Effect of Radiation on Segmental Distraction Osteogenesis in Rabbits

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Objective: To determine whether consolidation can occur during radiotherapy after segmental distraction osteogenesis. Segmental distraction osteogenesis has potential as a reconstructive option after oncologic resection of the mandible. However, postoperative radiotherapy has potentially deleterious effects on bone consolidation after distraction osteogenesis.

Methods: Tibial defects of 1.0 cm were created in 5 New Zealand white rabbits. After a 6-day latency phase, a 1.0-cm distraction segment was created in 0.3-mm increments every 12 hours. Within 24 hours of the distraction completion, the tibia received the biologic equivalent of 6000 rad (60 Gy). After 6 weeks of consolidation, the animals were humanely killed. Bone was analyzed radiographically, grossly (at autopsy), and histomorphometrically.

Results: Four rabbits completed the 6-week consolidation period. All specimens had evidence of calcified bone in the segmental defect on radiographic analysis. At autopsy, the volume of new bone equaled that of the removed segment. On histologic examination, the volume of new trabecular bone was similar to adjacent cortical bone.

Conclusions: Consolidation of segmental distraction osteogenesis defects can occur in rabbit tibia during external beam radiotherapy. To our knowledge, this study is the first to demonstrate successful consolidation of segmental distraction osteogenesis during external beam radiotherapy.

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Currently, the state of the art in mandibular reconstruction after oncologic resection involves the use of free revascularized fibular bone. However, the fibular free-flap procedure has shortcomings, including the need for increased medical resources and operative time and the risk of patient death.1-5

Distraction osteogenesis (DO) is a technique that involves the gradual lengthening of native bone under the tension of a distraction device. It is a straightforward procedure that has been used for mandibular reconstruction in humans for more than 15 years.6 In patients with head and neck cancer, its use has been limited because of several concerns, including consolidation in patients who require postoperative radiotherapy, difficulty with distraction of nonlinear segments, and likely a general focus on microvascular reconstruction. Several studies have analyzed the use of transport DO in previously irradiated animal mandibles, and reports of cases in humans have described mixed success.7-10

There are many potential benefits of performing DO at the time of oncologic resection. This timing allows a more rapid reconstruction and return to function for the patient, limits the number of delayed surgical procedures, and allows distraction to occur in a radiation-free bone bed. What remains unknown is the ability of distracted bone to consolidate, or mature and harden—a process that generally takes 8 to 12 weeks in adults—while undergoing radiotherapy. This study was designed to address that question.

METHODS

Departmental approval and Mayo Clinic institutional animal care and use committee approval were obtained before the study was undertaken. Animals were maintained under the supervision of Mayo Clinic veterinarians. Five New Zealand white male rabbits older than 8 months (ie, skeletally mature) were obtained. Water and pellet diets were supplied ad libitum. All animals were housed in individual cages with padded floors.

SURGICAL PROCEDURE

Anesthesia in each rabbit was induced with intramuscular injection of ketamine (50 mg/kg), acepromazine (1 mg/kg), and xylazine (10 mg/kg). The rabbit was intubated, and anesthesia was maintained with inhaled isoflurane, 1%. The animal was monitored by using
An oblique incision was made over the ipsilateral inguinal ligament and extended to the femoral sheath. The femoral nerve was dissected free of the femoral vessels, and a 3-mm segment was removed. The femoral incision was then closed with 4-0 polyglactin 910 sutures (Vicryl; Ethicon Inc, Somerville, New Jersey). A 4-cm incision was made on the lateral aspect of the lower extremity and over the tibia. The dissection was extended to the tibia, preserving the periosteum. A 2.0-mm stabilization plate was secured to the tibia with 2 or 3 bicortical screws proximal and distal to the planned osteotomies. A plate-guided distractor (KLS Martin LP, Jacksonville, Florida) was applied to the tibia, screwed in place, and then removed. Two osteotomies were performed with an oscillating saw, and a 1.0-cm section of bone was removed from the midportion of the tibia (Figure 1). A third osteotomy created a 1.0-cm transport segment on the proximal end of the distal tibial segment. The plate-guided distractor was then reapplied to the previously created screw holes (Figure 2). The external portion of the device was tunneled subcutaneously and brought to the surface via a separate skin incision. The wound was copiously irrigated; hemostasis was obtained with bipolar cautery. The incision was closed with a polyglactin 910 4-0 suture, and antibiotic ointment was applied. An Elizabethan collar and a splint fashioned from a 2-mm thermoplastic sheet (Aquaplast; WFR/Aquaplast Corp, Wyckoff, New Jersey) and a conforming bandage (Vetrap; 3M Corp, St Paul, Minnesota) were applied at the completion of surgery.

