Preclinical Evaluation of Zoledronate to Maintain Bone Allograft and Improve Implant Fixation in Revision Joint Replacement

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Background: Revision arthroplasty surgery is often complicated by loss of bone stock that can be managed by the use of bone allograft. The allograft provides immediate stability for the revision implant but may be resorbed, impairing subsequent implant stability. Bisphosphonates can delay allograft resorption. We hypothesized that zoledronate-impregnated allograft impacted around revision implants would improve implant fixation as characterized by mechanical push-out testing and histomorphometry.

Methods: Twenty-four axially pistoning micromotion devices were inserted bilaterally into the knees of twelve dogs according to our revision protocol. This produced a standardized revision cavity with a loose implant, fibrous tissue, and a sclerotic bone rim. Revision surgery was performed eight weeks later; after stable titanium revision components were implanted, saline solution-soaked allograft was impacted around the component on the control side and allograft soaked in 0.005 mg/mL zoledronate was impacted on the intervention side. The results were evaluated after four weeks.

Results: The zoledronate treatment resulted in a 30% increase in ultimate shear strength ($p = 0.023$), a 54% increase in apparent shear stiffness ($p = 0.002$), and a 12% increase in total energy absorption ($p = 0.444$). The quantity of allograft in the gap was three times greater in the zoledronate group compared with the control group ($p < 0.001$). The volume fraction of new bone in the zoledronate group (25%; 95% confidence interval [CI], 22% to 28%) was similar to that in the control group (23%; 95% CI, 19% to 26%) ($p = 0.311$).

Conclusions: The data obtained in this canine model suggest that pretreating allograft with zoledronate may be beneficial for early stability of grafted revision arthroplasty implants, without any adverse effect on bone formation. Clinical studies are warranted.

Clinical Relevance: The zoledronate treatment is simple to apply in the clinical setting. The treatment could increase early stability of revision joint replacements without impairing new bone formation. In the long term, this can potentially improve the longevity of revision joint replacements and reduce the number of subsequent revisions.

Because of the clinical success of total hip replacement, more patients are undergoing this procedure, and the mean age at surgery is decreasing. At the same time, patients have an increasing life expectancy and often engage in a higher physical activity level, resulting in higher demands with respect to mechanical loading and implant longevity.

Therefore, both the absolute and the relative number of revisions is expected to rise^{1,2}. Revision arthroplasty may be complicated by loss of bone stock. Bone allograft impacted around the revision implant can compensate for this, providing immediate implant stability that is crucial for long-term implant survival^{3,4}. However, the initial stability may be impaired if the

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bone allograft is resorbed. A strategy to avoid further revisions is to maintain the initial stability of the revision implant by preventing bone graft resorption.

The present study was designed to evaluate use of the bisphosphonate zoledronate for this purpose. After internalization by the osteoclasts, the nitrogen-containing bisphosphonates inhibit farnesyl pyrophosphate, an enzyme in the mevalonate pathway, resulting in reduced activity and/or viability of osteoclasts⁵.

Local treatment of the bone allograft with bisphosphonate may prevent adverse effects that can occur after systemic treatment. The treatment may reduce allograft resorption and reduce the risk of mechanical implant failure. Several studies have indicated that bisphosphonate-treated bone allograft is not as readily resorbed as untreated bone allograft⁶⁻¹³. However, despite preservation of the allograft, not all studies indicated a positive effect on mechanical implant fixation^{11,12}. In an earlier dose-response study¹³, we found that zoledronate caused a dose-dependent inhibition of the resorption of bone allograft. At higher doses, bone formation was blocked, whereas at the lowest dose allograft resorption was delayed without impairing new bone formation. That dose-response study formed the basis for our choice of the dose uses in the present study.

The overall aim of the present study was to preserve allograft impacted around stable revision implants to maintain initial stability and promote long-term implant fixation. The novel information that this study was designed to reveal was whether allograft soaked in zoledronate would enhance implant fixation in revision joint replacement. The specific objective was to investigate zoledronate-impregnated bone allograft around stable, loaded revision implants in a canine revision model by evaluating biomechanical fixation, allograft resorption, and new bone formation around the implant.

