

# Brachytherapy-Mediated Bone Damage in a Rat Model Investigating Maxillary Osteoradionecrosis

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**Objectives:** To assess clinical and radiologic findings from targeted radiotherapy to the rat maxilla and to compare findings with a recently developed rat model of mandibular osteoradionecrosis (ORN).

**Design:** A prospective, controlled animal study.

**Subjects:** Ten male Sprague-Dawley rats were divided into an experimental group receiving catheter-assisted high-dose-rate brachytherapy (n=6) and a control group with catheter-assisted sham therapy (n=4).

**Interventions:** The second left maxillary molar was extracted 1 week after radiation, and the maxilla was harvested 3 weeks after dental extraction.

**Main Outcome Measure:** We used a standardized method with micro-computed tomography to determine the ratio of bone volume to total volume of the dental extraction socket.

**Results:** On the gross clinical examination, all rats had mucosal coverage of the dental extraction site, whereas

only the brachytherapy group demonstrated scarring of the mucosa. The median bone volume to total volume was 0.21 for the brachytherapy group and 0.49 for the control group ( $P=.01$ ).

**Conclusions:** Similar to the mandible, the maxilla is susceptible to radiogenic bone injury as demonstrated by the significant decrease in bone volume of the radiated dental extraction socket. Despite radiologic similarities to mandibular ORN in the rat model, the maxilla demonstrated a more benign clinical course with a complete absence of bone exposure. Differences in the maxillary bone and microenvironment of the maxilla compared with the mandible may explain the subclinical response to radiation and lower incidence of maxillary ORN seen in patients. This maxillary model can be combined with our high-dose-rate mandibular ORN model to investigate these differences and better understand ORN.

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**O**STEORADIONECROSIS (ORN) of the mandible is a serious complication of radiotherapy to the head and neck. Despite more target-specific advances in radiation technology for the treatment of head and neck cancer, including intensity-modulated radiotherapy and high-dose-rate (HDR) brachytherapy, mandibular ORN continues to be a complication risk after radiation treatment of head and neck cancer. The incidence of maxillary ORN, especially in Western countries, appears smaller than the incidence of mandibular ORN. A study that retrospectively reviewed cases of ORN of the jaws during a 20-year period identified 99 cases of mandibular ORN vs only 5 cases of maxillary ORN.<sup>1</sup> Such observations have led to the belief that the

mandible is more susceptible to radiogenic bone injury and development of ORN. It has been postulated that the increased susceptibility of the mandible stems from its smaller blood supply and its compact bone structure.<sup>2-6</sup> To date, no study in humans or animals has compared maxillary and mandibular ORN to explain the smaller observed incidence of maxillary ORN.

In further searching the world literature, we found a subset of patients, especially in Asian countries, with an equivalent to a slightly greater incidence of maxillary ORN compared with its mandibular counterpart. In a review of 1758 patients undergoing radiotherapy for nasopharyngeal carcinoma, 48 cases of maxillary ORN vs 30 cases of mandibular ORN were identified.<sup>7</sup> In contrast to a predomi-



**Figure 1.** The external portion of the yellow brachytherapy catheter consistently measured 10 mm and served as a reproducible length to standardize and reproduce placement. This external distal portion of the catheter was secured to the non-hair-bearing neck and cheek skin using liquid adhesive and adhesive skin closure tape.

nance of mandibular ORN in all head-and-neck cancer patients, the maxilla may be at a higher risk of ORN in patients with nasopharyngeal cancer whose radiation ports are targeted closer to the maxilla. Moreover, ORN of the maxilla generally follows a more benign clinical course than the mandibular counterpart.<sup>7,8</sup>

Whether the maxilla is protected from ORN compared with the mandible remains to be studied. We investigated whether radiogenic bone damage can be produced in the rat maxilla by modifying a postradiotherapy dental extraction model already created in our laboratory for mandibular ORN.<sup>9,10</sup> Successful development of this maxilla model can serve as a platform to study possible differences between the maxilla and mandible with respect to radiogenic bone injury and development of ORN and further our understanding of this disease.

