Osteogenic enhancement of diaphyseal reconstruction

Comparison of bone grafts in the rabbit

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We investigated incorporation of autoclaved autografts in segmental defects of rabbit humeri for comparison with a previous study on similar grafts supplemented with demineralized allogeneic bone matrix (DABM). We also made similar reconstructions with frozen allografts—both DABM and nonsupplemented allografts.

Before the animals were killed at 8 months, they underwent scintigraphy, showing that all 28 humeral reconstructions were metabolically active. Faxitrone radiography showed nonunion in three of nine with autoclaved autografts and in two of eight with frozen allografts, whereas all 11 DABM-supplemented frozen allografts had incorporated. Taking into account only the 23 healed reconstructions, the mean torsional strength in relation to the contralateral nonoperated on humeri was 0.81 for all three groups. Histologically, new bone enveloping, partly replacing, the implants was more abundant in DABM-supplemented reconstructions.

Our study shows that osteogenic enhancement is more important than the type of nonviable bone chosen for diaphyseal repair. However, if healing is obtained, osteogenic enhancement per se does not increase the strength.

We recently reported that the incorporation of autoclaved autografts and frozen allografts in rabbit ulnar defects was enhanced by supplementing the reconstructions with demineralized allogeneic bone matrix (DABM; Köhler et al. 1987, Köhler and Kreicbergs 1987), which is known to induce new bone formation (Urist et al. 1967, Urist and Strates 1971). No nonunion occurred among 21 DABM-supplemented reconstructions; but without DABM supplementation, the incorporation rate of both autoclaved autografts and frozen allografts was less than 0.5. In another study on 8 rabbits, we resected the middle third of the humerus and reimplanted the specimen after autoclaving (Kreicbergs and Köhler 1987). All of these reconstructions were DABM-supplemented. In the humeral series, as opposed to the ulnar series, graft incorporation was assessed also by the torsional test. All the humeral reconstructions except one with nonunion proved to be mechanically adequate at 8 months.

In the present study, we repeated the experimental design described above for humeral reconstructions testing: 1) autoclaved autografts without DABM, 2) frozen allografts without DABM, and 3) frozen allografts supplemented with DABM.

Material and methods

Forty adult New Zealand White male rabbits were operated on with unilateral resection of the middle third of the humerus including the periosteum under full anesthesia. In 13 animals the resected specimen was autoclaved at 121 °C for 20 min and subsequently reimplanted. In another 13 animals the defect was reconstructed with frozen allografts, har-

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vested from adult female rabbits raised by a different breeder. In a third group of 14 animals, frozen allografts were supplemented with DABM (approx. 200 mg) as pieces along the entire reconstruction. The grafts in all three groups were fixed with two intramedullary AO pins of different length. The shorter pin was inserted across the wider proximal osteotomy to prevent tilting of the implant.

Allogeneic bone matrix was prepared (Urist et al. 1967) from cortical diaphyseal bone. The specimens were cut into pieces, demineralized in 0.6 M HCl for 24 hours, rinsed three times in water, defatted in chloroform-methanol (1:1) for 1 hour, rinsed three times in water, and finally lyophilized to constant weight. The whole process was carried out at 4 °C. The preparations were disinfected by exposure to dry ethylene oxide for 24 hours and subsequently ventilated in dry air for 1 week to remove residual gas (McGunnigle et al. 1975).

Postoperatively, the animals were allowed full weight bearing. At 6 months, both fixation pins were removed, and 2 months later the animals were killed.

The reconstructions were studied in vivo by scintigraphy 7.5 months postoperatively in a maxicamera 400T connected to a Gamma-11 image- processing system (Digital Equipment) 2 hours after i.v. administration of 50 MBq ^{99m}[Tc]MDP. The uptake of the reconstructed humerus was assessed semiquantitatively in each animal and classified as equal, moderately or markedly increased compared with the contralateral humerus.

Post mortem, the reconstructed humeri were examined by high-resolution radiography in a Faxitrone (Hewlett Packard) at 35 kV/144 mAS using Industrex M film (Kodak). The reconstructions were defined as healed if—apart from being stable, based on manual examination—no osteotomy lines were visible radiographically.

Strength and stiffness of the reconstructed (healed) and contralateral humeri were determined in a computerized torsion testing machine. After firm fixation of both ends, each specimen was twisted inwards at a constant speed (6°/sec) until fracture. Simultaneously, the torque-twist relationship was digitally recorded. For each specimen, torsional strength (maximum torque capacity) and stiffness (linear torque twist relationship) were determined. For each animal the ratio between the reconstructed and the nonoperated on contralateral humerus was calculated with respect to strength and stiffness.

After the torsional test, tissue specimens were collected for histologic analysis in Weigardt's hematoxylin and in van Gieson-stained sections.

Table 1. Isotope uptake in a reconstructed vs. an intact humerus

	Autogeneic (n 11)	Allogeneic (n 8)	Allogeneic + DABM (n 11)
Equal	1	2	0
Moderately increased	3	2	10
Markedly increased	5	4	1

Table 2. Structural properties of the humeral reconstructions. Ratio reconstructed/intact humerus. Mean *SD*

	Autogeneic (n 6)	Allogeneic (n 6)	Allogeneic + DABM (n 11)
Strength	0.80 *0.27*	0.81 *0.18*	0.76 *0.14*
Stiffness	1.26 *0.33*	1.33 *0.17*	1.24 *0.23*

Twenty-eight animals completed the experiment. Death caused by anesthesia, infection, intestinal obstruction, etc. was evenly distributed among the three groups, leaving for evaluation 9 animals with autoclaved autografts, 8 with frozen allografts, and 11 with frozen allografts supplemented with DABM.

