Rinsing morcellised bone grafts with bisphosphonate solution prevents their resorption

A PROSPECTIVE RANDOMISED DOUBLE-BLINDED STUDY

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During revision total hip replacement using morcellised compacted bone allograft, 16 patients were randomised to receive a graft which had been rinsed in either an ibandronate solution or in saline. Patients were assessed by dual energy x-ray absorptiometry after operation and at 3, 6, 12 and 24 months. A region of interest between the tip of the femoral stem and the distal plastic plug was chosen to measure the changes in bone density over time. The study was double-blinded. In all the control patients the bone density decreased during the first three months and then remained constant at this lower level. A large proportion of the mass of the bone graft was lost. In contrast, all patients with grafts treated with bisphosphonate showed a slight increase in bone density. The difference between the groups was highly significant at all points in time.

We conclude that rinsing the graft in a bisphosphonate solution prevents its resorption and may therefore reduce the risk of mechanical failure. The treatment is simple, inexpensive, and appears virtually free of risk.

Impaction grafting is an effective method of replacing missing bone at revision total hip replacement. The results from some surgeons approach those of primary replacement, whereas others, probably with more difficult cases, experience failures mostly from mechanical collapse of the graft. After the operation, the graft has to carry the entire weight of the patient and is soon reinforced by fibrous tissue forming within it.1 The resulting graft/scar composite appears to function for months or even years before being slowly replaced by host bone advancing from the periphery.2 It may be several years before the host bone can take over the entire load-bearing function, if ever.

If an impacted graft fails mechanically after the first few months it is likely to be an effect of resorption of the graft. Normally, a large percentage of the grafted bone is resorbed at the ‘frontier’ of host bone ingrowth,3 but, contrary to the remodelling of normal bone, there is no certain spatial coupling between resorption and formation when necrotic bone is involved.4 Therefore, resorption and mechanical failure is a constant threat to an allograft. This is clearly seen with structural allograft and is a likely cause of failure in morcellised grafts, although the problem may be less common and harder to demonstrate.

In order to improve the successful replacement of morcellised bone allografts with host bone, the addition of growth factors such as osteogeneic protein 1 (OP-1) has been tried.5 The results have varied, the growth factor has been seen to stimulate resorption more than formation in the early phase, with detrimental effects.6 Bisphosphonates can efficiently block resorption of bone, but systemic treatment will only reach the revascularised parts of the graft. Furthermore, blocking ‘pathological’ bone resorption, as in the case of a bone graft, may require higher local concentrations than other indications.7 This may be difficult to achieve with systemic treatment. Such problems would be solved if the graft could be treated before implantation, and as bisphosphonates bind almost irreversibly to the bone mineral this can be achieved. The action of the drug on the osteoclast comes when the latter starts to resorb the bone.8 We have previously shown in an animal model9 that local treatment of the graft with a bisphosphonate prevents resorption, and have now tested this principle in patients.

Patients and Methods

A total of 16 patients, seven of whom were women, underwent revision of a loose hip prosthesis using morcellised compacted bone allograft. The mean age of the patients at the time of revision was 70 years (57 to 84), with
no significant difference between men and women. None of the patients had taken bisphosphonates before surgery or during the two years for which they were followed up. One patient had bilateral revisions. All revisions were performed by one surgeon (UK) using a posterior approach and the Exeter X-change bone packing system and prosthesis (Stryker, Kalamazoo, Michigan). There was no specific selection of the patients; cases planned for revision with impaction grafting of the femoral component were taken consecutively from the waiting list. The study was approved by the Ethical Committee of Lund University, and all patients had given their informed written consent.

The hospital pharmacy prepared blinded bisphosphonate and buffer vials, each with a consecutive number, and the patients were randomised using sealed envelopes. In all cases the allograft consisted of fresh-frozen femoral heads, which were thawed in a bowl with 500 ml 0.9\% saline solution with 1 g gentamicin, and then morcellised in a Noviomagus bone mill (SMT, Nijmegen, The Netherlands) to chips of 2 mm to 5 mm in size. These were washed in the gentamicin solution for one minute, and then three times in 500 ml saline solution to eliminate as much fat as possible. The graft was then soaked in either 10 ml ibandronate (Bonefos) 60 mg/ml (Schering Nordiska, Järfalla, Sweden) or the placebo, in a bowl for three minutes. Finally, the graft was washed in 500 ml saline to remove any ibandronate not bound to bone. The bone harvested from one typical femoral head provided enough graft in all cases. If additional bone was needed for grafting the acetabulum it was prepared separately.

