequate dosimetric regimen by which therapeutic radiation minimizes the structural and functional degradation of bone and develop strategies to mitigate those effects.

MATERIALS AND METHODS

All studies were approved by the University of Michigan Committee for the Utilization and Care of Animals. Twelve adult male Sprague-Dawley rats (400 g) were obtained through the

University of Michigan Unit for Laboratory Animal Medicine. These animals were assigned randomly to three experimental radiation dosage groups: low-dose (5.91 Gy; $n = 4$), middle-dose (7 Gy; $n = 5$), and high-dose (8.89 Gy; $n = 3$). The animals had 7 days of acclimation before handling. They were placed in a pathogen-free restricted area on a 12-hour light/dark schedule, weighed, and fed standard rat chow and water ad libitum.

Radiation

The rats were anesthetized with an oxygen-isoflurane mixture and placed right side down with a custom-designed lead shield over the body with a window cut to expose the left posterior mandible. The left hemimandible received fractionated external beam radiotherapy at the above dosages over 5 days by means of a Philips RT250 orthovoltage unit (250 kV, 15 mA; Kimtron Medical, Woodbury, Conn.). This radiation protocol has been performed for several years at the Department of Radiation Oncology.^{5–8}

The rats were observed for 56 days and maintained on soft chow and water ad libitum. Subcutaneous infusion of buprenorphine (0.15 mg/kg) with 10 cc of lactated Ringer's solution was given to any rats exhibiting signs of radiation-induced discomfort.^{5–8}

Tissue Harvesting

Only one animal died in the high-dose group. The remaining animals were euthanized, and the left hemimandibles were harvested and frozen at -20°C until testing.

Mechanical Testing

Potting—In mechanical testing, the specimen to be tested is mounted in two molding cylindrical fixtures or pots (Fig. 1, *left*). Before potting, the hemimandibles were thawed in water at room temperature. Stainless steel reinforcement wires measuring approximately 1.5 cm in length were inserted perpendicularly through the hemimandibles at both anterior and posterior regions. The potting medium was a bismuth alloy (Cerrobend; Cerro Metal Products, Bellefonte, Pa.) that could be melted into an easy-to-use, conforming liquid. The posterior hemimandible was potted first at a depth determined to match the location of the region of interest that has been used by our laboratory in the past to look at distraction osteogenesis.^{5–8} Specifically, the region of interest was located 5.1 mm behind the last mandibular molar. The anterior hemimandible was potted next by fully burying the third molar in the potting medium. Potted mandibles were allowed to cool in an ice bath to solidify the potting medium (Fig. 1, *right*).

Tension Testing—Potted mandibles were loaded to failure in uniaxial tension at a constant displacement rate of 0.5 mm/second by means of a servohydraulic testing machine (858 Mini Bionix II; MTS Systems Corp., Eden Prairie, Minn.), with either a 10-lb or a 50-lb load cell (Sensotec, Inc., Columbus, Ohio). Grip-to-grip displacement was monitored using an external linear variable differential transducer (Lucas Schaevitz, Inc., Pennsauken, N.J.). Load and displacement data were acquired by means of the TestStar IIs System version 2.4; MTS Systems) at 2000-Hz sampling frequency. Results were expressed in load-displacement curves, which were analyzed for yield and breaking load by three independent reviewers.

Statistical Analysis

One-way analysis of variance with the appropriate post-Tukey test was used for yield and breaking load analysis (SPSS version 16.0; SPSS, Inc., Chicago, Ill.). Values of $p < 0.05$ were considered statistically significant.

RESULTS

During the 56 days of recovery, one death occurred in the high-dose group. All groups tolerated moist chow. The middle-dose and high-dose radiation groups demonstrated signs of severe stress and alopecia. All animals had mucositis and weight loss in proportion to the radiation dose starting at postirradiation days 4 through 11. Maximal weight loss from the pretreatment weight was 8 percent (32 g) in the low-dose group, 15.8 percent (63 g) in the middle-dose group, and 22.5 percent (90 g) in the high-dose group. All animals eventually gained weight and surpassed their pretreatment weight. At the time of harvest, the hemimandibles were atrophied noticeably in proportion to dosages. At tension testing, the breaking or fracture site varied among all hemimandibles (Fig. 2).