**POSTOPERATIVE CARE**

The rabbits were observed postoperatively in recovery cages until they completely recovered from anesthesia. Postoperative analgesia (buprenorphine, 0.05 mg/kg intramuscularly every 8-12 hours) was administered. If a rabbit’s behavior was conducive to wound healing, the Elizabethan collar was removed after 1 week. However, if self-trauma became apparent, the collar was put on again. A surgical and postoperative record was maintained and displayed on each animal’s cage.

**DISTRACTION**

After a 6-day latency phase, distraction was started at a rate of 0.3 mm every 12 hours for 17 days or until resistance was met.

**RADIOThERAPY**

The rabbits were sedated with ketamine (15 mg/kg) and xylazine (3 mg/kg) given intramuscularly. They were then secured in position using fluoroscopic simulator, and the target area (clinical target volume) with margin was isolated using independent collimators. The clinical target volume received a total treatment dose of 3600 rad (36 Gy) administered in 4 fractions. Therapy was given 3 to 4 days apart using an isocentric anterior-posterior and posterior-anterior beam arrangement. Six-millivolt photon beams were used at a dose rate of 400 rad/min (4 Gy/min) through a Varian 2100 EX linear accelerator (Varian Medical Systems, Palo Alto, California). In both anterior and posterior fields, 1-cm boluses were used as tissue equivalent. In addition, a 3-dimensional treatment plan was implemented using a computed tomography–guided simulator to confirm the proper dose coverage. Calculated with an α:β ratio of 10:1 for acute radiation reaction, a total dose of 3600 rad (36 Gy) in 4 fractions was determined to be approximately the radiobiologic equivalent of 6000 rad (60 Gy) in 30 fractions, which is a common postoperative radiation dose given to patients with head and neck cancer.

**EUTHANASIA AND SPECIMEN COLLECTION**

On postoperative day 65, the rabbits were humanely killed with pentobarbital given intravenously after they were sedated. Radiographs of the selected tibia were taken in anteroposterior and lateral planes. The entire tibia with the intact distraction device was then excised and inspected for the presence of distracted bone and osseous union. The distraction device was removed from the tibia. The tibias were stripped of soft tissue, placed in a fixative of 70% ethanol, and stored at 4°C. They were dehydrated in ascending concentrations (70%, 95%, and 100%) of ethanol until fully dehydrated.

**HISTOLOGIC EXAMINATION**

The dehydrated tibias were infiltrated and embedded in methyl methacrylate, and 5-µm sections were cut. The sections were stained with Goldner trichrome stain to differentiate between fully mineralized bone (staining green) and unmineralized osteoid (staining red). Four measurements of cortical thickness were taken from the surrounding native bone (from 2 proximal and 2 distal segments) and averaged. This average was compared with the average cortical thickness in 2 separate areas on the regenerate bone to estimate the volume of bone regenerated.

**RESULTS**

Five rabbits successfully underwent surgery. However, 1 rabbit was killed after it had an unstable fracture of the study extremity on postoperative day 14. The 4 other rabbits completed the distraction, radiotherapy, and consolidation phases.
RADIATION ADVERSE EFFECTS

The 4 rabbits that completed radiotherapy had mild cutaneous erythema after its completion. No major adverse effects of radiation occurred, and no animals showed signs of infection, plate extrusion, or wound breakdown.

MACROSCOPIC EXAMINATION

All 4 rabbits showed the presence of bone formation and osseous union at gross examination (Figure 3).

HISTOLOGIC EXAMINATION

Specimens from the 4 animals showed evidence of calcified bone that spanned the segmental defect (Figure 4). Newly formed bone was typically trabecular, but it had a greater diameter than the adjacent cortical bone (Figure 4A). All samples showed osteoid in the regenerate bone (Figure 4B), which suggested continued bone formation. The average regenerate bone was similar in thickness to the native cortical bone in all specimens (Table).