Dual energy x-ray absorptiometry (DEXA) measurements were undertaken with a Lunar Expert XL (Lunar Corporation, Madison, Wisconsin) scanner using Expert v 1.92 software (Lunar Corporation). A segment of the femoral diaphysis between the tip of the stem of the prosthesis and the distal plastic plug was chosen as the region of interest because in this area there was the largest measurable volume of bone graft. It was also the region most easily reproduced on the DEXA images (Fig. 1). The area was redefined for each DEXA measurement. The values of bone mineral density (BMD) in g/cm$^2$, bone mineral content in grams and the measured area in cm$^2$ were recorded. The cortical segment with the fewest osteolytic changes on either the medial or lateral sides of the region of interest was measured separately to see whether the ibandronate could have influenced the cortex surrounding the graft.

One DEXA reading was carried out at each follow-up, but the measurements from each recording, including definition of the region of interest, were repeated three times on different occasions. The mean value was used for statistical analysis.

All data from DEXA scans that were used in the analysis of the results were locked before the blinding was broken. However, only one author (PA) was unblinded by then, because the unexpected finding of a decreased area of the region of interest three months after operation in the bisphosphonate group required a fourth repeat measurement with a different definition of the region of interest, which was extended medially and laterally to include some of the adjacent soft tissue in order to be sure to include all bone. These measurements were carried out several months later by the other author (UK), who was still blinded. These data were used only as a second check and for analysis of the error of measurements.

**Statistics.** The main outcome variable, chosen in advance, was the change in BMD at the first follow-up and at subsequent post-operative examinations, with a mean of three measurements at each time point. This value was compared between the groups using Mann-Whitney’s non-parametric test. In each treatment group the number of patients with an increase as opposed to a decrease in bone density was analysed with a one-sample sign test. A $p$ value < 0.05 was considered to be significant.

**Results**

One patient refused the DEXA examinations. Another had a stroke before assessment at one year and did not continue the study after that, but the previous measurements were
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Table I. Bone mineral density (g/cm²) in the region of interest post-operatively and at follow-up. Each value is a mean of three measurements. Change refers to the difference from the post-operative measurement. Patients are listed in consecutive order.

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<th>Change</th>
<th>6 months</th>
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* one patient refused the dual energy x-ray absorptiometry examinations, so only 15 patients are shown here.

In spite of blinding, the chosen region of interest was smaller at subsequent follow-up than after operation in all bisphosphonate patients (p = 0.0002). The mean decrease was 8% (3% to 11%) at three months. This was not seen in the controls (controls p = 0.2; group difference p = 0.003). The decreasing region of interest was partly because of a decrease in the projected distance between the tip of the stem and the plug (controls 2%, bisphosphonate 4%, p = 0.02). Because the outer diameter of the femur is unlikely to have decreased considerably in three months, the difference between the groups must be because of either a decrease in the projected distance between the stem and the plug or a change in visual appearance of the region in the bisphosphonate patients. The bone mineral content measurements are highly sensitive to errors in defining the regions of interest and there was no group difference in bone mineral content change. However, when the change in this during the first three months was calculated using only the distance between the stem and the plug, assuming that the cortical periphery did not change, there was a significant loss of calcium in the controls but not in the bisphosphonate patients (p = 0.001). This difference was not because of increasing compaction. The blinded repeat measurement using a region of interest which extended medially and laterally to include some of the adjacent soft-tissue areas in order to be sure to include all bone, still showed a significant difference in BMD (p = 0.008), while the bone mineral content decreased by 1.9 g in the bisphosphonate group and 3.1 g in the controls (p = 0.13). There were no significant changes in the cortical region in either group.