The resulting biomechanical parameters in terms of yield and breaking load for all three groups are summarized in Table 1. The load-displacement curve defines the relationship between the load applied to a structure (here, the hemimandible) and the deformation in response to that load. The load-displacement curves were divided into two regions, the elastic (preyield) and the plastic (postyield). As expected, because of viscous effects caused by fluids within the bone matrix, and the natural viscoelastic properties of the bone, none of the curves resembled ideal elastic structures. As a result, interpretation of the Y point was based on a regression technique (10 percent deviation from linear regression fit) and an observer-chosen estimate of an inflection in the load-deformation curve. The load-displacement curve plots the force (in newtons) applied to the area of interest, against the corresponding displacement (in millimeters). The breaking load or ultimate load is the maximum load the bone can sustain resulting in partial or complete break.

The load-displacement curves from the low-dose specimens revealed an average breaking load of 215 ± 40.18 N, which was associated with displacements ranging from 0.26 to 0.59 mm (Fig. 3). The load-displacement curves from the middle-dose group showed a consistently lower breaking load of 211 ± 24.52 N and higher displacements ranging from 0.48 to 1.03 mm as compared with the low-dose group (Fig. 4). In stark contrast to both low-dose and middle-dose groups, the high-dose group had a much steeper slope, with an average lower breaking load of 141 ± 22.28 N, occurring at much higher displacements, ranging from 0.32 to 1.09 mm (Fig. 5).

More specifically, there was a significant difference in breaking load between middle-dose and high-dose groups (211 ± 24.52 N versus 141 ± 22.28 N; $p = 0.026$) (Fig. 6 and Table 1). Similarly, there was a statistically significant difference between the low-dose and high-dose groups (215 ± 40.18 N versus 141 ± 22.28 N; $p = 0.028$) (Fig. 6 and Table 1).

Yield is defined as an imaginary boundary above which stresses cause permanent damage to the bone structure (elastic properties become plastic and cannot revert to the original

architecture). The average yield for low-dose, middle-dose, and high-dose groups were 162, 136, and 69 N, respectively. There was a statistically significant difference between middle-dose and high-dose groups (136 ± 29.44 N versus 69 ± 14.34 N; $p = 0.004$), along with a corresponding difference between low-dose and high-dose groups (162 ± 30.67 N versus 69 ± 14.34 N; $p = 0.020$) (Fig. 7 and Table 1).

DISCUSSION

Therapeutic irradiation in head and neck cancer treatment severely attenuates bone healing, leading to a significant biomedical impediment and unforgiving sequelae, such as poor fracture and soft-tissue healing, osteoradionecrosis, and late pathologic fractures. Unfortunately, there are scarce data on the consequences or effect of irradiation dosage to the mandible, which is the most commonly affected and exposed bony structure in head and neck cancer radiation treatment. Bone changes related to irradiation were first reported in 1926 as “radiation osteitis” by Ewing⁹ or “radium necrosis of bone” by Phemister.¹⁰ Since then, the terms that characterized the effects of radiation on bone have evolved (including radiation osteitis, osteoradionecrosis, and avascular bone necrosis).¹ As irradiation of oral malignancies became a well-established practice, the first clinical reports of osteoradionecrosis of the jaw appeared in the 1950s.¹¹ Furthermore, since Marx described the “three-H” principle of radiation-induced tissue damage there has been increasing interest in better understanding the pernicious effects of radiation on living tissue. In a clinical study of 26 consecutive cases of osteoradionecrosis of the jaw secondary to external beam radiotherapy in the range of 6000 to 14,000 rads, Marx postulated that osteoradionecrosis is a valid entity related to a higher total radiation dose. Endothelium, bone, and periosteum are all important tissues that have been shown to become hypoxic, hypocellular, and hypovascular as a result of osteoradionecrosis.¹² Osteoradionecrosis leads to persistent cellular damage as the tissue oxygen, energy, and nutrition supply are reduced and the demands for basic components of tissue repair become overwhelmed in the less vascularized mandible.^{12,13}

Clinical associations of osteoradionecrosis have further prompted inquiry into the consequences of incremental radiation dosage. Experimental studies using animal models of radiation have demonstrated a higher incidence of biomechanical degradation in single-dose irradiated endochondral long bones. In Sprague-Dawley rats, a single fraction of 25 Gy was delivered to the middle part of the thigh, resulting in decreased cortical area and increased porosity by torsional testing.¹⁴ Similarly, Sugimoto et al. have shown a 50 percent decrease in bending strength (using a three-point bending test) following a single radiation dose of 50 Gy of external beam radiotherapy to the rabbit tibia at 24 weeks after irradiation.¹⁵ Although these clinical associations and experimental studies implicate radiation-induced changes to biomechanical properties of the bone, they are less relevant to present series of head and neck cancer because of their lack of fractionation, replacement with up-to-date radiotherapy techniques, and the important structural differences between endochondral and membranous flat bones such as the mandible.

