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ORIGINAL STUDY

Treatment of allograft nonunions with recombinant human bone morphogenetic proteins (rhBMP)

Christian DELLOYE, Sanjeev J. SURATWALA, Olivier CORNU, Francis Y. LEE

Investigation performed at

Cliniques Universitaires St-Luc, Brussels, Belgium and
College of Physicians and Surgeons of Columbia University, New York, NY, U.S.A.

Fractures and nonunions are the main complications associated with bone allografts. Although the osteogenic role of recombinant human bone morphogenetic proteins (rhBMPs) has been demonstrated in experimental models and human tibial nonunions, the results are unknown for allograft nonunions.

In this study, the efficacy of rhBMPs was evaluated in nonunions of femoral allografts. The results of six allograft nonunions in five patients who underwent resection of malignant bone tumours and allograft bone transplantation were analysed one to five years following application of rhBMPs at the nonunion site. There were two osteoarticular allografts and three intercalary allografts. Of three intercalary allografts, one demonstrated nonunion at both ends. Four patients received adjuvant chemotherapy and three had additional radiation therapy. There were two allograft fracture nonunions and four nonunions at the allograft-host junction. Two allograft fracture nonunions and one nonunion at the allograft-host junction were treated with 12 mg of rhBMP-2. The remaining three nonunions were treated with 7 mg of rhBMP-7 (Osigraft®). The outcome and radiological evidence of healing were evaluated at a minimal follow-up of twelve months.

There was neither healing of allograft fractures nor union of allograft-host junction.

There was elongation or enlargement of the callus from the host. One patient continued to develop resorption of the allograft, which led to allograft fracture. Two patients who were treated with rhBMP-7 and corticocancellous allografts developed sterile drainage. There was no tumour recurrence with the use of rhBMPs after a mean follow-up of 39 ± 25 months.

rhBMP's alone were not sufficient to achieve healing in allograft nonunions and fractures following wide resection including periosteum and soft tissues.

INTRODUCTION

Bone allografts have a long history as bone substitutes for large skeletal defects (*13, 15, 16*). Fractures of the allograft and nonunion of its junction to host bone are the most frequent compli-

■ Christian Delloye, MD, PhD, Professor of Orthopaedics, Chairman.

■ Olivier Cornu, MD, Orthopaedic Surgeon.

Department of Orthopaedic Surgery, Cliniques Universitaires St-Luc, Brussels, Belgium.

■ Sanjeev J. Suratwala, MD, Orthopaedic Surgeon.

■ Francis Y. Lee, MD, Chief of Tumor & Bone Disease Service, Director of Center for Orthopaedic Research.

Department of Orthopaedic Surgery, College of Physicians and Surgeons of Columbia University, New York, New York, U.S.A.

Correspondence : Christian Delloye, MD, PhD, Department of Orthopaedic Surgery, Cliniques Universitaires St-Luc, 10, avenue Hippocrate, B-1200 Brussels, Belgium.

E-mail : delloye@orto.ucl.ac.be.

cations encountered with structural bone allografts, occurring with a prevalence between 15% and 45% (*1, 7, 11, 19, 21*). Both complications are related to the non-viability of the allograft, even years after implantation (*6, 9*). In particular, fracture of a massive structural bone allograft is an unpredictable occurrence resulting from fatigue of the loaded non-revascularised allograft. Fracture through an allograft most often requires revision, with a new allograft or a prosthesis (*1, 7, 11, 19, 21*). Allograft nonunions do not heal well, even after autogenous iliac crest bone grafting and remain difficult clinical problems (*11*).

There are many potential avenues to improve the incorporation of a bone allograft (8). Among them, the use of osteogenic agents to promote healing of an allograft nonunion or fracture may be viewed as a potential alternative method to manage these allograft complications (*3, 4, 5, 17, 18, 24*).

