Effects of rhBMP-2 on cortical strut allograft healing to the femur in revision total hip arthroplasties: an experimental study

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Abstract We have studied the effects of recombinant human bone morphogenetic protein-2 (rhBMP-2) on cortical strut allograft healing and remodelling in revision hip arthroplasty. Thirty adult New Zealand rabbits underwent bilateral onlay allograft strut procedures to the femur using wires. The left femur (experimental side) received the rhBMP-2 device (1.0-mg rhBMP-2/gelatin composites) interposed between the allograft and host bone, while the right side was grafted with an allograft strut as the control. The femurs and implants were retrieved at 4, 6, and 8 weeks postoperatively. The healing of cortical strut grafts to the femur was enhanced dramatically by the addition of the rhBMP-2 device in radiographic examination, contact radiographic examination, non-decalcification sections, fluorescence tag, and computer-aided image analysis. The remodelling of cortical strut allograft was also accelerated. The new bone formation ratio and radiographic scores of the experimental side were also much higher than the control side at all times. Strut healing with the rhBMP-2 device at 4 weeks postoperatively was superior to the healing in control sides at 8 weeks. Our findings showed that the rhBMP-2 device improved and accelerated the course of cortical strut allograft healing and remodelling with host bone.

Résumé Nous avons étudié les effets de la BMP recombinante rhBMP-2 sur des allogreffes corticales dans les révisions de prothèses totales. 30 lapins adultes de Nouvelle

Zélande ont bénéficié d'une allogreffe bilatérale sur un fémur cerclé avec interposition entre l'allogreffe et l'os receveur au niveau du fémur gauche de rhBMP-2 (1.0 mg rhBMP-2 de gélamines composites), le fémur droit étant greffé avec une allogreffe simple (groupe contrôle). Les fémurs et les implants ont été prélevés à 4, 6, 8 semaines post opératoires. La consolidation par allogreffe corticale a été considérablement améliorée par l'addition de rhBMP-2, tant sur le plan radiographique que sur le plan histologique. La consolidation avec rhBMP-2 à 4 semaines post opératoires est supérieure au groupe contrôle à 8 semaines. Notre expérimentation nous permet de montrer que le rhBMP-2 améliore la consolidation et le remodelage au niveau de l'os receveur des allogreffes corticales.

Introduction

The number of revision total hip arthroplasties (THA) is increasing and accounts for up to 20% of all joint replacement procedures [6, 16]. Revision THA is often complicated by bone loss in the proximal femur, perforation, or periprosthetic fracture on the femoral side. In these cases, bone grafting is usually needed [1, 2, 10, 12, 13, 17]. Although autogenous bone graft is considered the gold standard material for bone grafting, we have to face problems such as limited supply, added surgical time, and morbidity of the donor site. Bone substitutes such as calcium phosphate and calcium sulphate ceramics are solely osteoconductive matrices and only have minimal tensile strength; their use in revision THA has not been well studied [5]. Therefore, various types of allografts are used in most cases. Cortical strut allograft has the biomechanical advantage over morselised bone allografts. It can serve as

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immediate structural support when severe bone loss presents in the proximal femoral site or periprosthetic fracture occurs. Cortical strut allografts were widely used in conjunction with hip arthroplasty and achieved good results [1, 2, 10, 12, 13, 17]. Barden et al. [1] and Kim [18] reported that all of the onlay strut grafts they used incorporated to the host femurs. However, it takes much longer to complete the union to host bone than through normal fracture healing. All grafts incorporate through sequential phases that may be active for years. In addition, delayed union and nonunion occasionally does occur. Hamadonche et al. [15] reported their histological findings in a proximal femoral structural allograft 10 years following revision THA. They found that the structural bone graft remodelled sparsely, and ingrowth of new bone into the graft rarely exceeded 5 mm. The slow healing, remodelling, and occasionally delayed union or nonunion greatly affect the patient rehabilitation and clinical results.

Since the discovery of BMP (bone morphogenetic protein) by Urist in 1965 [28], great progress has been made about its identification, characterisation and clinical applications. With the onset of molecular cloning techniques, they can be produced in large quantities and in a highly purified form as recombinant human BMPs. The translated BMPs in vitro have proved to be safe and efficacious in promoting and accelerating bone healing in animal models, clinical and preclinical research [3, 22–24]. One recent study demonstrated healing of an allograft osteotomy in animal models after augmentation with rhBMP-2 [19]. The purpose of our study was to investigate the effect of rhBMP-2 on onlay strut allograft healing and remodelling in a rabbit model.

