Osseous wound healing with xenogeneic bone implants with a biodegradable carrier

Jeffrey O. Hollinger, DDS, PhD, John P. Schmitz, DDS, MS, Deiren E. Mark, PhD, and Alan E. Seyfer, MD, FACS, Washington, D.C., and Bethesda, Md.

Human antigen-extracted, autolyzed (AA) bone and a bovine bone morphogenetic protein were prepared as implants within biodegradable carriers and compared with autogenous bone grafts and controls in the healing of critical-size bony defects in nonhuman primates. The treated craniotomy sites were studied 3 and 6 months after surgery; radiodensity and volume of newly deposited trabecular bone were assessed by radiomorphometric and histomorphometric methods, respectively. There was no evidence of adverse immunologic response to the experimental implants. The autografts resulted in the greatest volume of new bone formation (p < 0.01), but the AA implants elicited a significantly greater response than either the bovine bone morphogenetic protein derivatives or the controls (p < 0.05). By 6 months, the AA derivatives had healed with actively coalescing islands of new bone, displaying normal-appearing outer and inner tables along with well-developed marrow cavities. It appears that xenogeneic AA implants have the ability to elicit an excellent osseous response in critical-size calvarial wounds. In addition, the carrier polymer for the implants acted as an effective soft-tissue spacer before being absorbed. (SURGERY 1990;107:50-4.)

From the U.S. Army Institute of Dental Research, the Plastic Surgery Service, Walter Reed Army Medical Center, Washington, D.C., and the Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, Md.

To date, many of the surgical substitutes for autogenous bone have used agents derived from the organic matrixes of allogeneic bone. These human donor materials, however, invariably undergo resorption, are susceptible to the immunogenic responses of the host, and may carry the risk of transmitted viral agents. In addition, both autogenous and allogeneic grafts are not always available in sufficient quantity to repair the large defects that sometimes result from trauma, tumor ablation, or reconstructive procedures, especially in children. There is a need, therefore, for stable immunologically privileged bone repair materials.

The purpose of this investigation was to test the effectiveness of xenogeneic (human and bovine) bone derivatives in the healing of experimental osseous defects in nonhuman primates.

MATERIAL AND METHODS

Phase I: Determination of the critical-size defect. To obtain meaningful data, it was necessary first to establish the "critical-sized" defect in the test site of the appropriate animal model, that is, the dimensions of the experimental bony defect that would not heal spontaneously with bone. In an effort to establish this parameter, craniotomies of ascending sizes were prepared in 18 mature Macaca fascicularis (cynomolgus) nonhuman primate species (NHPS). This model was selected because of the close anatomic and physiologic similarity of the test site to the human calvaria. Skeletal maturity was confirmed radiographically in each subject by assessing closure of the epiphyseal plates of the distal femurs and proximal tibias.

Under general endotracheal anesthesia (Pentothal sodium induction, 20 mg/kg; maintenance: halothane/nitrous oxide/oxygen), both parietal bones were generously exposed through a midline cranial vertex incision. Three full-thickness bony defects were established with
trephines and dental burs: on the right side, one 20 mm defect; on the left side, separate 15 mm and 8 mm defects. No grafts or foreign materials were placed in the defects, and the scalp was reapproximated over the craniotomy sites. After 6 months, the defects were reexposed and completely excised, incorporating a 2 mm rim of undisturbed bone around the craniotomies. The harvested specimens were subjected to radiographic and histologic analysis, and it was concluded that any defect over 15 mm would not heal spontaneously.

Phase II: Testing of xenogeneic materials. For the experimental phase, the NHPS were subjected to an identical operative approach, establishing a single 24 mm circular, full-thickness osseous defect in one of the parietal bones. Four experimental groupings, each consisting of six NHPS, were randomized for treatment. The fresh craniotomy defect was then repaired with materials according to one of the following variations: (1) autogenous particulate corticocancellous bone recovered from the iliac crest of the same subject; (2) xenogeneic (human) antigen-extracted, autolyzed (AA) bone in a biodegradable carrier of 50:50 DL-polylactide coglycolide; (3) xenogeneic (bovine) bone morphogenetic protein (BMP), also in a biodegradable carrier of 50:50 DL-polylactide coglycolide; and (4) untreated controls in which the defect was identical to that of the other groups.