Recombinant human bone morphogenetic proteins (rhBMP's) are osteoinductive proteins that will result in the appearance of new bone formation where they have been delivered. These proteins cause differentiation of precursor cells from osteoblastic and chondrocytic lineage (*22*). rhBMP-2 has been used successfully to both accelerate and ensure healing of open tibial fractures in human patients (*23*) while rhBMP-7 has been successfully used to manage tibial nonunions (*10*). rhBMP's have been used successfully in various animal models (*3, 4, 5, 17, 18, 24*) and there are several arguments which support their value in presence of a bone allograft. The healing of anastomotic junctions was greater in presence of BMP-2 than without BMP-2 in an intercalary femoral allograft in dogs (*17*). A similar finding was made in dogs using either a strut allograft model or an ulnar defect treated with osteogenic protein-1 (*3, 4, 18*). In an allograft osteotomy model in rats, Lee *et al* (*12*) noted healing of a bone allograft fracture only in the presence of rhBMP-2. Conversely, rhBMP-2 was not found to increase the allograft porosity and incorporation at six months postoperatively in a canine segmental femoral defect model while OP-1 soaked allografts had an increased porosity in the same animal model at three months (*5, 24*). However, the efficacy and safety of rhBMP's for the treatment of human allograft nonunions are not known. This study was conducted in order to determine whether rhBMP's are effective and safe for allograft nonunions following wide resection of malignant bone tumours and massive allograft transplantation.

MATERIALS AND METHODS

The results of treatment of allograft nonunions with rhBMP's between 1999 and 2004 were analysed in 5 patients listed in table I. There were three women and two men. The mean age of the patients was 32 years (range : 14-56). The mean follow-up is 39 ± 25 months.

Criteria for inclusion in the study were presence of a nonunion following wide resection of a malignant bone tumour and allograft replacement, absence of active or recurrent tumour and absence of active infection. Nonunion was defined as absence of healing at the interface between host and allograft bone, requiring additional surgery. There were two osteosarcomas, one Ewing sarcoma, one plasmocytoma and one malignant fibrous histiocytoma. All primary tumours occurred in the femur and were treated with wide resection including the periosteum and a cuff of normal soft tissue. Four patients received adjuvant chemotherapy and three received adjuvant radiation therapy. There were two osteoarticular allografts at the distal and proximal femur (Cases 1, 4). Three other cases concerned an intercalary allograft at the femur (Cases 2, 3 and 5). Fracture was the concern in two patients while nonunion with host bone was the main problem in three others. The clinical history is briefly summarised as follows :

– Case 1 had an osteosarcoma of the distal femur which was treated with adjuvant chemotherapy, wide resection and reconstruction using a 41 cm osteoarticular allograft. A fatigue fracture of the allograft was observed at 3 years. Dull pain at the thigh was experienced. The patient underwent implantation of 12 mg rhBMP-2 at the fracture site.
– Case 2 had a 23-cm long intercalary allograft at the femur for treating an Ewing sarcoma. The patient received adjuvant chemotherapy and 50 Gy preoperative radiation therapy. Five years after surgery, the allograft fractured and was augmented with a vascularised fibular graft placed along the medial side of the allograft. Twelve years after the initial surgery, the fracture of the allograft did not heal and progressed. The fracture was then treated with local application of bone morphogenetic protein (12 mg rhBMP-2).

Table I. — Demographics and Summary of Cases

Case	Sex/Age	Diagnosis	Adjuvant Treatment	Allograft Type Location	Nonunion Type	Treatment	Results (Union)	Follow up (months)	Remarks
1	M / 18	Osteosarcoma Femur	Chemotherapy	Osteoarticular Distal	Allograft Fracture at 3 yrs	rhBMP-2	–	63	None
2	F / 28	Ewing Sarcoma Femur	Chemotherapy Radiation	Intercalary Proximal	Allograft Fracture at 5 yrs	rhBMP-2	–	61	None
3	M / 16	Osteosarcoma Femur	Chemotherapy	Intercalary Proximal	Junction	rhBMP-2	–	44	None
4	F / 36	Plasmacytoma Femur	Radiation	Osteoarticular Proximal	Junction	rhBMP-7	–	14	Resorption of allograft Allograft fracture (+) Revision with metal prosthesis
5	F / 56	Malignant Fibrous Histiocytoma Femur	Chemotherapy Radiation	Intercalary Proximal	Junction Fracture of intramedullary rod	rhBMP-7	–	12	Sterile drainage

M : male F : female.