## METHODS

### ANIMAL MODEL

Approval for the research protocol was obtained from the Chancellor's Animal Research Committee of the University of California, Los Angeles. Ten male Sprague-Dawley rats (Charles River Laboratories; weight range, 160-222 g), 7 weeks of age, were used in this study following institutional guidelines regarding animal experimentation. The rats were kept in pairs in metal cages, given a standard pellet rodent diet and water ad libitum in accordance with the requirements of the US Animal Welfare Act and the Policy on Humane Care and Use of Laboratory Animals from the US Public Health Service.

The 10 rats were divided into 2 groups. The experimental group underwent brachytherapy to the left maxilla (n=6), whereas the control group had placement of an HDR catheter without irradiation (n=4). The second left molar was extracted 1 week after radiotherapy and the maxilla was harvested 3 weeks after dental extraction.

### BRACHYTHERAPY

Under inhalational isoflurane anesthesia, the left cheek skin of the rat was shaved and prepared with povidone-iodine (Betadine; Alcon Pharms Ltd). A sterile plastic HDR catheter (Alpha Omega Services) was implanted across the left maxillary second molar. The catheter was inserted via a stab incision with a sharp wire probe through the buccal mucosa just lateral to the left second maxillary molar and exited the skin halfway be-

tween the left lateral canthus and the left external auditory canal. The catheter was then advanced submucosally over the wire probe. The external portion of the catheter consistently measured 10 mm and served as a reproducible length along with the other landmarks to standardize and reproduce placement. This external distal portion of the catheter was secured to the non-hair-bearing neck and cheek skin using liquid adhesive (Mastisol; Ferndale Laboratories) and adhesive skin closure tape (Steri-Strips; 3M) (**Figure 1**).

A 3-dimensional (3D) treatment plan for brachytherapy was devised that encompassed a depth of 5 mm from a point 1 cm from the distal aspect of the catheter tip. A single dose of 20 Gy was applied with an HDR after loading the remote machine (Gammamed 12it; Varian Medical Systems, Inc). The catheter was removed after brachytherapy was complete in the experimental group and immediately removed after placement in the control group.

### TOOTH EXTRACTION

Under inhalational isoflurane anesthesia, all rats underwent atraumatic extraction of the left second maxillary molar 1 week after brachytherapy (or sham catheter placement). Extreme care was taken to avoid breaking the tooth roots from the crown. Postoperative pain management was achieved with 24 hours of buprenorphine hydrochloride administration (Buprenex; Reckitt Benckiser Healthcare Ltd) at a dose of 0.03 mg/kg given subcutaneously twice daily.

### PROCUREMENT

The animals were humanely killed 28 days after brachytherapy (21 days after tooth extraction). The maxillae were extracted and photographed using a commercially available camera (Rebel Ti digital single-lens reflex camera; Canon). Maxillae were placed in 10% formalin for 24 hours and then placed in 70% isopropyl alcohol.

### RADIOLOGIC ANALYSIS

Computed tomography (CT) of the extracted maxillae was performed using a desktop cone-beam micro-CT scanner ( $\mu$ CT 40; Scanco Medical). Three-dimensional reconstruction and volume analysis of the microradiographs was accomplished using micro-CT evaluation software (version 6.0; Scanco Medical). A standard method for evaluation of bone volume to total volume (BV:TV) of the mandible has been used previously in our laboratory.<sup>9</sup> From our prior pilot studies on the rat model of ORN, we performed a power calculation to determine the sample size. Based on this calculation we continued with a sample size of 10 rats, which is expected to provide 80% power to detect an effect size of 1.80 using a 2-sample *t* test with a 2-sided significance of .05 when comparing the percentage of BV remaining between the brachytherapy and control groups. We applied the same method in evaluating the maxillae.