Xenogeneic implant preparation

**Bovine bone morphogenetic protein.** An insoluble, noncollagenous fraction of bone matrix was quantitatively extracted according to the procedures described by Urist et al. to yield a BMP. The main components of the extract corresponded to a molecular weight of 23,000, 18,000, and 12,000 daltons compared with standards. The biodegradable carrier material for the BMP was prepared by dissolving 50:50 DL-polylactide coglycolide in acetone. One hundred five milligrams of BMP was added evenly to the carrier solution in a glass dish. Dishes were placed under a hood at room temperature for 48 hours to allow for evaporation of the acetone. The cured implants, premolded to fit the craniotomy defect, was sterilized at room temperature with ethylene oxide. The specimens were aerated and degassed under vacuum (25°C; 20 mtorr) for 48 hours to remove residual ethylene oxide and resultant products (ethylene chlorohydrin and ethylene glycol).

**Human chemosterilized, antigen-extracted, autolyzed bone.** The preparation of the AA Bone was a modification of the 10-step protocol for block implants described by Urist. This method was modified to include grinding of the donor diaphyses before demineralization in 0.6N HCl. The resulting particle size ranged from 75 to 420 μm. The carrier polymer was again prepared by dissolving 50:50 DL-polylactide coglycolide in acetone with 16 gm of the human particulate bone. The solution was cured under a hood for 48 hours at room temperature, sterilized in ethylene oxide, aerated, and degassed as described above.

Each batch of the experimental preparation (both AA bone and BMP) was subjected to bioassay before use. In this test, either 20 mg of AA bone or 6 mg of BMP was inserted into the pectoralis major muscle of 28-day-old Long-Evans rats. After 28 days, the test site was evaluated histologically for the presence of an active ossicle of new bone. Only positive-assayed batches were used in the experiments.

Retrieval of specimens. The craniotomy sites of three subjects from each group were harvested with a 2 mm rim of undisturbed bone at 3 and 6 months. Specimens were recovered and radiographed with Kodak X-Omat TL x-ray film and a Minishot Cabinet x-ray system (TFI Corp., New Haven, Conn.) at 25 kVp, 3 MA, 1 second. The film was developed in an automatic x-ray film processor. Specimens were placed immediately into 70% alcohol, followed by ascending alcohol s, and into polymethyl methacrylate for undecalcified sectioning. Serial sections were stained with modified Masson-Goldner trichrome and von Kossa stains and assessed.
Histomorphometric analysis. For each 24 mm craniotomy, 12 histologic fields, at 2 mm intervals from host margin to host margin, were studied. The defects were sectioned to a thickness of 4.5 μm, and the image analysis system interfaced with a universal microscope (Carl Zeiss, Inc., Thornwood, N.Y.) and Newvicon camera (Cambridge Instruments) was used to measure the volume of new bone (trabecular bony volume) that was forming across each craniotomy site. The trabecular bony volume was quantitated with a digitizing board and light cursor to compute the percentage of each microscopic field that was occupied by new bone. Data analyses for between-group differences and differences over time were performed by a one-way analysis of variance computer model. A multiple-comparison test was used to determine treatment differences within the same temporal group.

RESULTS

There was no clinical or histologic evidence of adverse immunologic reaction to the experimental implants or grafts. At 3 months, there was evidence (palpable hardness) that the autogenous graft had elicited a partial healing. Both the bovine BMP and human AA bone...
implants, however, were palpably softer. At 6 months, clinical bone healing was readily apparent at the autogenous graft site. The AA bone was also palpably firm. The BMP, however, exhibited only a fibrous union with a soft-tissue consistency.

The 3- and 6-month radiomorphometric analyses are displayed in Fig. 1. At 3 months, the percent area fill was greatest for the autogenous graft, followed by the AA bone, BMP, and control specimens, respectively. The autogenous graft also elicited a significantly greater response than did the other experimental treatments (p < 0.05). In addition, the response to the AA bone was significantly greater than the response at the BMP and control sites (p < 0.05). The radiopacities of both the autogenous graft and the AA bone were also greater at 6 months than at 3 months (p < 0.01) and were significantly greater for the autogenous graft-treated craniotomies than for the AA bone implants (0.01 < p < 0.05). The BMP implants and the untreated controls displayed no statistical difference in radiopacity.

At the histomorphometric study at 3 months, the AA bone implants showed islands of bone that by 6 months, in one animal, had coalesced into well-developed inner and outer tables with an intervening marrow cavity (Fig. 2). Overall, the autogenous grafts were superior to the xenogeneic treatments. The trabecular bony volumes measured in the graft-treated recipient sites were significantly greater than the implant-treated and control sites at both 3 and 6 months (p < 0.01; Fig. 3). Also, the trabecular bony volume associated with the AA bone was significantly greater than that of the BMP implants and untreated controls (p < 0.05). As with the radiomorphometric assessment, there were no significant differences in bony trabecular volume between the BMP implants and the untreated controls at either 3 or 6 months (Fig. 3).