Materials and Methods

The study involved twelve female American Hounds with a mean weight of 22.5 kg (range, 17.4 to 26.7 kg) and a mean age of 46.7 months (range, 19.3 to 88.3 months). A paired study design was used, with each animal receiving two implants, one in each of the medial femoral condyles (see Appendix). The bone allograft used on the intervention side was soaked in zoledronate prior to impaction around the implant, and the allograft used on the control side was soaked in saline solution. The treatment was alternated systematically between left and right femora, with a random start. The number of animals included was based on an a priori sample size estimation. We wished to detect relative differences of $\geq 30\%$ in mechanical and histomorphometric variables between the groups, and we assumed a 30% coefficient of variance in the paired differences (based on data from previous studies), an alpha level of 0.05, and a beta level of 0.80. This yielded a sample size of approximately ten, and two additional animals were included to counteract loss of power in case animals or implants were lost for follow-up and analysis. The animals had been bred for scientific purposes, and the Institutional Animal Care and Use Committee approved the study.

We implemented our established surgical revision protocol^{14,15} to create an environment representative of implant loosening. This protocol creates a reproducible response to mechanical implant instability in the presence of polyethylene particles.

Implants and Bone Allograft

Twenty-four polymethylmethacrylate (PMMA) implants were used for the primary surgery, and twenty-four porous-coated titanium-alloy (Ti-6Al-4V)

implants were used for revision surgery. All implants were cylindrical, with a 6-mm length and 10-mm height. The porous coating had a mean volume porosity (and standard deviation) of $64\% \pm 3\%$ as specified by the manufacturer (DePuy, Warsaw, Indiana).

Bone allograft was harvested from two animals not included in the study. The harvesting was performed under sterile conditions immediately post mortem, and the bone was stored at -80°C until processing. Following complete removal of soft tissue and cartilage, the proximal aspects of the humeri, distal aspects of the femora, and proximal aspects of the tibiae were morselized with use of a standard bone mill (Biomet, Warsaw, Indiana) to create bone chips 1 to 3 mm in size. To ensure a uniform foreign-body response in the recipient animals, the allograft material from the two animals was mixed and then underwent three one-minute rinses in 0.5 L of sterile 37°C saline solution. Finally, the allograft was divided into twenty-four portions and stored in sterile containers at -80°C .

Zoledronate Impregnation of the Bone Allograft

At the time of each surgery, two portions of bone allograft were thawed for ten minutes prior to soaking. The zoledronate solution was made by diluting Zometa (Novartis, Basel, Switzerland) in sterile saline solution. One allograft portion was soaked in 5 mL of 0.005 mg/mL zoledronate solution for three minutes and then underwent three one-minute rinses in saline solution with gentle stirring to ensure complete removal of unbound zoledronate. The control portion underwent identical soaking and rinsing with saline solution only.

Surgical Procedure

All surgical procedures were performed under sterile conditions with the animals under general anesthesia. The revision protocol began with the primary surgery, in which a micromotion device was inserted into the medial condyle of each femur (see Appendix). A PMMA implant was attached to the micromotion device to mimic a loose cement mantle. Polyethylene particles applied in the implant-bone gap represented wear debris. A polyethylene plug attached to the piston articulated with the tibial plateau, thereby inducing controlled micromotion of the intra-articular PMMA implant with each gait cycle.