– Case 3 had an osteosarcoma of the distal femur, which was treated with chemotherapy and surgery at age fourteen. A 9-cm intercalary allograft was implanted and fixed with a locked intramedullary nail. A proximal nonunion developed ; it was first treated with iliac crest bone autograft and additional internal fixation with plate and screws. No healing was observed and 12 mg of rhBMP-2 was implanted.
– Case 4 had a pathologic fracture of the proximal femur secondary to plasmocytoma. The patient was treated with wide resection, reconstruction using a proximal femur osteoarticular allograft and internal fixation and postoperative radiation therapy. The patient did remarkably well six months after the initial procedure but then developed increasing pain and muscle weakness. Radiographs demonstrated nonunion, which was treated with 7 mg of rhBMP-7 and corticocancellous allografts.
– Case 5 had a malignant fibrous histiocytoma of the distal femur, which was treated with wide resection, intercalary femoral allograft, intramedullary nailing, chemotherapy and radiation therapy. The patient developed nonunion of the proximal allograft-host junction and a small incomplete fracture line in the intramedullary nail. The patient was able to ambulate with crutches and elected to wait rather than having bone grafting and revision of intramedullary nailing. He presented an acute fracture through a nonunion site ten years after the initial treatment and became bedridden. He was treated with revision of internal fixation, additional plating and topical application of 3.5 mg of rhBMP-7 mixed with corticocancellous allografts at the proximal and distal allograft host junctions.

Application of rhBMP-2 or rhBMP-7 was approved by the Institutional Review Board at either sites. All the patients were fully informed and signed an informed consent. A dose of 1.5 mg /ml (total dose of 12 mg) of rhBMP-2 in a type I collagen carrier (Genetics Institute, Cambridge, Massachusetts) was applied at the fracture or nonunion site through a limited exposure (cases 1-3). The absorbable carrier sponge that was used as a delivering matrix (7.5 $\times$ 10 cm) was laid down directly in contact with the allograft as an onlay graft and was covered by muscles. No hardware was changed. Micromotion was observed in two cases (case 1, 2) but no additional fixation was performed. A serum sample was taken 6 months after surgery for testing the presence of antibodies to rhBMP-2 as well as to bovine and

human collagen type I by enzyme-linked immunosorbent assay (ELISA). A dose of 3.5 mg of rhBMP-7 (Osigraft®, Stryker, Hopkinton, Massachusetts) in a type I collagen carrier and additional corticocancellous allografts was applied at the nonunion sites through a large exposure (Cases 4, 5). Revision of the fixation was performed in one patient (case 5). Serologic tests to detect antibodies against rhBMP-7 or carriers were not performed for this group. The patients were followed with plain radiographs every month. Two patients (Cases 4, 5) underwent ^{99m}Tc scintigraphy at 6 months in order to determine the presence of reparative bone at or around the allograft-host junction. The final outcome was graded according to Mankin's criteria (*13*). The result was classified as excellent for patients who had no evidence of disease, had normal function of the grafted part, and had returned to normal activities with minimum limitations. The result was considered good for patients who had no evidence of disease, had reduced function of the part, and needed neither a brace nor a support to return to most daily activities. A fair result was recorded if the tumour had not recurred but the patient had a substantial deficit that necessitated the use of a brace or a support. The result was considered a failure if the graft had been removed or the limb had been amputated because of recurrence of the tumour, fracture of the allograft, or infection.

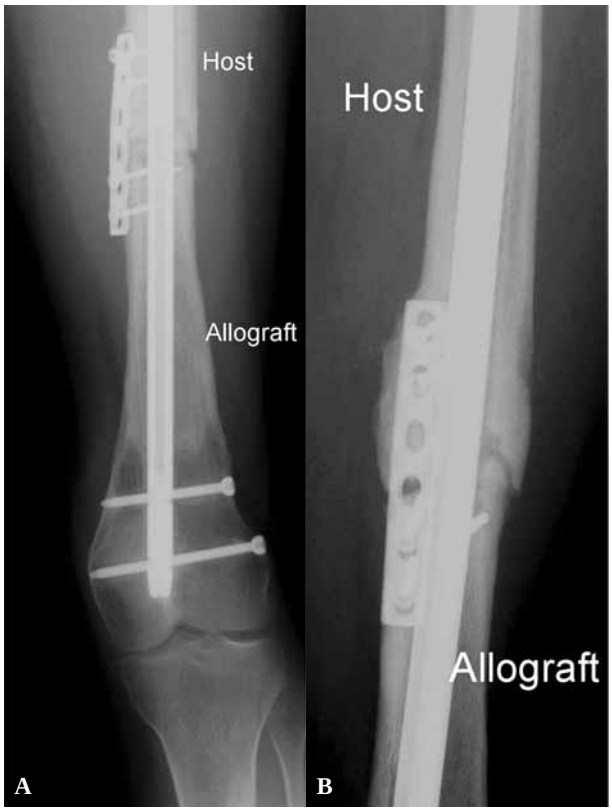

Fig. 1. — Radiographs of Case 3. **A.** Nonunion of the proximal allograft-host nonunion, one year following wide resection of osteosarcoma, intercalary allograft transplantation and adjuvant chemotherapy. The patient underwent implantation of 12 mg of rhBMP-2 at the nonunion site. **B.** One year follow-up radiograph demonstrating persistent nonunion and hypertrophy of the host bone at the allograft-host bone junction.