Based on micro-CT evaluation of these maxillae, we created a cylinder with a defined TV to determine the volumes of bone in the extracted single tooth sockets. A consistent landmark where at least 3 roots of the third maxillary molar were seen was set as the first image to be analyzed. We determined that 45 sections 36  $\mu$ m apart and a circular area of 0.0746 cm<sup>2</sup> sufficiently included the area of interest around the extracted dental socket with appropriate height of the maxilla. The depth of 45 sections was calculated from the average depth from our landmark to the base of the root socket (0.16 cm). With these length, width, and height measurements, the software was able

to create a reproducible, standardized cylindrical volume measuring 11.157  $\mu\text{m}^3$  for the TV of analysis in all the animals.

## RESULTS

### CLINICAL (VISUAL/PHOTOGRAPHIC)

All rats survived the study period of 4 weeks. The rats from the brachytherapy group clearly demonstrated adverse effects of target-specific HDR radiotherapy, evidenced by unilateral left cheek skin alopecia in all subjects in the group, whereas the control group had complete hair regrowth.

Evaluation of the intraoral alveolar ridges using high-resolution digital photography demonstrated complete mucosal coverage at the dental extraction site in all rats in both groups. All the rats in the irradiation group, however, demonstrated scarring at the HDR catheter insertion site (**Figure 2**).

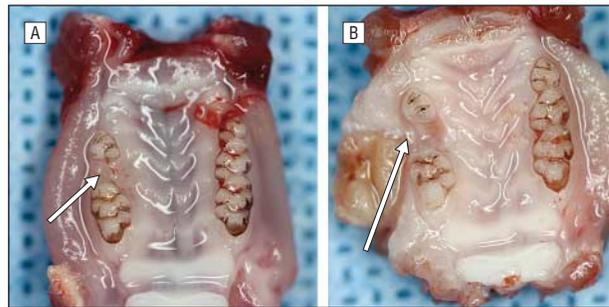
### MICRO-CT EVALUATION

Bone volume was measured in relation to the TV of the maxillary second molar extraction sockets and expressed as a ratio (BV:TV), as described in the "Methods" section (**Table**). We used a 2-tailed *t*-test to compare each of these variables between experimental and control rat maxillae. The level of significance was set at .05. The median BV:TV in the brachytherapy group was 0.21 (range, 0.20-0.33) compared with 0.49 (range, 0.39-0.57) for the control group ( $P = .01$ ).

Evaluation of the 3D micro-CT reconstructions of the rat maxillae allowed for ultrastructural viewing of bone regrowth and qualitative comparisons between experimental and control samples. The 3D reconstructions demonstrate gross reduction in cortical width, decreased bone formation in extraction sockets, and increased resorption in traumatic and atraumatic areas of irradiated maxilla compared with control samples (**Figure 3**).

## COMMENT

Mandibular ORN is a serious irreversible complication of radiotherapy to the head and neck. Mandibular ORN is a clinically defined entity described as exposed bone for at least 2 months.<sup>9,10</sup> The incidence varies in the literature from 2% to 22% and occurs most commonly in the mandible. Osteoradionecrosis can be early onset (occurring <2 years after radiotherapy) or late onset.<sup>11</sup> Recent theories on the pathophysiologic processes of ORN have diverged from the role of trauma and infection and instead focused on the fibroatrophic therapy to explain radiation-induced injury.<sup>11</sup> Despite advances in surgical treatment involving free tissue transfer, tumor-free patients are at increased risk of perioperative complications, recurrence, and even mortality.<sup>12</sup> Short of removing the entire mandible as the definitive cure for this disease, another alternative is needed to improve the lives of patients with head and neck cancer who are cured of their malignant neoplasms yet at indefinite risk of ORN. We recently developed a mandibular model of ORN in



**Figure 2.** Maxillae photographed 21 days after extraction of the second left molar (arrow) after sham therapy (A) or experimental brachytherapy (B). The experimental group demonstrated scarring at the high-dose-rate catheter insertion site.

the rat to serve as a platform to study the pathogenesis of this poorly understood disease.<sup>9,10</sup> In this model, HDR brachytherapy to the mandible caused radiogenic bone damage and mucosal breakdown with bony exposure, analogous to clinically defined mandibular ORN in human patients.