With the knee in full flexion, a 2.1-mm guidewire was placed in the central portion of the medial condyle perpendicular to the articulating surface. A 30-mm-deep cavity was made with use of a cannulated step drill, creating a superficial cavity with a 7.5-mm diameter and 20-mm depth and a deep cavity with a 6-mm diameter and 10-mm depth. The outermost 3 mm of the superficial cavity was tapped for placement of a subcortical centralizing ring. The anchor section of the micromotion device was placed in the deep cavity. The centralizing ring was screwed into the subchondral bone of the superficial cavity, and the PMMA implant was mounted onto the piston of the micromotion device. The internal spring of the micromotion device controlled implant movement. The implant was displaced 500 μm (tolerance, $\pm 15 \mu\text{m}$) in the axial direction during knee loading, and the internal spring pushed the implant back to its initial position when the knee was unloaded. The 0.75-mm concentric peri-implant gap was filled with 0.5×10^8 polyethylene particles (0.5 to 50 μm in diameter, with 85% [by number] being $< 12 \mu\text{m}$ in diameter) administered in 0.2 mL of hyaluronic acid (Lifecore Biomedical, Chaska, Minnesota). Finally, a polyethylene plug was mounted onto the piston superficial to the PMMA implant. The incision was closed in layers with resorbable sutures. The procedure was repeated for the other knee.

Eight weeks after the primary surgery, revision surgery was performed under identical conditions. The PMMA implant and the centralizing ring were removed, and the fibrous membrane was meticulously cleared with a curet. The superficial cavity was reamed with an 8.2-mm cannulated reamer, removing the neocortex (sclerotic shell of bone) that had formed during the eight weeks of implant pistoning. A new set of threads for a revision centralizing ring was tapped, and the cavity was irrigated with saline solution. The revision implant was screwed onto the piston. This implant incorporated a flange that prevented further micromotion and ensured stability. Saline solution-treated allograft was impacted in the reamed 1.1-mm gap surrounding the implant on the control side, and zoledronate-soaked allograft was used on the intervention side. The

Fig. 1
Representative histological sections from the two groups (toluidine blue, $\times 10$). The sections are from two implants inserted in the same animal. The bone allograft (G) appears as lightly stained lamellar structures with empty lacunae. New bone (N) appears as a dense, dark purple, disorganized substance with embedded cells. Bone marrow (MAR) appears as a less dense, cell-rich, disorganized conglomerate with empty areas representing dissolved fat. Fibrous tissue (not evident) would appear as well-organized bundles of fibers with spindle-shaped cells. The control specimen (shown on top) has a sparse amount of bone allograft (11%, similar to the group mean of 9%) and some new bone formation in the defect between the implant and the surrounding bone (22%, similar to the group mean of 23%). Compared with the control specimen, the zoledronate-treated specimen (shown on the bottom) has a greater amount of retained bone allograft (29%, similar to the group mean of 27%) and a comparable amount of new bone formation (26%, similar to the group mean of 25%).

revision centralizing ring was mounted, and the revision polyethylene plug (0.25 mm shorter than the primary plug) was mounted and was adjusted to provide secure loading during each gait cycle. The same surgeon, skilled in these procedures, performed both surgical procedures. To avoid zoledronate contamination, the procedure on the control side was always performed first.

One gram of ceftriaxone was administered before and at three days after both surgical procedures. Postoperative analgesia was provided by 2 to 3 mL of 0.5% bupivacaine administered at the incision sites and a transdermal fentanyl patch (75 $\mu\text{g/hr}$). At the end of the four-week observation period, the animals were anesthetized and killed with an overdose of hypersaturated barbiturate.

Specimen Preparation

Immediately after euthanasia, the distal aspects of the femora were removed and stored at -20°C . To provide consistency and avoid any end effects, the outermost 1 mm of the specimen closest to the subchondral plate was cut off and discarded. The remaining implant-bone specimen was divided into two sections perpendicular to the long axis of the implant with use of a water-cooled diamond band saw (EXAKT, Norderstedt, Germany). The implant section closest to the subchondral plate was cut to a thickness of 3.5 mm and stored at -20°C until mechanical testing. The remaining section was prepared for histomorphometry.