Materials and methods

Experimental design

Thirty New Zealand rabbits aged 6–8 months with a mean weight of 2.9 (2.5–3.6) kg were used. They were screened radiographically to verify skeletal maturity in our animal research centre before the experiment. The operative procedures and animal care were approved by the regional animal research ethics committee. The rabbits from a previous unrelated study were sacrificed as onlay strut allograft bone donors. Three-centimeter-long femoral intercalary allografts were harvested from adult New Zealand rabbits. All soft tissue including the periosteum and surrounding muscles were stripped from the bone, and allografts were kept in a freezer at -80°C . Each rabbit underwent bilateral onlay allograft strut procedures to the proximal femur. An onlay graft was secured to the lateral femur with two wires. The left femur of each rabbit

received a rhBMP-2/gelatin device interposed between the allograft and host bone; no device was used on the other side. Animals were sacrificed at 4, 6, and 8 weeks postoperatively.

Grafting materials

For the bone allograft, the femur (length of approximately 3 cm) for cortical strut allografts was harvested aseptically from the donor rabbits of the same size and weight range as the study rabbits. The femur was debrided of all soft tissues (the periosteum was removed, and the medullary canal was completely curetted of marrow), then washed with quantities of sterile saline. The allograft bone was stored in sterile conditions at -80°C for at least 3 weeks before being implanted.

The rhBMP-2 device consisted of 1.0 mg of rhBMP-2 combined with 10 mg of gelatin. The rhBMP-2 device was manufactured and supplied sterile for implantation (Hua-dong Gene Technology Institute, Hangzhou, China).

Surgical procedure

The rabbits were given general anaesthesia with sodium pentothal (50 mg/kg, intraperitoneal injection). A longitudinal incision was made at the lateral aspect of the thigh, and the femur was exposed. The periosteum of the femur was incised and elevated. The strut allograft was cut longitudinally into two halves, washed with sterile saline, and shaped to conform to the exposed femur, and secured in place with two wires. On the left side, the rhBMP-2/gelatin device (the net weight of the rhBMP-2 was 1.0 mg) was interposed between the allograft and the cortical of host bone. The role of the rhBMP-2 carrier in allograft incorporation was not investigated in this study as the bone formation effect of gelatin alone has been reported to be minimal [29, 30]. On the other side, no device was implanted. The surgical wounds were closed routinely in layers. Postoperatively, all animals were permitted free exercise within their runs. Antibiotics were administered to prevent wound infection for 4 days after surgery. Tetracycline (25 mg/kg) was given by intramuscular injections of

Table 1 Radiographic scoring system

Items	Points		
	Total	Partial	No
Radiolucent line at allograft-host junction	0	2	4
Allograft bone appearance	No reaction	Partial absorption	
		0	1
	Moderate remodelling	Complete remodelling	Total organisation
	2	3	4

Fig. 1 Gross appearance of the specimen showed that the allograft-host interface was obscure, and allograft bone was surrounded by new bone on the A side (a) 4 weeks after surgery, on the B side (b), the allograft-host junction was recognised easily

bone labels 2 weeks and 3 days before the rabbits were sacrificed.

Radiography

Anteroposterior (AP) radiographs of the femurs were taken immediately postoperatively and at weekly intervals after surgery to evaluate new bone formation at the allograft-host junction and allograft bone remodeling. Radiographs were graded independently by two observers according to a modified scoring system [27] (Table 1). Then the proximal femurs with allograft bone in situ were resected from the host bone, and contact radiographs were taken vertical to the longitudinal axis of the femur.

Histological studies

After taking radiographs, the specimens were fixed in 70% ethanol solution, dehydrated in increasing concentrations of ethanol, degreased in acetone, and embedded in methylmethacrylate. The transverse sections were cut vertical to the longitudinal axis of the femur using a Leica 1600

Diamond Saw (Leica, Germany) with a thickness of 60 μm . Then some of the sections were stained with toluidine blue, and six sections of each specimen were prepared for histomorphometric analysis. The sections were examined by epifluorescence microscopy (Zeiss, Germany) to quantify bone formation in areas of allograft-host junction. The ratio of new bone to total bone volume was analysed using Leica QWin Software (Leica, Germany). Three images were taken for each section.