DISCUSSION

In 1965 Urist11 discovered consistent osteoinduction through the use of demineralized bone matrix. After his work, Glowacki et al.12 and Mulliken et al.13 used similar preparations in conjunction with the correction of craniomaxillofacial deformities. Urist modified the processing of bone matrix to include chemical sterilization, a reduction in antigenicity, and an enhancement of the osteoinductive potential by preserving the bone morphogenetic activity.1 The resulting product has been called a chemosterilized, antigen-extracted, autolyzed bone (AA bone). Urist et al.10,14 also described a BMP extracted from the noncollagenous matrix of bone that induces differentiation of perivascular mesenchymal cells into osteoprogenitor cells, a process he defined as osteoinduction. It was the purpose of our study to determine if these two xenogeneic bone matrix derivatives (i.e., particulate AA and BMP) could successfully regenerate bone in the osseous wounds of nonhuman primates.

Autogenous iliac crest was clearly the most effective treatment for the craniotomies. This repair material continues to be the “gold standard,” and its performance is most likely related to the direct transfer of viable osteoblasts, preosteoblasts, and marrow stem cells, as originally described by Axhausen15 and Phemister,16 in the early phases of bone healing. The release of bone-inductive agents during later healing probably augments the osseous response.17 The AA bone stimulated new bone growth, but to a lesser degree than the autogenous graft. This partial success may have been related to the smaller particle size resulting from our modified preparation protocol, the improved provision for cell attachment as a result of this modification, and the release of bone-inductive factors that normally initiate the differentiation of nearby mesenchymal cells into active osteoblasts.10 At no time did the AA bone or BMP appear to elicit an adverse tissue response, and there was no evidence of residual carrier polymer at either 3 or 6 months, confirming its biocompatibility and biodegradability.6 Contrary to previous reports of successful bone regeneration of dogs and human patients with BMP, our study could not support that data.18,19 Sato and Urist19 demonstrated bone repair of 14 mm cranio-
omes in dogs with bovine BMP and a carrier matrix, γ-carboxyglutamic acid-rich protein. They showed that with a 200 mg bovine BMP dose, 89.9% healing occurred at the defects within 8 weeks and 86.8% healing was present at 11 weeks. For 50 mg doses, there was 57.1% healing at 8 weeks and 61.9% healing at 11 weeks. With 105 mg bovine BMP in a flexible film of 50:50 DL-polylactide coglycolide, our data revealed less than 10% healing. There may be several reasons for these differences. Sato and Urist may have not used a critical-sized defect. They stated that in dogs about half of a 14 mm trephine defect will repair, whereas a 24 mm craniotomy defect in a nonhuman primate will not go on to osseous repair. This is of critical importance because partial regeneration of calvaria in the Sato and Urist study may have been a result of normal bone repair. Notably, they reported 29.4% to 41.5% bone healing in their controls. In addition, prolapse of soft tissue into the 24 mm nonhuman primate defect may have been a significantly greater deterrent to bone regeneration than prolapse into the smaller, 14 mm canine wound. The ratio of the nonhuman primate defect to its skull surface is substantially greater than the ratio of the dog defect to its skull surface. Furthermore, even though the dogs in the Sato and Urist study and the NHPS in our study were treated with bovine BMP, there may have been an early, acute, and intense inflammatory response at the NHPS' recipient sites that resulted in a marked loss of osteoinductive capacity.

The positive results with human BMP reported by Johnson et al. in treating six human patients for segmental tibial continuity defects may be attributed to species specificity of human BMP, the dose (50 to 100 mg), and concurrent autogenous graft augmentation. Furthermore, Johnson et al. administered human BMP plus autogenous graft to a functional, long bone site in contrast to the passive, cranial site that we used in our study. Thus differences in responses to graft materials at functional versus passive sites and differences as a result of embryonic origin (membranous bone versus endochondral bone) may explain why our results with bovine BMP were not successful.

From this study, it appears that a xenogeneic particulate derivative of bone (AA) in a biodegradable carrier may be useful for initiating new bone formation in cranio-tomy defects. Theoretically, an allogeneic material might produce an even more profound healing response. Perhaps in the higher species, a species-specific (allogeneic) BMP must be administered for osteoinduction, and by incorporating the protein within a biodegradable delivery system similar to the one we used for the AA bone, soft-tissue prolapse would not occur and osteogenesis rather than fibrogenesis would take place.

We selected a dose of BMP based on extrapolation of the data available in the literature. Optimization of the BMP doses through a systematized dose-response protocol is needed to validate the efficacy of BMP-induced bone regeneration in the critical-sized defect.

REFERENCES