An obvious factor that has not been investigated previously is the susceptibility to ORN of the mandible vs other facial bones, such as the maxilla, which is also exposed to radiation during treatment of head and neck cancer. In reviewing ORN in the literature, the incidence of maxillary ORN, especially in Western countries, appears lower than the incidence of mandibular ORN.<sup>1</sup> It has been postulated that the increased susceptibility of the mandible stems from its lower blood supply and its compact bone structure,<sup>2-6</sup> but this remains to be proved. To study this postulation, we first needed an experimental model of maxillary ORN to compare with the mandible. This led to our present study, which is based on a modification of our recent mandibular ORN animal model.

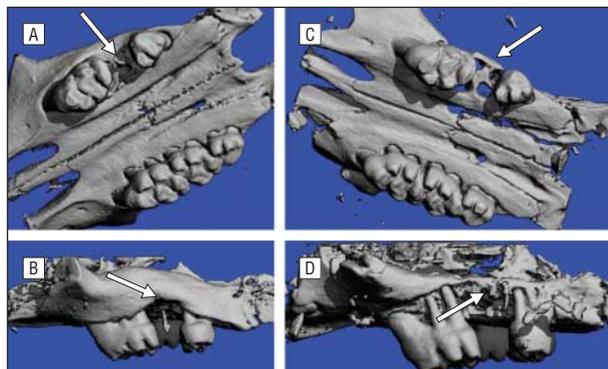
Based on our study, we found that the maxilla is highly susceptible to radiogenic bone injury as demonstrated by the significantly reduced BV:TV ratio in the experimental group compared with the control group. This finding implies that the lower incidence of maxillary ORN seen in clinical practice is partly due to the selective targeting of tumors more common in Western countries. As seen in large series of patients with nasopharyngeal carcinoma in Asian countries, the maxilla is in fact susceptible to ORN when included in the radiation fields.<sup>7,8</sup> The clinical presentation of maxillary ORN, however, does seem to differ from mandibular ORN and in general follows a more benign course. Radiotherapy for nasopharyngeal carcinoma often requires the administration of high doses of radiation and reirradiation in some cases. In addition, the requirement for microvascular reconstruction in cases of maxillary ORN is generally lower. In our model, all rats undergoing brachytherapy similarly demonstrated mucosal coverage (although scarred mucosa) of the wound defect, whereas with our mandibular model, mucosal breakdown and exposed bone were observed in all rats receiving radiotherapy.<sup>9,10</sup> Such differences between the mandible and maxilla can partly be explained by the improved vascularity of the surrounding tissue of the maxilla as reported in the literature.<sup>2-6</sup> Based on our study, however, it appears that ra-

**Table. Micro-Computed Tomography Volume Analysis of the Maxilla<sup>a</sup>**

Measurement	Rat No.									
	Brachytherapy Group						Control Group			
	1	2	3	4	5	6	7	8	9	10
BV, $\mu\text{m}^3$	2.68	2.20	2.52	2.22	3.66	2.21	6.06	4.36	6.31	4.97
TV, $\mu\text{m}^3$	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15
BV:TV ratio	0.24	0.20	0.23	0.20	0.33	0.20	0.54	0.39	0.57	0.45

Abbreviations: BV, bone volume; TV, total volume

<sup>a</sup>The TV was standardized at  $11.157 \mu\text{m}^3$ . Brachytherapy consisted of 20 Gy of radiation. The median BV:TV ratio was 0.21 (range, 0.20-0.33) for the brachytherapy group and 0.49 (range, 0.39-0.57) for the control group ( $P = .01$ ).