Statistical analysis

The Wilcoxon signed-rank test was used to determine interobserver reliability between observer grades of the radiographic appearance. The effect of the treatment and postoperative time on the radiographic scores and new bone formation ratio was determined using two-way analysis of variance (ANOVA). Kruskal-Wallis multiple comparisons were performed for each implant type at each period. We consider the results to be significant when $P < 0.05$. All statistical analyses were performed with commercially available software (SAS Institute, Cary, NC).

Fig. 2 Series of radiographs showed the allograft bone union and remodeling of the A side (a–c) and the B side (d–f) at the 4th (a, d), 6th (b, e), and 8th (c, f) weeks after surgery

Table 2 Results of radiographic scoring (mean $\pm$ SD*)

Time	4th week	6th week	8th week	All times
Side A	3.60 $\pm$ 0.70	5.00 $\pm$ 0.67	6.00 $\pm$ 0.67	4.87 $\pm$ 1.20
Side B	2.30 $\pm$ 0.95	3.00 $\pm$ 0.67	3.50 $\pm$ 0.74	2.83 $\pm$ 1.15
<i>P</i> value	<0.05	<0.05	<0.05	<0.05

*SD: standard deviation

Results

All animals made an uneventful recovery to normal walking. There was no evidence of infection, and all wounds healed normally.

Gross appearance

As early as 4 weeks postoperatively, on the left side, the junction of allograft-host became obscure; we could see new bone formation around the allograft bone, and also some ectopic bone formation (Fig. 1). At the 8th week after surgery, the new bone became mature, and in some areas, we could not distinguish the allograft bone from the host, particularly at the two extremes of allograft; they were healed to the host by new bone. On the control side, until the 8th week after surgery, we could see little new bone formation at the extremes of the allograft. At an earlier time period, we could easily distinguish the allograft bone from the host.

Radiographic evaluation

Compared with the control sides, the sides treated with the rhBMP-2/gelatin device had rapid and a greater quantity of new bone formation at the allograft-host junction, especially at two extremes. Superior bone bonding between the graft and host increased cortical thickness, and graft incorporation was evident in the left sides. The allograft bone resorption and remodelling procedure were also

accelerated compared with the control side and occurred at the 4th week after surgery in some cases. On the contact radiographs, we can see the new bone formation mostly at the allograft border, where the graft-host conformity was generally better, and where the rhBMP-2 gathered after gelatin degeneration (Fig. 2).

Statistical analysis revealed excellent reliability between two observers about the radiographic scores ($P=0.8486$). The effect of treatment (rhBMP-2) and time in situ both were statistically significant ($P<0.05$). The radiographic scores among the 4th, 6th, and 8th week groups all had significant differences between the two sides. The sides treated with rhBMP-2 had significantly higher radiographic scores than the control sides at all periods. The scores of the experimental sides at 4 weeks postoperatively are superior to those of the control sides at 8 weeks after surgery (Table 2).

Histological evaluation

On the left sides, graft-host bone bonding occurred as early as 4 weeks after surgery. At the same time, there was almost no new bone on the right sides. At the 6th and 8th weeks postoperatively, new bone formation was shown to invade the resorbed cavities of the allografts, or paralleled at the surface area of allograft bone on the left sides. The allograft bone was almost encased by new bone. The new bone and graft incorporation were less on the control sides (Fig. 3).

New bone formation was more evident when examined using fluorescent microscopy examination of the tetracycline-labelled technique (Fig. 4). There was more labelled new bone at the allograft surface and in the Haversian canal and Volkman canal on the left sides than that on the right sides. The new bone formation ratio on the rhBMP-2-treated sides was highly significant compared with the control sides at all times, as was the effect of time (Table 3). It showed more new bone formation surrounding and invading the allograft, indicating better allograft-host bone incorporation and allograft bone remodelling.

Fig. 3 Undecalcified bone sections at the 6th week after surgery showed that on the A side (**a**) there was more new bone formed and bridged the allograft bone with host bone (toluidine blue staining, original magnification $\times 20$)

Fig. 4 Fluorescent microscopy of unstained sections showed tetracycline labelled new bone around and in the allograft bone on the A side (**a**, **b**) and B side (**c**, **d**) at 4th (**a**, **c**), 8th (**b**, **d**) week after surgery. (unstained, original magnification $\times 200$)

Discussion

Revision total hip arthroplasty has not enjoyed the success of primary THA. Femoral bone loss, in case of some loss of component support, has usually been associated with femoral fracture, stem subsidence and component loosening. The incidence of femoral bone loss is increasing steadily with time, probably related to osteolysis due to polyethylene wear. The femoral support is an important factor leading to increased durability of the femoral component in revision THA. Cortical strut allografts are possible for the return of lost femoral bone stock at the time of revision. They are used widely at the time of revision

when biomechanical support was required or large femoral defects existed.

