Zoledronate reduces unwanted bone resorption in intercalary bone allografts

Sung W. Seo · Samuel K. Cho · Steven K. Storer · Francis Y. Lee

Received: 1 January 2009 / Revised: 2 February 2009 / Accepted: 12 February 2009 / Published online: 3 April 2009
© Springer-Verlag 2009

Abstract Bone allografts are often hampered by graft incorporation and poor host bone formation. Bisphosphonates, synthetic pyrophosphate analogs, have shown promise in inhibiting bone resorption in human and animal trials. Some *in vitro* studies have suggested that high dose bisphosphonate may also inhibit bone formation, leading to our hypothesis that an ideal dose of bisphosphonate in allografts could protect allografts from resorption. We transplanted intercalary allografts in to the segmental defect of the rat femurs after soaking each allograft in zoledronate solution (30 μ M) and then analysed bone density of the allografts six to 12 weeks after transplantation. At six and 12 weeks, the bone mineral density was higher in the experimental group compared with the control group. Qualitative radiographic and histological analysis also revealed more allograft resorption in the control group than in the zoledronate-treated group. Our data indicate that pharmacological modification of intercalary allografts with zoledronate solution can decrease osteoclast-mediated allograft resorption.

Francis Young-In Lee has received funding from the National Institutes of Health, Orthopaedic Research Society, Orthopaedic Research and Education Foundation and the Musculoskeletal Transplant Foundation.

All procedures involving animals were approved by the Institutional Animal Care and Use Committee in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care guidelines.

S. W. Seo
Department of Orthopaedic Surgery, Samsung Medical Center,
Sungkyunkwan University,
Seoul, South Korea

S. K. Cho · S. K. Storer · F. Y. Lee (✉)
Center for Orthopaedic Research,
Department of Orthopaedic Surgery, Columbia University,
630W 168th Street PH11,
New York, NY 10032, USA
e-mail: FL127@columbia.edu

Introduction

The use of bone allografts is one of the most common treatment options for reconstructing segmental skeletal defects incurred from tumour, trauma, or total joint arthroplasties [5, 10, 15, 21]. The ideal allograft incorporation involves the envelopment of the necrotic graft by the new host bone containing a remodelling unit consisting of haematopoietic cells and osteoblasts [4, 7, 11]. Allograft integration takes place through ingrowth (creeping substitution) or apposition of new host bone [7]. This requires optimal osteoclast-mediated bone resorption as well as bone formation.

The long-term success of cortical allografting is often hampered by fatigue failures, graft-host nonunions [3, 16], and in pathological conditions such as osteolytic bone tumours and wear debris-induced osteolysis, whereby host osteoclastogenesis and osteoclastic function are overly activated. Berrey et al. reported that the fracture rates of bone allografts after tumour resection range from 9.1 to 31.3% each year [3]. Furthermore, the use of immunologically unmatched allografts may trigger host immune response against the graft resulting in inflammation and bone resorption [6, 11]. Therefore, optimal regulation of osteoclast-mediated bone resorption is a crucial step in enhancing favourable allograft remodelling, incorporation, and long-term survival.

At the cellular level, bisphosphonates (BPs) target osteoclasts and interfere with specific biochemical processes ultimately inducing apoptosis. BPs are stable synthetic pyrophosphate analogs whose selective action on bone is based on the binding of the BP moiety to the bone mineral. At the tissue level, all BPs inhibit bone destruction and lead to an increase in bone mineral density (BMD) by decreasing bone resorption and turnover [14, 18]. Cancellous allograft bone pretreated with alendronate did not

undergo resorption in the distal femur in a rodent model [1]. Zoledronate, the most potent of the commercially available BPs when given systemically through subcutaneous injections, decreased the resorption of both allograft and newly formed host bone during bone graft remodelling in a rat model [2]. In another study, morselised bone allografts presoaked in an ibandronate solution during revision THA [12] increased BMD in patients at a two-year follow-up. It was concluded that rinsing the graft in a BP solution prevents its resorption and may reduce the risk of mechanical failure. However, other studies caution against soaking the allograft bone in BP. One study suggested morsellised allograft presoaked in alendronate and impacted into titanium implants substantially decreased biomechanical implant fixation in a canine model [9]. The alendronate treatment blocked formation of new bone while inhibiting resorption of the graft material. The authors acknowledged the limitations of their study resulting from alendronate dosage and omission of excess BP.

Indeed, some studies showed that osteoblast proliferation was also affected by zoledronate at the concentration of 0.5 μM or more and that osteoblast survival was significantly decreased at 50 μM of zoledronate [17, 19]. As such, knowing the optimal BP dose is critical in the context of obtaining a biological benefit by soaking allograft in BP.