**Figure 3.** Three-dimensional micro-computed tomographic reconstruction of maxillae 21 days after extraction of the second left molar. Bony regrowth of the dental socket (arrow) is seen in the control group (A and B); however, lack of bony growth (arrow) is demonstrated in the exposed socket of the irradiated maxilla in the experimental brachytherapy group (C and D).

diotherapy has similar effects on the bony mandible and maxilla that may be independent of vascularity.

One limitation of our study is that we studied radiogenic bone injury and assessed ORN clinically as occurs in patients in present clinical practice. The process of ORN, however, is more complex than radiogenic bone injury, and we cannot equate radiogenic bone injury with ORN. The quality of the surrounding tissue and other local factors, such as blood supply, inflammation, possible infection, and osseous cellular imbalances, may contribute to or be critical to the pathogenesis of ORN. Because clinical differences were noted in the radiated maxilla vs the radiated mandible, undiscovered factors in the surrounding osseous milieu may be responsible for these differences and could further help explain the pathogenesis of ORN. This model of maxillary ORN can serve as a platform for further studies of these factors.

Another limitation of our study was the use of brachytherapy in our model, whereas in clinical practice fractionated external beam radiation is used. With the use of brachytherapy, the rats are exposed to an acute radiation insult that may not completely represent the same insult our patients receive with fractionated therapy. However, an effective animal model is simple and cost-effective and reduces unnecessary pain and discomfort to the subjects. It would not be feasible to have the rats undergo a course of fractionated external beam radiotherapy because they would require daily general anes-

thesia and therefore create a high cost. Although fractionated intensity-modulated radiotherapy would be more representative, it would not be feasible. By studying the acute effects of radiotherapy, other contributing factors to ORN may also be overlooked. For example, polymicrobial exposure may contribute to the differences between the maxilla and the mandible because they possess differing microenvironments.

Several theories for the pathogenesis of ORN have been postulated in the past, with recent theories focusing on a cellular defect underlying the disease process.<sup>13,14</sup> The possible role of osteoblast and osteoclast interaction has been brought to light, but these theories have yet to be validated by in vivo models.<sup>9,10</sup> By comparing radiogenic bone injury between the maxilla and the mandible, we hope to shed further light on the cellular basis of ORN through our in vivo model. In the future, we also plan to perform a histological analysis of our maxillae and compare osteoblast and osteoclast activity in the maxilla and the mandible. Longer-term studies investigating multiple factors theorized to play a role in ORN can be performed in our mandibular and now maxillary animal models.

Optimally treating and preventing ORN is premature because the pathogenesis of ORN remains uncertain. By creating a maxillary rat model analogous to clinical ORN, we have been able to show that radiogenic damage, when targeted, occurs in the maxilla and the mandible. The maxilla does not appear to be protected from radiogenic bone damage as might have been thought. Because we described clinical ORN, we noted differences between the maxilla and mandible when exposed to radiotherapy and dental extractions. Whether this less severe response or greater recovery ability is unique to the maxilla needs to be further studied. Because we now have an animal model of maxillary and mandibular ORN, further studies can be conducted and compared in order to challenge the age-old question of whether ORN is a vascular issue or more likely a local adverse effect of radiotherapy triggering sequelae of necrosis. Moreover, by elucidating possible differences between the maxilla and mandible with respect to the development of ORN, we hope to further our understanding of this disease that affects patients with cured head and neck cancer.

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**Author Contributions:** Drs Armin and Nabili had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Armin, Nishimura, Beumer, Steinberg, Abemayor, and Nabili. *Acquisition of data:* Armin, Hokugo, Tamplen, and Nabili. *Analysis and interpretation of data:* Armin. *Drafting of the manuscript:* Armin, Tamplen, and Nabili. *Critical revision of the manuscript for important intellectual content:* Armin, Hokugo, Nishimura, Beumer, Steinberg, Abemayor, and Nabili. *Statistical analysis:* Nabili. *Obtained funding:* Nabili. *Administrative, technical, and material support:* Armin, Hokugo, Tamplen, and Nabili. *Study supervision:* Nishimura, Beumer, Steinberg, Abemayor, and Nabili.

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