RESULTS

Clinical Outcomes

Prior to the application of rhBMPs, one patient (case 3) was graded as 'Good', three patients (cases 1, 2, 4) as 'Fair' due to increasing pain and the use of a cane, and the remaining one patient (case 5) as 'Poor' due to fracture of the intramedullary nail through a proximal allograft-host nonunion site. Overall, there was no improvement in clinical outcome following the application of rhBMP-2 or rhBMP-7. Patients with rhBMP-2 had an unchanged clinical and radiological outcome (fig 1). Case 1 had further surgical revision with another allograft whereas case 3 had revision with autograft. Case 4 had a worse outcome due to resorption of the allograft and subsequent allograft fracture twelve months after surgery (fig 2) ; the outcome following rhBMP-7 application was graded as 'Poor'. Case 5 did not demonstrate allograft incorporation but had a good outcome mainly due to rigid internal fixation. The mean follow-up period is thirty-nine months.

Radiographic Results

Standard radiographs were obtained in the anteroposterior and lateral projections every four weeks for the first six months and then every three months thereafter. In Cases 1, 2 and 3, no new bone formation was observed at the allograft-host interfaces or allograft fracture sites at one year postoperatively. The only noticeable change on radiographs was an enlargement of the host bone but not of the allograft in the nonunion patient (Case 3). In

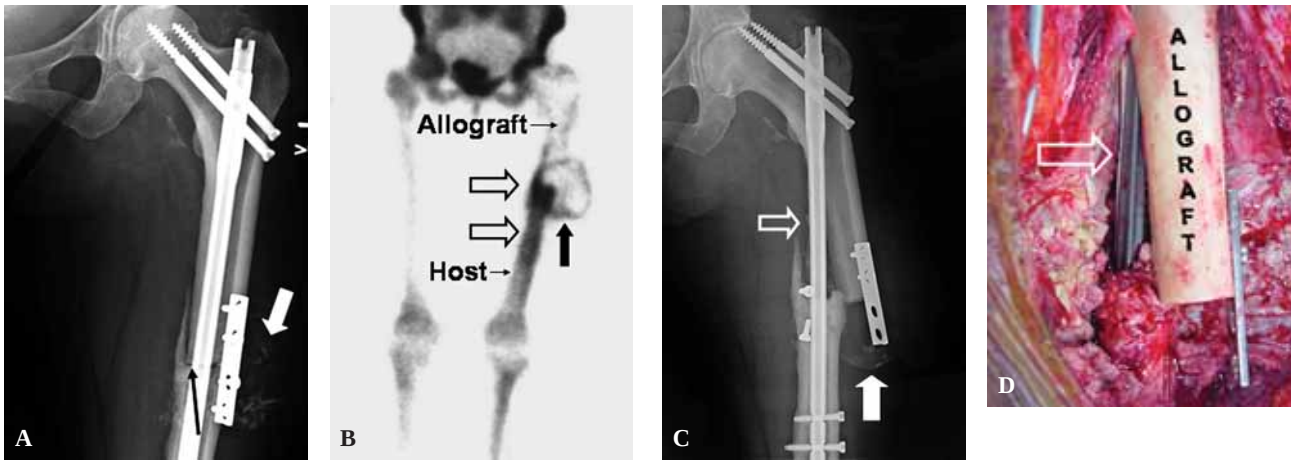

Fig. 2. — Radiographs of Case 4. Radiographs of a thirty-six-year-old female patient with allograft-host nonunion following proximal femoral allograft transplantation. **A.** Postoperative radiograph four weeks following the application of 7mg of rhBMP-7 and morselized allografts (white solid arrow) around the allograft-host junction nonunion site (black arrow). **B.** ^{99m}Tc bone scintigraphy at 6 months demonstrate increased uptake in the callus originating from the host bone (open arrow), in the host bone (open arrow) and in the soft tissue (solid arrow). **C.** Radiograph 11 months following treatment with rhBMP-7 demonstrates marked resorption on the medial aspect of the allograft (open arrow) and allograft fracture (solid arrow). **D.** Intraoperative photograph demonstrating resorption of the medial cortex of the allograft and fracture through the allograft (arrow).