We postulated that an optimal BP dose will prevent the resorption of allograft in vivo in a biologically conducive way that also prevent osteoclasts activity without inhibiting bone formation.

Materials and methods

To decide the optimal dose of zoledronate for an in vivo study, we reviewed the literature and tested different doses of zoledronate in our own in vitro studies. A previous study showed that osteoblast survival on a zoledronate-treated calcified surface up to 50 μM was not significantly affected, while this dose reduced osteoblast viability on the plastic culture plate [19]. Since osteoclast viability was reduced significantly at 10 μM , we chose a dose between 10 and 50 μM to maximise suppression of osteoclast viability without compromising osteoblast viability. We decided to soak the allograft in 30 μM of zoledronate, which theoretically would reduce osteoclasts without affecting the function of osteoblasts.

We conducted a rat allograft experiment to translate the use of zoledronate into clinical practice in the context of a pharmacological modification of allografting. We tried to simulate clinical situations of human-bone allograft. To maximise the difference between donor and receiver, we choose different strains of rats. Thirty-two femoral allografts from both legs of 16 adult Sprague-Dawley male rats

(400 g, 16 weeks; Charles River Laboratories, Wilmington, MA) were transplanted to both femurs of 16 adult Lewis male rats (400 g, 16 weeks; Charles River Laboratories). Sixteen of the right allografts were inserted without treatment, whereas 16 of the left allografts were pretreated with 30 μM zoledronate at room temperature for five minutes.

Eight rats were sacrificed at six weeks using CO_2 gas; the other eight were sacrificed at 12 weeks to evaluate bone density and histology. All procedures involving animals were approved by the Institutional Animal Care and Use Committee in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care guidelines.

To provide allograft samples, we harvested 2-cm long femoral intercalary allografts from both femurs of 16 adult Sprague-Dawley male rats after euthanasia by carbon dioxide inhalation. To simulate deep freezing, the allografts were stored in a freezer at -80°C . After induction of general anaesthesia using ketamine (60–80 mg/kg intraperitoneally) and xylazine (5–8 mg/kg intraperitoneally), we made a longitudinal incision over the anterolateral aspect of each thigh of 16 adult Lewis male rats (400 g, 16 weeks; Charles River Laboratories). A 2-cm diaphyseal segment of the femur was resected with a cuff of surrounding periosteum and muscle. The intercalary skeletal defects were replaced with the allograft femur pieces that were already harvested from the donors. Before reconstruction of the left hind limb, we thawed each allograft in 10 mL of zoledronate solution (30 μM) for five minutes at room temperature. The allograft used for the right hind limb reconstruction was simply thawed in a saline solution for five minutes. The proximal femur, allograft, and distal femur were stabilised with an intramedullary threaded 1.6-mm Kirschner wire in a retrograde manner to provide a stable construction and to allow early ambulation. We closed the wounds with absorbable suture material in muscle and skin layers. Buprenorphine (0.3–0.5 mg/kg subcutaneously) was administered in the early postoperative period for postoperative analgesia. The rats were sacrificed at six- and 12-week intervals each, and the reconstructed femurs were harvested (eight experimental zoledronate-soaked allograft constructions, eight control allograft constructions for each time period). The specimens were kept moist in 70% ethanol at 4°C until further analysis.

We qualified the effect of zoledronate-soaked allograft compared to the simple allograft by analysing radiographs and bone density.

We assessed healing of the host–allograft junction and bone density of the allograft by radiography. Radiographic images of the retrieved femur specimens were analysed to

answer the integrity and incorporation of the intercalary allografts. We counted the number of samples that had incorporated at either side of the host–allograft junction and compared them with the independent sample *t*-test (SPSS version 11.5; SPSS Inc. Chicago, IL, USA).

Bone density of each allograft was analysed by dual-energy X-ray absorptiometry (DEXA) at six and 12 weeks to compare the amount of bone resorption between zoledronate-soaked allograft versus control allograft. On harvesting the femurs with the allograft, we removed intramedullary Kirschner wires from the femurs (Fig. 1) and separated the allografts from the host bone. We subjected each allograft sample to DEXA analysis. The average BMD of the experimental group and the control group were compared by one-way analysis of variance Scheffe test, for which we used SPSS version 11.5 (SPSS Inc.).