COMMENT

Cancer of the oral cavity accounts for 30% of all cancers of the head and neck, and squamous cell carcinoma of the alveolar ridge alone accounts for 10% of these tumors. Resection of mandibular defects after head and neck ablative surgery remains a challenge. The goals of head and neck reconstruction are to restore function and cosmesis. In mandibular reconstruction, this includes the restoration of speech, mastication, swallowing, and normal mandibular contour.

Contemporary mandibular reconstruction uses free revascularized fibular bone. The fibular free-flap technique provides an excellent length of bone that can be contoured to fit the shape of the native mandible, has an excellent blood supply, and can be harvested with a skin pedicle for soft-tissue reconstruction when needed.

Although its results in microvascular reconstruction are reliable; this technique presents challenges. Harvest and placement of the flap require a surgeon skilled in microvascular reconstruction and, often, 2 separate surgical teams. Use of microvascular reconstruction can increase operative time, hospitalization time, and costs. Donor-site morbidity may also occur, including wound breakdown, ankle weakness, nerve damage, and gait alterations. Furthermore, although fibular bone provides an appropriate length for mandibular reconstruction, it invariably provides insufficient height, and additional bone grafting may be required to allow dental implantation.

Distraction osteogenesis is a technique that involves the gradual lengthening of native bone at a surgically created osteotomy under the tension of a distraction device. As new bone forms at the osteotomy site, the bone is distracted further, slowly increasing the length of the new bone at a rate of 0.5 to 1.0 mm daily. After the desired length of bone has been attained, the distraction device is removed.

Table. Average Cortical Thickness in Native Cortical Bone and Regenerate Bone

<table>
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<th>Specimen</th>
<th>Native Bone</th>
<th>Regenerate Bone</th>
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<tr>
<td>1</td>
<td>1314.9</td>
<td>1968.6</td>
</tr>
<tr>
<td>2</td>
<td>1543.7</td>
<td>1452.0</td>
</tr>
<tr>
<td>3</td>
<td>2289.3</td>
<td>1804.9</td>
</tr>
<tr>
<td>4</td>
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Figure 3. Rabbit tibia and distractor device. Scale indicates centimeters, and union between distraction segment and proximal native tibia is present at the 1-cm mark.

Figure 4. Specimens show evidence of calcified bone spanning the segmental defect (modified Goldner trichrome stain, original magnification ×20). A, Rabbit tibia regenerate bone (left side) and adjacent native cortical bone (right side); calcified bone stains dark blue, while osteoid stains red. B, Regenerate bone with osteoid area (large arrow) lined by osteoblasts (small arrow).
vice is left in place for several weeks as the new bone matures and consolidates, and then the distractor device is removed.

Application of the distraction device is straightforward and does not require specialized surgical training. It adds little to the operative time, causes no donor-site morbidity, and requires no specialized postoperative monitoring. Although the distraction process is time-consuming, patients and families can be taught to do the distraction themselves at home.

Distraction osteogenesis has been used for mandibular reconstruction in humans for 15 years, and its applications and frequency of use are continually increasing. It is most frequently used in craniofacial reconstruction in patients with mandibular developmental abnormalities, such as those found in Pierre Robin syndrome. Costantino and associates introduced the concept of mandibular segmental DO in 1990, demonstrating its efficacy in repairing segmental defects in dogs. New techniques and materials allow curvilinear distraction, which can provide a more natural mandibular contour. Furthermore, curvilinear distractors have been designed to allow complete submersion after the completion of distraction, and this can allow for long-term stabilization during the consolidation phase.

The height of the distracted mandible segment is equal or nearly equal to the height of the native mandible, and the distracted segment can accept dental implants.

Muhonen and colleagues performed several studies on rabbit mandibles that were pretreated with radiotherapy. They specifically evaluated the effects of hyperbaric oxygen treatment on bone formation, angiogenesis, and osteoblastic activity in the distraction of previously irradiated rabbit mandibles. Although radiotherapy had deleterious effects in the irradiated bone, some of which were mitigated by hyperbaric oxygen, DO was successfully performed in previously irradiated rabbit mandibles with or without the use of hyperbaric oxygen. Gantous and colleagues successfully performed DO in 4 of 5 canine mandibles 6 months after external beam radiotherapy was completed.