Further evaluation of the recordings confirmed the differences between the two groups. The error of measurement for the BMD, assessed as the standard deviation (SD) for difference between measurements, divided by the \( \sqrt{2} \), was 1%. The error of measurement for the range of interest was 2%. There were no significant differences between the means of the original and the repeat measurements. How-
ever, women had a lower bone density, although there were no differences between the genders in the change in density over time.

Clinical follow-up showed that all the revised stems were still in situ and had no radiographic signs of loosening. One patient in the bisphosphonate group has been revised for loosening of the acetabular component three years later, probably as a result of repeated trauma because of two falls from a height of 2.5 m, and a bicycle accident.

Discussion
In the control patients the grafts were already partially resorbed after three months, whereas the grafts treated with bisphosphonate increased in density in the beginning and then remained unchanged for two years. Compacted allograft has a high density and when it is replaced by host cancellous bone this density is likely to decrease. In both the treated patients and the controls it is theoretically possible that the graft had already been penetrated by new host bone at three months, thereby explaining the decrease in density in the controls. However, allograft replacement is usually a slow process and it is more likely that the decreased density at three months reflects resorption initiated by post-operative inflammation.

BMD was chosen as the primary outcome variable as it is less sensitive to errors in the definition of area of measurement than is bone mineral content. The results showed significant changes in the area of measurement in the bisphosphonate group which we cannot explain. Possibly an increase in density made the blinded examiner put the limits at a slightly darker point in the gray scale at the border between bone and soft tissue, or at the distal plug. Blinding and a high degree of statistical significance, with a small error of measurement, imply a true effect of bisphosphonate. The reduced projection of the distance from the tip of the stem to the plug could theoretically be caused by an increased sinking of the prosthesis in the bisphosphonate group of 0.5 mm to 1 mm. However, we can think of no biological explanation for this. The observations of BMD cannot be explained by increased compaction because of sinking, because the bisphosphonate had a significant effect on change in bone mineral content when this was based on the distance from the tip of the stem to the plug. This calculation is also less dependent on errors created by variation in external rotation of the hip. The decrease in calcium mass of 3 g in the control group corresponds to about 6 cm³ of compact bone. This suggests that a large proportion of the untreated graft was resorbed in three months. A bisphosphonate-treated graft can serve as a scaffold for the formation of host bone, which can explain the increase in density during the first three months.

We have seen this in our animal model, where there was not only a preservation of the graft bone with bisphosphonate treatment, but also more host bone in the area. The bisphosphonate-treated graft had become covered with a layer of host bone which also interconnected the graft fragments. The measurements of density could have been influenced by changes occurring in the cortex surrounding the graft. However, the bisphosphonate could only influence the graft to which it was adsorbed, so the difference between the groups is because of a difference in graft behaviour. Furthermore, no changes occurred in our measurements of the cortex.

The total dose of bisphosphonate was minimal. Only the bisphosphonate which had adhered to the graft surface was implanted; the remainder was rinsed away in order to avoid any possible local toxicity from a high concentration. However, even if all the bisphosphonate used in the entire treatment bath had entered the patient, this would correspond to just one single approved dose for intravenous treatment. A possible problem with our treatment may be a longer handling time of the graft in the operating room, but rinsing of the graft is a part of the normal procedure and has been shown to improve incorporation.

The inability to resorb could cause a problem with fatigue in a structural allograft. However, the granular nature of morcelled graft precludes this, as it acts as a porous filler similar to hydroxyapatite granules. As long as there is no ingrowth of host bone, fatigue cracks are preferable to resorption. If there is such ingrowth, the new host bone will surround the fragments of the graft and adapt to mechanical demands. This new bone will be able to remodel. Should there be a need to accelerate bone ingrowth by adding a bone morphogenetic protein (BMP), the potential drawbacks of increased resorption induced by BMPs may be eliminated. The cost of the graft treatment is less than 0.5% of the cost for a second revision. This makes the treatment economically justifiable. Rinsing of morcelled bone grafts with bisphosphonates prevents resorption and is likely to reduce the risk of mechanical failure. It appears safe, simple and inexpensive.

References