Indeed, modern radiation oncology has evolved to develop methods of dose delivery that would increase tumor control without an increase in side effects to the adjacent normal

tissue. Published data on mouse lethality after bone marrow irradiation have helped establish operational value of the parameters α and β . The linear quadratic or α/β model is a mathematical quantification of biological response to different fractionation schedules. The biological effect (E) of a dose (d , in Gy) is determined uniquely by the surviving fraction (S) of a target cell population:

$$E = \text{Effect} = -\log S = \alpha d + \beta d^2$$

where α is the cell kill per gray of the initial linear component (on a log-linear plot) and β is the cell kill per gray squared of the quadratic component of the survival curve.³ The lower the α/β ratio, the greater the rate of change in the total dose required for a certain biological effect E with change in fraction size. The ratio α/β is measured in gray. The radiobiological characteristics of bone, because it is a late-responding tissue, has an estimated α/β value of 3 Gy.⁴ Such a low value indicates a markedly increased probability of injury associated with an increased fraction dose. This has led to the concepts of fractionation schemes, total radiation dosage, timing of the initiation of radiotherapy, and volume effects in normal tissue and tumors. Perez et al. postulated that the tolerance dose of external beam fractionated radiotherapy for bone (as a whole organ) is higher than 70 Gy and not well established.¹⁶ However, radiation dose to the mandible is not assessed routinely in standard radiotherapy for head and neck cancer or in clinical trials.¹⁷

In 1964, Currey considered the concept of bone as a two-phase material, mineral and collagen matrix.¹⁸ It is well thought that collagen and mineral contribute differently to the elastic/plastic properties of bone.¹⁹ Specifically, in an elastic/perfectly plastic model, the mineral phase of bone would contribute the major share of the elastic, linear portion of the low-dose curve, and the mineral constituent would provide the major share of tensile strength properties. Conversely, collagen is dominantly responsible for the postyield elastic biomechanical properties.¹⁹ Currey et al. also speculated that the biomechanical aspects of strength were diminished by radiation-induced denaturation of protein (collagenous) matrix.²⁰ Cheung et al. have corroborated that gamma radiation caused cleavage of the alpha chains in the collagen helix in a dose-dependent manner.²¹ More recent molecular studies have assessed the chemical changes occurring in both mineral and organic components after irradiation. They have validated the concept of high x-ray energy-induced side chain decarboxylation of the tissue, thus altering the binding or interaction between the organic matrix and the hydroxyapatite mineral.^{22,23} Consequently, there is a decrease in mechanical parameters with higher radiation doses.²⁴

Previous studies in our laboratory examining the effects of fractionated radiation dosing on the murine mandible have demonstrated ultrastructural changes consistent with a substantial influence of radiation on mandible biomechanics.⁶ Specifically, the studies reported a statistically significant decrease in breaking load compared with the nonirradiated mandible. The studies used a subclinical total radiation dose of 40 Gy. The purpose of our subsequent studies was to expand on our previous work by determining a dose that would limit bone healing without altering its structure and function and approaching levels that would correlate with human cancer treatment. Using quantitative histomorphometry, we initially

demonstrated that radiation-induced dose-dependent alterations in bone cellularity where a decrease in osteocytes and an increase in empty lacunae were observed with increasing radiation dosages.⁵ We also set out to analyze the potential dose dependence of radiation on the biomechanical properties of the bony mandible. Our global hypothesis is that radiation dosage and regimen are both critical and play a crucial role in bone mechanical properties. In this study, we have successfully induced radiation damage with the application of a controlled fractionation dosage regimen equivalent to human head and neck cancer treatment. We have also quantified radiation-induced degradation of the biomechanical properties of the mandible in a dose-response fashion (Table 1 and Figs. 6 and 7). Because breaking load measures the force value at which bone actually breaks and was found to be significantly diminished in the high-dose group, bone submitted to a lower radiation dosage can support a greater load. Likewise, because yield corresponds to the ability of a material to return to its normal shape (i.e., elastic properties), our findings demonstrated that increasing radiation dosage led to a lesser load than would be necessary before the material would become irreversibly deformed or would break. We could also speculate that more radiation significantly alters the essential binding between the mineral and organic phases of the mandibular bone, thus resulting in the postyield behavior demonstrated in our experiment. Our findings at the tissue level are in keeping with molecular studies whereby radiation has demonstrated (1) delays in the normal temporal progression of biomechanical parameters of fracture healing and (2) ultrastructural alterations in mineral/organic matrix interactions resulting in changes in the structural integrity of bone.^{20–25}