Mechanical Testing

The thawed specimens were evaluated, in a blinded fashion, by push-out to failure with use of an MTS 858 Mini Bionix test system (MTS Systems, Eden Prairie, Minnesota). All specimens were tested during a single session. Specimens were placed on a metal support jig with the side closest to the articulating surface facing upward. The implant was centered over a 7.4-mm opening under a 5-mm cylindrical test probe. A preload of 2 N was applied to define contact with the implant. Axial push-out of the implant was performed at a speed of 5 mm/min.

Load versus implant displacement was recorded for every 10 μm of displacement, and the mechanical parameters were calculated as described previously¹⁶. The specimen thickness and implant diameter were used to normalize the data and derive the ultimate shear strength (maximum force at failure), apparent shear stiffness (slope of the load-deformation curve), and total energy absorption (area under the load-deformation curve until failure).

Histomorphometry

Each specimen for histomorphometry was dehydrated in 96% ethanol, 100% 2-propanol, and finally xylene. It was then embedded in methylmethacrylate (product no. 800590; Merck, Darmstadt, Germany). The embedded specimen was rotated randomly around its vertical axis, and four serial sections (approximately 30 μm thick) were produced from the central part of the implant. All sections were cut parallel to the vertical axis of the implant with use of a microtome (KDG-95; MeProTech, Heerhugowaard, The Netherlands). Finally, the specimens were stained with 0.1% toluidine blue (pH 7) (Sigma-Aldrich, St. Louis, Missouri), rinsed, and mounted on glass. This staining method aided distinction among the different types of tissue on the basis of morphological characteristics (Fig. 1).

Quantitative histomorphometry was performed, in a blinded fashion, with use of a light microscope (Olympus, Ballerup, Denmark) and software (newCAST version 3.0.9.0; Visiopharm, Hoersholm, Denmark). In the axial direction of the implant, the region of interest spanned the entire histological section except 500 μm at the end of the implant closest to the anchor house. In the transverse direction, the region of interest spanned the mean radius of the porous coating and an additional 1000 μm into the initial surgical gap. The volume fractions of new bone, bone allograft, fibrous tissue, and marrow space in the gap were quantified with use of a point-counting technique¹⁷. The area fractions of the same tissues on the surface of the implant were quantified with use of a line-intersection technique¹⁸. Since it is not possible to measure the actual surface coverage of the implant with use of this technique, the relative surface coverage by the various tissues was assessed on the basis of the surface area fractions. The same principles apply to the volume fraction measurements. These techniques provide highly reliable results with negligible bias¹⁹.

Statistical Methods

Both the mechanical and the histological data were normally distributed, as evaluated on Q-Q plots of the residuals, and were evaluated with use of the paired Student *t* test. A *p* value of <0.05 was considered significant.

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Fig. 2
Paired plot of ultimate shear strength in MPa. The mean was 2.7 (95% CI, 2.1 to 3.2) in the control group and 3.5 (95% CI, 3.0 to 4.1) in the zoledronate group. The absolute difference between the means (zoledronate – control) was 0.87 (95% CI, 0.14 to 1.60).

Astrid Jacobsens Fond (Denmark), The Danish Rheumatism Association (Denmark), Civilingeniør Frode V. Nyegaard og hustrus Fond (Denmark), Aarhus University (Denmark), and Orthopaedic Research UK (United Kingdom). None of the private foundations played any role in the investigation. Zometa was purchased through the hospital pharmacy without interaction with the manufacturer.

Results

All animals recovered from each surgical procedure within three days and completed the observation period. There were no clinical signs of infection. The presence of a membrane consisting of fibrous tissue surrounding each implant was verified at revision. All twenty-four specimens were available for analysis.

Implants impacted with zoledronate-treated allograft had better mechanical fixation compared with those in the control group. There was a 30% increase in ultimate shear strength ($p = 0.023$) (Fig. 2), a 54% increase in apparent shear stiffness ($p = 0.002$) (Fig. 3), and a 12% increase in total energy absorption ($p = 0.444$) (see Appendix).