Cases 4 and 5, there was neither incorporation of the allograft – rhBMP-7 composite around the allograft-host interface nor union of allograft-host junction. Case 4 developed an eccentric area of mineralisation on the lateral aspect while the medial wall of the distal portion of the allograft demonstrated marked resorption and thinning of the cortex. It is of note that the reparative bone from the proximal end of the host bone on the medial side elongated further after the application of the rhBMP-7 but healing did not take place. Bone scan did not reveal any radioisotope uptake in the allograft while it demonstrated relatively increased radioisotope uptake in the host bone and ectopic sites where allograft – rhBMP-7 composite induced heterotopic ossification in the soft tissue (fig 2).

Case 5 demonstrated gradual resorption of corticocancellous allograft bone. There was no bridging callus following rhBMP-7 application. Bone scan did not demonstrate radioisotope uptake in the cortico-cancellous allograft-rhBMP-7 composite.

Complications

There was no tumour recurrence. There were no associated skin rashes, erythema, pus drainage, fever or chills. Of three patients who had rhBMP-2 implantation and serologic tests, none developed antibodies against rhBMP-2. One patient developed a very low titer of anti-bovine collagen antibodies ; he was clinically asymptomatic. Two patients who received rhBMP-7 and corticocancellous allograft developed sterile drainage which stopped spontaneously

DISCUSSION

Revascularisation of an allograft occurs as part of the incorporation process but this process remains very limited, leaving a bulk of unremodeled bone that is prone to nonunion and fracture (*6, 8, 9*). Management of these allograft complications is still challenging. Osteoinductive proteins could offer an alternative option to bone autografting or allograft revision for treating these complications.

Bone morphogenetic proteins have been shown to induce differentiation of mesenchymal cells into osteoblasts (*22*) and as such have been considered as a potential agent to promote bone formation in bone allografts. In this limited series of five patients, BMP's delivered from a collagen carrier at the nonunion site did not promote allograft healing. No new bone formation was observed on the allograft side while host bone showed enlargement of the callus. Many variables can be considered to explain the lack of healing in response to rhBMP's. These variables include the dose, the delivery system, antibodies against BMP and collagen and prior adjuvant treatments such as chemotherapy or radiation therapy. One of possible reasons might be the paucity of living cells in the immediate vicinity of the allograft. The fibrous tissue surrounding an allograft does not appear to contain the critical amount of responding cells to become bone forming cells. Follow-up radiographs in the current study demonstrated some evidence of bone formation only by the host bone suggesting that for bone induction to occur, responding cells are more likely present in or near a living bone rather than near the allograft. The presence of the allograft might also have had a negative effect on the responding cells. This observation is in contrast to the results of the rodent allograft osteotomy healing following the application of rhBMP-2 (*12*). Several studies have demonstrated that higher doses or concentrations of rhBMPs are required to induce bone formation in the higher animals such as human and nonhuman primates (*2, 14, 20*). Recent clinical trials of rhBMP-7 for tibial nonunions demonstrated results that are comparable to the results of autogenous bone grafting (*10*). Clinical trials of rhBMP-2 for open tibial fractures showed less complications and faster wound healing (*23*). Overall, the results of human clinical trials using rhBMP's did not demonstrate the consistent bone formation that was described in lower animal studies (*5, 17, 18, 24*). The results of application of rhBMPs at the allograft nonunion site were not satisfactory in the current series. The doses that were used in the current study were comparable to those used in other published studies (*10, 23*). However, it should be noted that all our cases had wide resection including the periosteum and a cuff of surrounding soft tissue and they also received adjuvant therapy such as chemotherapy and radiation therapy. All these factors provided poor healing environments in comparison with open tibial fractures or tibial nonunions. The current study does not provide any data on the possible therapeutic role of rhBMP's in allograft-host nonunions without prior history of adjuvant therapies. Lastly, the use of rhBMP's may raise theoretical concerns regarding tumour recurrence . RhBMP's were applied one to ten years following the index procedure and adjuvant therapies. The current cases did not demonstrate any local recurrence or distant spread of original tumours. In summary, a single application of standard recommended doses of rhBMP-2 or rhBMP-7 was not effective in allograft fractures or allograft nonunions that occurred after wide resection and adjuvant therapies. Further studies would be necessary to reproduce the successful results that were demonstrated in experimental studies in allograft nonunions.

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