Results

Radiographic analysis

At six weeks, both control and experimental allografts showed well-defined cortical bone. By 12 weeks, most of the control allografts showed irregular cortical surface and lower radio-opacity. On the other hand, the majority of the experimental group pretreated with zoledronate showed well-preserved allografts (Fig. 2). Radiographically, 37.5% of experimental allografts and 25% of control allografts were incorporated in the distal metaphyseal host–donor junction at 12 weeks, but there was no measurable or statistical difference ($p=0.62$). The proximal diaphyseal host–donor interfaces were not incorporated in either group.

DEXA analysis

Zoledronate-soaked allograft had less allograft resorption than control allograft. At six weeks, the average BMD was higher ($p=0.012$) in the experimental group ($0.8\pm 0.054\text{ g/cm}^2$) compared with the control group ($0.64\pm 0.031\text{ g/cm}^2$). This trend was maintained at 12 weeks; the average BMD for the experimental group ($0.7\pm 0.042\text{ g/cm}^2$) was higher ($p=0.028$) than that of the control group ($0.84\pm 0.051\text{ g/cm}^2$) (Fig. 3).

Discussion

In this study, we questioned whether an allograft soaked in the optimal dose of zoledronate would prevent the resorption of allograft in vivo. Our BMD results demonstrated that the average BMD of allograft significantly increased by soaking the allograft in $30\text{ }\mu\text{M}$ zoledronate. A limitation of our study is the lack of biomechanical testing that would determine whether the allograft–host bone construction treated with zoledronate would be structurally stronger than the non-treated allograft.

The benefits of soaking morsellised bone grafts in a BP solution have been established in both animal and human trials [1, 9]. Osteoclast-mediated bone resorption is decreased in response to BP treatment, allowing better integration of the allograft with the host bone. One potential concern is excess BP hindering complete inhibition of osteoclasts resulting in a negative impact on new host bone formation, which is essential for the successful incorporation of the allograft and the long-term survival of the host–graft construct. These problems can be mitigated by rinsing the graft and eliminating excess BP, thereby improving

Fig. 1 Intercalary allograft transplantation surgery was performed by obtaining 2-cm intercalary allografts from Lewis rats and transplanting a 2-cm segmental defect after removing 2 cm of segmental femur including periosteum. The intercalary allografts were stabilised with threaded Kirschner wires in both femora

Fig. 2 In radiographs and photomicrographs of 12-week specimens, the control group shows irregular cortical surface and osteoclastic resorption (*white arrows*), whereas the experimental group shows a well-defined cortical surface

bone and tissue ingrowth [20]. In this study, union rates of BP-soaked allograft with host bone were not significantly different than in the control group, meaning that BP did not inhibit new bone formation under our protocols. However, further evaluation will be needed to analyse local concentrations of zoledronate when it is released from BP-soaked allograft in vivo.

A unique feature of our rodent model is the use of both hind limbs, which effectively decreases by half the number of animals needed for statistically significant experiments and allows the allograft-host construction to be conducted in the same immunological environment. Radiographic analysis indicated that zoledronate pretreatment before graft installation can decrease the amount of bone resorption by 12 weeks in an animal model (Fig. 2). In addition, healthy callus formation was enhanced in the experimental group, indicating the BP treatment not only preserved the graft bone from resorption, but also allowed for increased new bone formation in comparison to the control group. Our finding was consistent with previous studies [1].

DEXA analysis was undertaken to quantify the loss or preservation of bone in our animal model. There was a statistically significant difference in BMD between the zoledronate and saline groups at both time points (Fig. 3). This finding supported the conclusions drawn from qualitative radiographic analysis. One limitation of this part of the study was that there was no way of deciphering whether the difference was primarily the result of BP-mediated prevention of bone resorption or enhancement of host-bone formation. A BMD measurement of the allografts could be determined before installing them into Lewis rats and comparing preoperative values versus postoperative values. This experiment would determine if BMD actually de-

creased or increased during the initial six-week period and whether the observed increase in BMD from week six to week 12 (Fig. 3) brought BMD values back to preoperative levels. Overall, DEXA was a useful quantitative tool that showed the beneficial effects of BP treatment in our rodent model.

Taking the current experimental design one step further, there may be other ways of encouraging successful allograft incorporation. For instance, the addition of bone morphogenetic proteins can promote a more extensive callus formation [13]. The use of viral vector-mediated gene therapy to enhance allograft remodelling has been reported [8]. Clinically, the cost of using allografts is minimal compared with the total cost for a revision surgery. To that end, it makes economic sense to optimise allograft incorporation by using BPs. Our data suggest that presoaking cortical allografts in a BP solution may reduce unwanted bone resorption and subsequent mechanical failure.

