Osteoinduction Using Bone Morphogenic Protein in Irradiated Tissue

Brian K. Howard, MD; Karla R. Brown, MD; Joseph L. Leach, MD; Cheng-Hui Chang, PhD; David I. Rosenthal, MD

Objective: To prove the efficacy of bone morphogenic protein as an osteoinductive agent in irradiated tissue.

Design: Prospective randomized controlled trial designed to test the effectiveness of recombinant bone morphogenic protein 2 (rBMP-2) combined with solid hydroxyapatite disks in an irradiated tissue bed.

Subjects: Eighteen adult, male, white New Zealand rabbits weighing 3.0 to 3.5 kg.

Intervention: The rabbits were randomly divided, with 9 receiving radiation treatment and 9 receiving no radiation treatment. Each animal underwent implantation of 2 hydroxyapatite disks onto the snout at 9 weeks following radiation treatment. One disk was impregnated with rBMP-2 and the other with buffer only. The animals were killed at 3, 6, or 20 weeks after implantation for analysis.

Results: Histological analysis demonstrated that rBMP-2 was equally effective as an osteoinductive agent in the irradiated and nonirradiated tissue. We also found significantly increased new bone formation in the rBMP-2 group vs the buffer group.

Conclusions: This study supports the potential clinical utility of rBMP-2 and solid hydroxyapatite in irradiated tissue beds. These findings have interesting implications for patients with head and neck cancer who have undergone radiation therapy and need bony reconstruction.


NEW TECHNIQUES for craniofacial tumor ablation have improved the ability to surgically remove difficult tumors. While these techniques offer potential long-term survival and locoregional control, surgeons are now faced with more difficult reconstruction dilemmas. Furthermore, curative treatment for many of these malignancies involves adjuvant therapeutic irradiation. Reconstruction in this environment creates a compounded problem due to radiation-induced soft tissue fibrosis, cell necrosis, and compromised vascular supply.1-3 To date, a variety of materials have been used to reconstruct defects after extirpation. The use of autologous bone and cartilage is susceptible to long-term resorption and donor site morbidity.4-7 Allograft materials have the same limitations in addition to the potential transmission of infectious diseases.4,5,7 Synthetic materials have been used with limited success owing to infectious morbidity, extrusion, migration, and foreign body reaction.8-10 The use of hydroxyapatite (HA) has shown some promise in reconstructing the skeletal support of the defects. Although nonantigenic, HA has had limited application due to its lack of osteointegration. Poor osteointegration has resulted in migration, fragmentation, and long-term resorption.11,12 The brittleness of HA blocks makes them difficult to use with plating techniques.13 Hydroxyapatite slurry is available but cannot maintain any structural stability in the short-term.13 Ideally, surgeons would want to reconstruct using a nonantigenic substance that could be easily shaped and immobilized, undergo complete host osseous incorporation, be used safely in irradiated tissue, and show minimal resorption with time.

Bone morphogenic protein (BMP) was discovered relatively recently as a substance belonging to the family of transforming growth factor β. This class of molecules has been shown to reliably induce osteoneogenesis. It appears to be a highly conserved molecule, which to date has no known antigenicity and no adverse local or systemic effects.14 Bone morphogenic
MATERIALS AND METHODS

Twenty adult, male, New Zealand white rabbits weighing 3.0 to 3.5 kg were included in the study group. Two animals were used to develop the initial working model. A detailed protocol was followed after approval by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center, Dallas. All rabbits were individually caged throughout the study, and food and water were provided ad libitum.

The experiment was initiated after a successful animal model was established. One half (9) of the animals were randomly selected to receive XRT. The surgical bed over the snout of each rabbit was irradiated with five 4-Gy fractions of 6-MeV electron beams. The dose was prescribed to the 90% isodose line with 1 cm of bolus, which administered a full dose to the operative bed but spared the oronasal mucosa. The output factor used to monitor unit calculation was measured with a diode system. A 10-cm electron cone was used. There was secondary collimation with a cerrobend electron cutout with an opening of 6 × 7.3 cm. The field was further shaped by the tertiary collimation using a trapezoidal 3-mm-lead thick lead cutout that was 4 cm in height and had parallel sides of 4.5 cm and 5 cm. The lead cutout was bent to fit the snout contour of the rabbits.