Results

Nonunion occurred in three of nine reconstructions with autoclaved autografts and in two of eight reconstructions with frozen allografts, whereas all 11 reconstructions with frozen allografts supplemented with DABM healed.

Isotope uptake was increased in the majority of reconstructions regardless of type (Table 1). Notably, all five pseudoarthrotic reconstructions showed a marked increase.

The assessment of relative strength and stiffness, taking into account only the 23 healed reconstructions, showed no decisive differences between the groups (Table 2).

Histologically, there was no clear difference between nonsupplemented reconstructions with autoclaved autogeneic and frozen allogeneic grafts. Thus, in both types of reconstructions, a thin appositional layer of viable bone was observed in intimate contact with the implants, which themselves appeared to be nonviable. The borderline between vi-

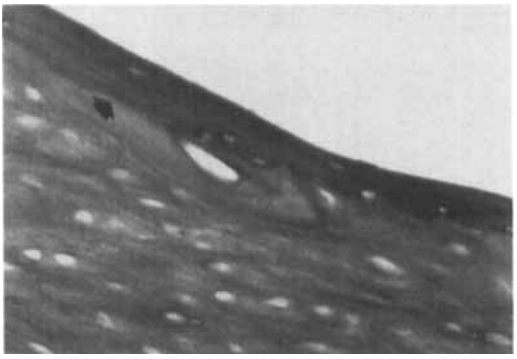

Figure 1. Nonsupplemented allogeneic graft. High magnification of periosteal region. Note the clear-cut border between the thin layer of viable and the nonviable bone. x400

Figure 2. DABM-supplemented allogeneic graft. Abundant new bone in the periosteal region (up) of the graft, more sparse endosteally. New bone around vessels in deeper parts of the nonviable graft. Note the absence of a clear-cut border between viable and nonviable bone. x120

able and nonviable bone was distinct (Figure 1). In the healed osteotomies, there was some new cancellous bone showing sprouty ingrowth into the cortical ends, which, however, still were discernible. In those osteotomies that radiographically exhibited nonunion, there was abundant fibrous tissue.

In the DABM-supplemented reconstructions with frozen allogeneic bone, abundant new bone surrounded the entire length of the grafts (Figure 2). As opposed to the nonsupplemented reconstructions, new bone enveloping the supplemented grafts appeared to grow diffusely into the nonviable bone. Thus, scattered areas of viable bone were found in the implants, seemingly invading from the outer appositional layer, but not from the inner endosteal layer. In the osteotomies, there was abundant cancellous bone that almost completely replaced the cortical ends of the grafts.

Discussion

The present study strongly supports that promotion of new bone formation is more important than type of nonviable graft chosen in the reconstruction of large diaphyseal defects. DABM supplementation, unequivocally enhanced graft incorporation. However, if healing is obtained, there is no difference between DABM-supplemented and nonsupplemented reconstructions with regard to structural properties and metabolic activity.

In our previous study of DABM-supplemented autoclaved autografts in rabbit humeri, the mean torsional strength of the reconstructions was 0.8 (Kreicbergs and Köhler 1987). These values do not differ from those recorded for the three reconstruction groups in the present study. Although the incorporation rate of DABM-supplemented grafts was clearly higher than that of nonsupplemented grafts, it appears that structural properties of diaphyseal reconstructions once healed are not related to the amount of new bone formed. In fact, healed reconstructions, irrespective of DABM supplementation and graft type, were found to have the same structural properties. Presumably, a certain amount of new bone is necessary for graft incorporation, but any excess beyond this critical amount does not contribute significantly to strength and stiffness.

Histologic analysis clearly showed that new bone enveloping the grafts was more abundant in reconstructions supplemented with DABM. Moreover, in the latter, new bone appeared to replace the implants by a highly active creeping substitution, presumably as a result of induction. As to the nonsupplemented reconstructions, the thin appositional layer of new bone and the clear-cut borderline between viable and nonviable bone seem to reflect only conduction from adjacent recipient bone.

The present study seems to confirm our previous findings on ulnar reconstructions, where we applied a similar experimental design, although the grafts were inserted without any internal fixation (Köhler et al. 1987, Köhler and Kreicbergs 1987). Summing up our previous results on ulnar and humeral recon-

structions, and the results of the present study, only one single nonunion has occurred among 40 DABM-supplemented reconstructions including 18 with frozen allografts and 22 with autoclaved autografts. The incorporation rate (0.97) is clearly superior to that (0.55) obtained by corresponding reconstructions without DABM supplementation, consisting altogether of 15 with frozen allografts and 23 with autoclaved autografts. As to nonsupplemented reconstructions, there does not seem to be any decisive difference in incorporation between frozen allografts and autoclaved autografts. Collective data on 21 ulnar and 17 humeral nonsupplemented reconstructions from previous studies and the present show show an incorporation rate of 0.53 for frozen allografts and of 0.56 for autoclaved autografts. Thus, for skeletal reconstruction, biologic differences between frozen allografts and autoclaved autografts seem negligible. The combined results clearly show that enhancement of new bone formation is more crucial than type of nonviable graft chosen for repairing large diaphyseal defects.

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