Fig. 3 Transplanted allografts that were pretreated with zoledronate show a higher bone density than untreated allografts in the measurement of bone density in the transplanted allografts

Acknowledgments The authors thank the Musculoskeletal Transplant Foundation and the Orthopaedic Research and Education Foundation for the initial funding of this study.

References

- Aspenberg P, Astrand J (2002) Bone allografts pretreated with a bisphosphonate are not resorbed. *Acta Orthop Scand* 73:20–23
- Astrand J, Harding AK, Aspenberg P, Tagil M (2006) Systemic zoledronate treatment both prevents resorption of allograft bone and increases the retention of new formed bone during revascularization and remodelling. A bone chamber study in rats. *BMC Musculoskelet Disord* 7:63
- Berrey BH Jr, Lord CF, Gebhardt MC, Mankin HJ (1990) Fractures of allografts. Frequency, treatment, and end-results. *J Bone Joint Surg Am* 72:825–833
- Burchardt H (1983) The biology of bone graft repair. *Clin Orthop Relat Res* 174:28–42
- Clohisey DR, Mankin HJ (1994) Osteoarticular allografts for reconstruction after resection of a musculoskeletal tumor in the proximal end of the tibia. *J Bone Joint Surg Am* 76:549–554
- Delloye C (2003) How to improve the incorporation of massive allografts? *Chir Organi Mov* 88:335–343
- Enneking WF, Mindell ER (1991) Observations on massive retrieved human allografts. *J Bone Joint Surg Am* 73:1123–1142
- Ito H, Koefoed M, Tiyyapatanaputi P, Gromov K, Goater JJ, Carmouche J, Zhang X, Rubery PT, Rabinowitz J, Samulski RJ, Nakamura T, Soballe K, O’Keefe RJ, Boyce BF, Schwarz EM (2005) Remodeling of cortical bone allografts mediated by adherent rAAV-RANKL and VEGF gene therapy. *Nat Med* 11:291–297
- Jakobsen T, Baas J, Bechtold JE, Elmengaard B, Soballe K (2007) Soaking morsellized allograft in bisphosphonate can impair implant fixation. *Clin Orthop Relat Res* 463:195–201
- Jofe MH, Gebhardt MC, Tomford WW, Mankin HJ (1988) Reconstruction for defects of the proximal part of the femur using allograft arthroplasty. *J Bone Joint Surg Am* 70:507–516
- Kandel RA, Pritzker KP, Langer F, Gross AE (1984) The pathologic features of massive osseous grafts. *Hum Pathol* 15:141–146
- Kesteris U, Aspenberg P (2006) Rinsing morsellised bone grafts with bisphosphonate solution prevents their resorption. A prospective randomised double-blinded study. *J Bone Joint Surg Br* 88:993–996
- Lee FY, Storer S, Hazan EJ, Gebhardt MC, Mankin HJ (2002) Repair of bone allograft fracture using bone morphogenetic protein-2. *Clin Orthop Relat Res* 397:119–126
- Madsen JE, Berg-Larsen T, Kirkeby OJ, Falch JA, Nordsletten L (1998) No adverse effects of clodronate on fracture healing in rats. *Acta Orthop Scand* 69:532–536
- Mankin HJ, Doppelt SH, Sullivan TR, Tomford WW (1982) Osteoarticular and intercalary allograft transplantation in the management of malignant tumors of bone. *Cancer* 50:613–630
- Matejovsky Z Jr, Matejovsky Z, Kofranek I (2006) Massive allografts in tumour surgery. *Int Orthop* 30:478–483
- Pan B, To LB, Farrugia AN, Findlay DM, Green J, Gronthos S, Evdokiou A, Lynch K, Atkins GJ, Zannettino AC (2004) The nitrogen-containing bisphosphonate, zoledronic acid, increases mineralisation of human bone-derived cells in vitro. *Bone* 34:112–123
- Reszka AA, Rodan GA (2003) Bisphosphonate mechanism of action. *Curr Rheumatol Rep* 5:65–74
- Schindeler A, Little DG (2005) Osteoclasts but not osteoblasts are affected by a calcified surface treated with zoledronic acid in vitro. *Biochem Biophys Res Commun* 338:710–716
- van der Donk S, Weernink T, Buma P, Aspenberg P, Slooff TJ, Schreurs BW (2003) Rinsing morselized allografts improves bone and tissue ingrowth. *Clin Orthop Relat Res* 408:302–310
- Vastel L, Lemoine CT, Kerboul M, Courpied JP (2007) Structural allograft and cemented long-stem prosthesis for complex revision hip arthroplasty: use of a trochanteric claw plate improves final hip function. *Int Orthop* 31:851–857