All 18 animals underwent disk implantation 9 weeks following the completion of XRT. We decided to wait 9 weeks after XRT for implantation because rabbits have a bone and tissue turnover rate approximately 3 times faster than that of humans. Therefore, the 9-week waiting period for rabbits is equivalent to a 6-month waiting period for humans. Six months is the minimum recommended waiting time prior to attempting delayed reconstruction in humans, and we wished to mirror the human model as closely as possible. Anesthesia was induced with 30 mg/kg of intramuscular ketamine hydrochloride and 5 mg/kg of intravenous xylazine (Rompun, German) and had parallel sides of 4.5 cm and 5 cm. The lead cutout was bent to fit the snout contour of the rabbits.

All 18 animals underwent disk implantation 9 weeks following the completion of XRT. We decided to wait 9 weeks after XRT for implantation because rabbits have a bone and tissue turnover rate approximately 3 times faster than that of humans. Therefore, the 9-week waiting period for rabbits is equivalent to a 6-month waiting period for humans. Six months is the minimum recommended waiting time prior to attempting delayed reconstruction in humans, and we wished to mirror the human model as closely as possible. Anesthesia was induced with 30 mg/kg of intramuscular ketamine hydrochloride and 5 mg/kg of intramuscular xylazine hydrochloride. The snouts were shaved, prepared with povidone-iodine solution, and draped in sterile fashion. A midsubgaltal 4-cm incision was made through the peristeum of the snout, and 2 isolated subperiostal pockets were elevated along the midline (Figure 1). The pockets were separated by a 10-mm gap to avoid overlap of individual osteoneogenesis centers. A solid HA disk measuring 5 × 10 mm (200-µm pore size; Interpore International, Irvine, Calif) was placed into each pocket. Recombinant bone morphogenic protein 2 (Genetics Institute, Cambridge, Mass) or buffer solution was applied. One disk in each animal received 144 µg of rBMP-2 in 0.180-mL buffer solution, while the second disk received an equal amount of control buffer. This dosage of rBMP-2 was recommended by the supplier and has proven effective in other animal studies. All solutions were directly applied to the disks with a single-use micropipette syringe. The incisions were closed using 4-0 chronic sutures.

The animals received 7.5 mg/kg of intramuscular chloramphenicol for 3 days after surgery, and 100 µg/kg of subcutaneous buprenorphine hydrochloride 3 times per day for 2 days for analgesia following surgery. At 4 weeks after implantation, the animals received the described doses of ketamine and xylazine and were killed by cardiac puncture exsanguination. The snouts with HA were then sharply harvested en bloc. The specimens were placed into a 10% formalin solution, dehydrated in an ascending concentration of acetone, and embedded in methylmethacrylate. The implants were cut on a low-speed wafering saw (Buehler, Evanston, Ill) to expose their interior surfaces. The surfaces were carbon coated in a vacuum evaporator (Denison Vacuum, Cherry Hill, NJ) and documented by random selection using a scanning electron microscope (JEOL JSM 840A, JEOL Inc, Tokyo, Japan) at an accelerating voltage of 30 kV in the backscattered electron imaging mode. Images were standardized by adjusting the wave-form monitor in backscattered electron imaging mode so that the HA contributed the maximum intensity (white) and the methylmethacrylate contributed the minimum (black).

New bone growth appeared as intermediate intensity (gray). Polaroid Type 55 Land Film (Polaroid Corp, Cambridge, Mass) was used for documentation at a magnification of ×50. Six representative sections were photographed from each harvested disk. A counting grid was used to determine the percentage of new bone growth in each section. The calculations from the grids were performed separately by 2 examiners in a blind fashion. Animals were killed at 3, 6, or 20 weeks after implantation. Three animals from both groups in the study (the group with XRT and the group without XRT) were randomly selected at each time interval.

Data were evaluated using a 3-factor analysis of variance (ANOVA) with 1 of the factors, rBMP-2, repeated within animals. The factors rBMP-2 and XRT were observed at 2 levels (present and absent), and time was observed at 3 levels (3, 6, and 20 weeks after implantation). P ≤ .05 was considered statistically significant.

RESULTS

All animals completed the study. Radiated animals were examined on every treatment day and then every other day for evidence of corneal or oral/nasal mucosal changes from XRT injury. No animal exhibited mucosal damage, and all maintained dietary intake equivalent to that of their non-irradiated counterparts. Two animals developed tempo-
ratory sciatic nerve palsies as a result of intramuscular ket-amine injections in the hindquarter. Both animals recovered without sequelae and completed the study.