Our findings complement and corroborate the handful of experiments that have ventured to examine the general effects of radiation on the mandible, as we used yield and breaking load, whereas previous attempts gleaned data from other methods to test the strength of bone. Nonetheless, our findings were consistent with a scenario whereby the effects of radiation on bone microarchitecture are not only dose-dependent but also quantifiable.⁵

We also found that our murine model of fractionated radiation treatment to the mandible was associated with a concomitant weight loss, as mirrored in patients undergoing head and neck cancer radiotherapy. Furthermore, we demonstrated that the weight loss in our model also exhibited a dose response. Such findings are encouraging, as they help to validate our model as reasonable reflections of constitutional findings also found in the human clinical scenario. Even more importantly, both our findings and our model can now be used to formulate targeted therapies aimed at both remediating those devastating side effects and fostering the development of strategies to augment the osteogenic regenerative processes of bone healing and repair in patients subjected to radiation treatment.

CONCLUSIONS

A reproducible murine model of human biologically equivalent fractionated radiation to the mandible has been devised. Our findings demonstrate a dose-dependent statistically significant reduction in both yield and breaking load with increasing radiation dose. We have also demonstrated a dose-dependent weight loss in our model mirroring the human condition. Our data would imply increasing risk of late pathologic fractures and losses of structural integrity with increasing doses of radiation. Our data clearly show the importance

of defining dosimetry and have established quantifiable bone healing metrics. Future studies can now be performed using these outcome metrics to determine the efficacy of therapies aimed at remediating the harmful effects of radiation and optimizing bone regeneration.

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Fig. 1. Biomechanical potting and testing. The posterior hemimandible is potted first in the lower fixture, with care taken to avoid the region of interest, followed by the potting of the anterior hemimandible.

Fig. 2.

Left hemimandibles with various failure points (breaking) at the end of tension testing.

(Above) The majority of the low-dose specimens broke behind the third molar. (Center) The majority of the middle-dose specimens broke before the first molar. (Below) The majority of the high-dose specimens broke behind the third molar.

Fig. 3. Load-displacement curve of low-dose irradiation (*XRT*) tested to failure. For this specimen, the yield (approximately 124 N) occurs at a displacement of 0.12 mm, whereas the breaking load (approximately 251 N) occurs at a displacement of 0.45 mm.

Fig. 4.

Load-displacement curve of middle-dose irradiation (*XRT*) tested to failure. For this specimen, neither the yield (approximately 107 N) nor the breaking load (approximately 222 N) was significantly lower than for the low-dose group, and they occur at displacements of 0.18 mm and 0.51 mm, respectively.

Fig. 5. Load-displacement curve of high-dose irradiation (*XRT*) tested to failure. For this specimen, both yield (approximately 74 N) and breaking load (approximately 138 N) are significantly lower than for the low- and middle-dose groups and occur at approximately 0.05-mm and 0.24-mm displacements, respectively.

Fig. 6. Breaking load at increasing radiation dosage (comparison among low-, middle-, and high-dose groups, with mean values in newtons of applied load on the y axis and standard deviations as y error bars). The breaking load is significantly lower in the high-dose than in both low-dose ($\ddagger p < 0.05$) and middle-dose groups ($\dagger p < 0.05$).

Fig. 7.

Yield at increasing radiation dosage (low-, middle-, and high-dose groups with mean values in newtons of applied load on the y axis and standard deviations as y error bars). Although the high-dose group has a significantly lower yield than either the low-dose ($\ddagger p < 0.05$) or the middle-dose group ($\dagger p < 0.05$), there was no statistically significant difference between the low- and middle-dose groups.

Table 1

Results of Tension Testing for All Mechanical Parameters after Statistical Analysis

Radiation Dosage Groups	Mean (N)	SD (N)	<i>p</i>
Yield			
Low-dose	162	30.67	0.375 [*]
Middle-dose	136	29.44	0.004 [†]
High-dose	69	14.34	0.020 [‡]
Breaking load			
Low-dose	215	40.18	0.976 [*]
Middle-dose	211	24.52	0.026 [†]
High-dose	141	22.28	0.028 [‡]

* Low- and middle-dose groups.

[†] Middle- and high-dose groups.

[‡] Low- and high-dose groups.