The most common indication for cortical onlay grafting has been to reinforce a structurally compromised proximal femur [1, 2, 10, 12, 13, 17, 18]. Cortical allografts have the disadvantage of slower graft incorporation and a high rate of delayed union or nonunion [7, 12]. The average time to union, however, was 8.4 months as reported by Emerson et al. [10]. Undoubtedly, these problems would greatly affect patient rehabilitation and extend the time of protected weight bearing, as well as increase the fracture ratio postoperatively.

In order to get better graft incorporation, multi-perforations of the allograft bone have been advocated as a means to increase the interface between living soft tissues of the host and the allografted bone and to achieve a higher new bone formation [8, 27]. However, perforation of the cortical allograft bone weakened the biomechanical properties and increased the risk of fracture. Freeze-dried [17], surface demineralisation [28], perforation and surface demineralisation [20, 21] of the graft were also used to augment the incorporation of bone allograft. However, these techniques all reduced the structural support proper-

Table 3 Results of the new bone formation ratio (mean $\pm$ SD)

Time	4th week	6th week	8th week	All times
Side A	9.37 $\pm$ 2.63	15.27 $\pm$ 3.00	21.13 $\pm$ 3.42	15.26 $\pm$ 5.69
Side B	4.11 $\pm$ 1.32	4.47 $\pm$ 1.35	7.15 $\pm$ 1.84	5.24 $\pm$ 2.03
P value	<0.05	<0.05	<0.05	<0.05

SD: standard deviation

ties and could not accelerate the allograft bone healing and remodelling greatly. On the other hand, they retained more BMP in the allograft. Although it cannot be routinely used because of the shortcomings of using autogenous bone, autogenous cancellous grafting at allograft-host junctions was used by some surgeons [2, 12], and it improved the allograft union rate, but did not greatly accelerate the healing procedure. The osteoinductive agents in the autogenous bone are likely to be responsible for it, which raises the hypothesis that the use of more recently developed osteoinductive substances may be similarly useful.

Recombinant human BMP-2 has been proved to induce bone formation at ectopic and orthotopic sites in small and large mammals [3, 22, 26]. Recombinant human BMP-2 was reported to accelerate normal fracture healing and healing of an allograft osteotomy in animal models [3, 19, 22, 26], and rhBMP-2 has also been shown to be a safe and efficacious replacement for autogenous bone graft in lumbar interbody fusion [14, 23]. Djapic et al. [9] found that BMP-7 has a potential to stimulate osteogenesis in a comparable fashion to autologous bone marrow when it was used with compressed homologous cancellous bone for treating long-bone critical defects in an animal study. Clinically, rhBMP-2 has also been shown, when applied to open tibia fractures, to reduce the frequency of secondary interventions, accelerate fracture and wound healing, and reduce the infection rate [11]. RhBMP-7 has been successfully used to treat resistant tibial non-union and scaphoid non-union [4, 25].

Our study shows that the rhBMP-2/gelatin device placed at the allograft-host junctions greatly improves the quality of, quantity of, and time required for new bone formation and graft healing. The healing of cortical strut allografts to the femur is accelerated significantly. Graft incorporation and remodelling were dramatically enhanced by the examination of plain radiographs, fluorescent microscopy and new bone formation ratio. The radiographic scores were significantly higher in the femurs treated with the rhBMP-2 device. Speeding the procedure of graft incorporation would really increase the thickness of the femur and restoration of the bone stock. New bone encasing the allograft bone would provide earlier stability of the graft bone. Undoubtedly, the rapid strut allograft incorporation and increased bone formation with the use of the rhBMP-2 device could provide earlier biological and mechanical stability to the femur, shorten the time of the patient's protected weight-bearing and rehabilitation, and minimise the risk of postoperative fracture.

We conclude that rhBMP-2 placed between the cortical strut allograft bone and host femur would gain substantial clinical benefits, and the rhBMP-2 device may take the place of the autogenous cancellous bone in this situation. There are, however, several limitations in the current study.

The tissue conditions in the study are better than those in the revision cases. There were no bone defects created in the host femur. Only one dosage of rhBMP-2 was used in this study. Further biomechanical testing will be conducted in large animals and primates to explore the effect of rhBMP-2 on allograft remodelling and resorption.

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