A significant relationship was noted between bone growth and time from surgery. Animals that received rBMP-2 had a significant increase in bone growth at 6 weeks and again at 20 weeks ($P < .05$). Those that received buffer only had an increase in bone growth from 3 to 6 weeks ($P < .05$), but the increase from 6 to 20 weeks was not statistically significant (Figure 2). In addition, the amount of bone growth over time was significantly greater in the group that received rBMP-2 than in the controls ($P < .05$). The effects of rBMP-2 with XRT were statistically significant at all time intervals ($P < .05$). With rBMP-2, there was no significant difference in bone growth between the group that received XRT and the group that did not ($P = .61$, 1-way ANOVA). Surprisingly, in the animals that received buffer only, more bone growth was recognized in those that received XRT than in those that did not ($P < .05$). Little or no growth was seen in the control disks (Figure 3). With rBMP-2, osteoneogenesis was limited to the HA disks. No islands of new bone growth were noted beyond the implanted disks. Furthermore, there was uniform growth of newly formed bone in the HA, so no skip areas were found (Figure 4).

**COMMENT**

Head and neck reconstruction remains a challenging and often aesthetically disappointing task. Reconstructing defects involving skeletal components is particularly difficult, and irradiated tissue beds further complicate the reconstructive outcome.

Reconstruction with autologous tissue has evolved as the criterion standard for most head and neck defects. Although donor-site morbidity with autologous grafts is always a consideration, donor sites for soft tissue reconstruction are relatively abundant and diverse. On the other hand, skeletal reconstruction presents more problems. The complexity of the facial bony skeleton makes accurate reconstruction extremely difficult owing to the paucity of available bone donor sites, the frequent need for complex osteotomies, and limitations of the vascular pedicles of the bone-containing flaps. Hence, surgeons are often faced with achieving less-than-ideal results. Free bone grafts of autologous or homologous origin are readily available, but they are susceptible to long-term resorption, migration, and infection. When free bone grafts are placed in a previously irradiated tissue...
bed, the risk of these complications is likely to increase. The use of synthetic materials under these conditions is also risky because of the possibility of migration, infection, and rejection.

Taking advantage of the osteoconductive properties of HA and the osteoinductive properties of rBMP-2, this report demonstrates the in vivo potential of creating form-controlled autogenous bone in irradiated tissue. One factor not addressed in this study was the need for an intact overlying periosteum. All implants were placed in a subperiosteal pocket. However, other work has shown that rBMP-2 induces bone growth from primitive mesenchymal cells in the absence of periosteum. It would be interesting to determine if osteogenesis could occur in this irradiated model in the absence of periosteum. Such a finding might have clinical relevance since periosteum is often missing where hard tissue needs to be replaced over a facial prominence. This study demonstrates the feasibility of introducing rBMP-2 by direct application onto the HA conduit. Although this may seem to be an inaccurate method of applying the osteoinductive substance, it was demonstrated that osteogenesis is limited to the HA conduit. We also noted that the HA block was uniformly replaced by new bone growth and that there were no skip areas lacking new bone. The latter finding would be important for load-bearing areas such as the mandible, where structural incontinuity may result in areas of weakness and possible fracture. Clinically, a combination of rBMP-2 and HA could eliminate the need to rely on autogenous free bone flaps. Skeletal platforms could be created to accurately reestablish the missing framework before or during surgery. Further study will hopefully provide cost-efficient means of obtaining sculptable or presculpted HA in block form. One promising method is the use of mineralized bone in a paste form, which could be sculpted during surgery. The paste hardens within minutes, at which time rBMP-2 could be directly applied. It is interesting to note that bone growth was significantly greater in irradiated specimens with or without rBMP-2 than in their nonirradiated counterparts. The mechanism by which this increased bone growth occurred is unclear.

CONCLUSIONS

We have demonstrated that osteogenesis induced by rBMP-2 is unimpeded by previous irradiation in an animal model. This finding has important clinical implications for delayed head and neck reconstruction. Reconstruction of bony defects could be accomplished by the use of an HA skeletal framework combined with rBMP-2. This would create an autologous, host-integrated, bony structure, which could be sculpted during surgery. The paste hardens within minutes, at which time rBMP-2 could be directly applied. It is interesting to note that bone growth was significantly greater in irradiated specimens with or without rBMP-2 than in their nonirradiated counterparts. The mechanism by which this increased bone growth occurred is unclear.

REFERENCES