More zoledronate-treated bone allograft was preserved compared with control allograft. There was a twofold increase in the fraction of the surface area covered by allograft, from 0.63% (95% confidence interval [CI], 0.27% to 0.99%) to 1.5% (95% CI, 0.97% to 2.1%), with zoledronate treatment ($p = 0.007$). There was a threefold increase in the volume fraction of zoledronate-treated bone allograft ($p < 0.001$) (Fig. 4). New bone formation was not significantly affected by the zoledronate treatment. The surface area fraction of new bone was 21% (95% CI, 19% to 24%) in the zoledronate group compared with 24% (95% CI, 21% to 27%) in the control group ($p = 0.239$) (see Appendix). The volume fraction of new bone was 25% (95% CI, 22% to 28%) in the zoledronate group compared with 23% (95% CI, 19% to 26%) in the control group ($p = 0.311$) (see Appendix). Only small amounts of fibrous tissue were present in both groups (<3% in terms of both the surface area fraction and the volume fraction) except in the case of one implant from the control group that had abundant formation of fibrous tissue (surface area fraction, 41%; volume fraction, 42%).

Fig. 3
Paired plot of apparent shear stiffness in MPa/mm. The mean was 13 (95% CI, 11 to 16) in the control group and 20 (95% CI, 16 to 23) in the zoledronate group. The absolute difference between the means (zoledronate – control) was 6.47 (95% CI, 2.9 to 10).

TABLE I Correlation Between Mechanical Fixation and Histomorphometric Parameters*

	Surface Area Fraction		Volume Fraction			
	New Bone		Allograft		New Bone	
	Spearman Rho	P Value	Spearman Rho	P Value	Spearman Rho	P Value
Strength	0.16	0.463	0.44	0.031	0.1	0.642
Stiffness	0.22	0.302	0.55	0.006	0.26	0.221
Energy	0.12	0.568	0.19	0.368	0.001	0.997

*The mechanical fixation parameters are the ultimate shear strength, apparent shear stiffness, and total energy absorption. The histomorphometric parameters are the surface area fraction of new bone and the volume fractions of bone allograft and new bone.

A mean of 583 ± 103 points were evaluated in the tissue area fraction calculations and a mean of 397 ± 54 were evaluated in the tissue volume fraction calculations, allowing us to rule out the possibility that inadequate sampling density could be a reason for the lack of differences between the groups.

The histological observations were consistent with the mechanical findings. A positive correlation was observed between the volume fraction of retained allograft and two of the three mechanical parameters. No correlations were found for any other pairs of parameters (Table I).

Discussion

We hypothesized that zoledronate would result in retention of the allograft without impairment of bone formation at a site in which a loose implant was revised. Implants with zoledronate-treated bone allograft had significantly greater mechanical fixation as determined on the basis of two of three parameters. The change in the energy to failure was not significant, which is not unexpected since this parameter is expressed as the energy per unit area, and an increase in stiffness that is greater than the increase in strength can result in a similar area. There was a threefold increase in the allograft volume fraction, and the zoledronate treatment did not negatively affect new bone formation. Both strength and stiffness were positively correlated with the bone allograft volume fraction in the peri-implant gap.

Although this study indicates an effect of zoledronate on the early mechanical fixation of revision implants, the results must be interpreted with the limitations of this experimental model in mind. To standardize conditions and ensure high reproducibility, we used a canine revision model in which the implants were not functional arthroplasties but merely components with a simple cylindrical shape. The surface of the experimental revision implant was intended to represent a part of an uncemented stem in the setting of implant loosening and revision. The revision cavity was created over a short period (eight weeks) and does not reflect the spectrum of revision settings encountered by orthopaedic surgeons. However, the experimental protocol used in this study does produce an environment and tissue response representative of aseptic implant loosening^{14,15}. The conditions represent the mechanical conditions at a clinical bone-implant interface and the intra-articular loading conditions that implants can undergo.