In contrast to the successes reported in animal studies, results in human studies have been discouraging. Holmes et al reported 2 cases of failed distraction in previously irradiated mandibles in humans; an absence of bone formation was noted during the surgical procedure to remove the distraction devices. Girod and colleagues performed bilateral mandibular distraction osteogenesis in goats, with 12 of 32 animals receiving irradiation during the consolidation phase. The researchers found no significant difference between the irradiation group and the control group, with the exception of a smaller osteoid surface of the regenerate bone in the experimental group. To our knowledge, no studies have been published on transport DO for segmental defects with irradiation during the consolidation phase.

Performing DO at the time of oncologic resection allows a more rapid reconstruction and return to function for the patient, limits the number of delayed surgical procedures, and allows distraction to occur in a radiation-free bone bed. One of the major limitations to success is the patient’s need for postoperative radiotherapy.

Many studies have demonstrated decreased local and regional control of head and neck cancer when radiotherapy is delayed after diagnosis. These studies have led to the current treatment paradigm in which the patient completes radiotherapy ideally within 13 weeks of diagnosis. Because most patients receive approximately 6 weeks of radiotherapy, this regimen necessitates the institution of radiotherapy within 5 to 6 weeks of surgery.

Distraction osteogenesis can be divided into 3 stages: latency, distraction, and consolidation. The latency phase lasts for several days and allows for the formation of soft callus. The duration of the distraction phase varies depending on the length of regenerate bone needed and the distraction rate. In adults, the rate of distraction in the mandible is 0.5 to 1.0 mm/d. The duration of the consolidation phase varies depending on the patient’s age. This phase is critical because during it, the distracted bone is allowed to mature and gain strength while the distractor is left in place to provide rigid support for the healing mandible. The following consolidation periods in the mandible have been recommended: 4 to 5 weeks in a child, 6 to 8 weeks in a teenager, and 8 to 12 weeks in an adult.

Several studies have evaluated methods to decrease the duration of the consolidation phase, including the use of local factors such as calcium sulfate, chitosan microsphere–encapsulated human growth hormone, pulsed ultrasound, and other growth factors. At a distraction rate of 1.0 mm/d and with a 3- to 4-day latency phase, a 4-cm defect would complete the distraction phase in about 7 weeks. Therefore, to not diminish the benefits of radiotherapy on survival and local and regional control, patients would need to start radiotherapy during the critical bone consolidation phase of the DO process.

Distraction osteogenesis has excellent potential to both simplify and improve mandibular reconstruction after head and neck ablative procedures. However, it remains unknown whether the distracted bone can tolerate radiotherapy and mature while undergoing the treatment. Our study was designed to answer this question.

Four rabbits completed the study protocol, and all 4 animals had calcified bone and osteoid formation at the site of the segmental DO. The regenerate bone had a greater caliber than the adjacent native cortical bone, although it was trabecular in nature. Macroscopic examination showed the presence of osseous union between the distraction segment and the proximal native tibia.

Because of the short period from placement of the DO device to euthanasia, a conclusion about whether the regenerate bone would have matured to cortical bone is not possible. The presence of osteoid is promising, however. The shortcomings of this study include its small number of animals and lack of a control group. A control group would have assisted in elucidating what changes, if any, concurrent radiotherapy causes on consolidation. Also, a larger sample size would have allowed euthanasia to occur at several different stages of maturation, thus allowing the documentation of the rate and extent of consolidation. Another limiting aspect of this study was the animal chosen. Our preliminary studies (results not published) have demonstrated the ability of rabbits to break reconstruction plates with their pow-
erful hind leg, and thus, we sectioned the femoral nerve. Future directions in DO include the use of chemotherapeutic agents and assessment of the viability of dental implants in the regenerate bone.

The present study successfully showed that consolidation and osseous union can occur during radiotherapy of segmental tibial defects in rabbits. To our knowledge, it is the first study to show successful consolidation of segmental DO during external beam radiotherapy. Although further controlled studies are warranted, segmental DO may be an alternative to microvascular reconstruction of oncologic segmental mandibular defects.

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REFERENCES