We focused on a problem that is often encountered by surgeons performing revision arthroplasty: insufficient bone stock. Allogeneic bone graft is usually used to compensate for such an insufficiency. Although the allograft provides immediate

Fig. 4
Paired plot of bone allograft volume fraction. The mean was 9% (95% CI, 7% to 11%) in the control group and 27% (95% CI, 23% to 32%) in the zoledronate group.

support for the implant, stability is often challenged when the pace of allograft resorption is greater than that of remodeling of newly formed woven bone into structurally strong lamellar bone. This can result in a transient period of mechanical weakening, during which time a fibrous membrane could form and prevent osseointegration and long-term implant stability.

We utilized fresh-frozen allograft because that is standard in some countries²⁰. The graft from the two donors was combined and refrozen to tightly control the conditions of the bone allograft source and preparation. We cannot extrapolate the findings to allograft that is processed and sterilized prior to the morselization process.

We chose the lowest zoledronate dose used in the dose-response study by Jakobsen et al.¹³, and the allograft was rinsed thoroughly after soaking in the zoledronate solution. Our results are in agreement with the results from that dose-response study. We could not demonstrate any anabolic effect of zoledronate, as has been suggested by other authors²¹.

We chose to soak the allograft in zoledronate even though studies have also shown that a local effect on bone allograft can be achieved by systemic administration of bisphosphonate^{22,23}. Restricting the bisphosphonate exposure to a local site will limit potential side effects in individuals who are not taking bisphosphonate for a bone disease. We aimed for a local effect of the zoledronate and wished to be certain that the bisphosphonate was delivered to the bone allograft site. With systemic administration, the amount of bisphosphonate reaching the bone graft would have been expected to be a small percentage of the administered dose because the impacted graft is not initially vascularized, and the local dose would also have been difficult to control. By local administration, we ensured that the zoledronate would reside primarily on the bone allograft surface. Additionally, we assumed that the zoledronate soaking would result in protective coating of the bone allograft. Once all zoledronate is released by the osteoclasts, we expect that the normal resorption rate will resume. If that is indeed the case, such a result would be beneficial for treatment purposes, as bone remodeling would not be affected in the long term but only temporarily until osseointegration is sufficient to ensure early implant stability.

In clinical practice, zoledronate treatment of bone allograft will be a simple procedure to perform prior to impaction. This may result in retention of the allograft and maintenance of the structural support of an allografted implant until osseointegration is adequate. On the basis of previous experiments, topically administered zoledronate appears to have a therapeutic dosage window and can be detrimental to mechanical implant fixation and new bone formation if an excessive dose is used. The proapoptotic effects of bisphosphonates are not restricted to osteoclasts; any cell that is able to internalize them, including osteoblasts, may be affected. Idris et al. showed that nitrogen-containing

bisphosphonates could cause osteoblast apoptosis²⁴. Osteoclasts are most likely exposed to higher concentrations than osteoblasts and osteocytes are because bisphosphonates bind to exposed bone mineral and are released and internalized by the osteoclasts during bone resorption. This results in higher concentrations in the osteoclast cytoplasm. To protect other cells from bisphosphonate, removal of unbound bisphosphonate after soaking of the allograft (as was done in the present study) appears important.

Clinical trials that incorporate the spectrum of conditions present in revision arthroplasty are needed before implementation of this promising and practical procedure in humans.

In summary, impaction of zoledronate-treated bone allograft around revision implants in a canine model resulted in a significant increase in mechanical implant fixation that could be explained by increased preservation of the bone allograft. New bone formation was not impaired. Zoledronate treatment of bone allograft may help maintain the stability of allografted revision implants, potentially improving the longevity of revision joint replacements and reducing the risk of subsequent revisions. Clinical trials with appropriate protocols are needed before this approach is implemented as a standard regimen in humans.

Appendix

Figures outlining the surgical protocol and comparing the mechanical parameters and amount of new bone in the two groups as well as a video showing the surgical procedure are available with the online version of this article as a data supplement at jbjs.org. ■

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