■ CASE REPORT

Histology of vancomycin-supplemented impacted bone allografts in revision total hip arthroplasty

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Vancomycin-supplemented allografts provide biological restoration of bone stock and sound fixation with a low incidence of re-infection. Experimental incorporation of these grafts is similar to allografts without vancomycin. However, the underlying biology remains unknown.

We report the first histological observations of vancomycin-supplemented impacted bone allografts in two reconstructions performed 14 and 20 months after revision surgery because of a periprosthetic fracture.

Areas of active bone remodelling (creeping substitution), as well as calcified bone trabeculae and graft particles embedded in dense fibrous tissue, were observed with osteoid and fibroconnective tissue surrounding polymethylmethacrylate particles.

These pathological findings are similar to those reported in allografts without vancomycin and support the hypothesis that high levels of vancomycin do not affect the incorporation of bone graft.

Cancellous bone can act as a delivery vehicle for vancomycin *in vitro* and *in vivo*, reaching high peak concentrations in the surrounding medium initially, followed by steady levels for longer periods.¹⁻³

The addition of vancomycin to morcellised bone allografts does not impair incorporation of graft in pigs.⁴ High local levels of this antibiotic without nephrotoxicity can be obtained in revision hip surgery.⁵ Vancomycin-supplemented allografts also provide biological restoration of bone stock and sound fixation with a low incidence of re-infection.⁶ Although the clinical and radiographic results have been very promising, the underlying biology remains unknown.

We report the first histological observations in one femoral and one acetabular reconstruction with vancomycin-supplemented impacted bone allografts.

Case 1. A 75-year-old man was treated with a two-stage protocol after an infected total hip arthroplasty. At the second stage, acetabular and femoral reconstruction with vancomycin-supplemented impacted bone allografts and a plain cemented Exeter (Stryker-Howmedica-Osteonics, Rutherford, New Jersey) total hip arthroplasty were performed.

Three femoral heads from our bone bank were used for the femur. They had not been irradiated and were manually fragmented into 0.4

to 0.6 cm particles and mixed for 15 minutes with 1000 mg of powdered vancomycin (Lilly, Indianapolis, Indiana) for each femoral head. Impaction grafting was performed as described by Gie et al.⁷ The graft was packed with special instruments (X-Change Revision Instruments System, Stryker-Howmedica-Osteonics).

On post-operative radiographs, a cement leakage in Gruen zone 3⁸ was observed. The patient fell 20 months after revision and sustained an undisplaced periprosthetic fracture at the tip of the stem (Fig. 1). The stem, cement, and allograft were clinically and radiographically stable. The fracture was treated with a locking compression plate (LCP, Synthes GmbH, Umkirch, Switzerland) and vancomycin-supplemented cancellous allografts at the site of the fracture. Before surgery, the patient's consent was obtained to take biopsy specimens from the reconstructed femur.

Six intact biopsy specimens were taken with a Yanshini needle (diameter 4 mm; Jamshidi, Deerfield, Illinois) from the proximal and distal parts of the femoral reconstruction by the same surgeon (MAB) who performed the two-staged procedure.

Case 2. A one-stage aseptic acetabular and femoral reconstruction with vancomycin-supplemented impacted bone allografts and a plain cemented C Stem (De Puy, Warsaw, Indiana) total hip arthroplasty was performed in a

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Fig. 1

Radiograph showing a periprosthetic fracture at the tip of the stem in case 1, 20 months following femoral reconstruction with vancomycin-supplemented impacted bone allografts.

Fig. 2

Radiograph showing a displaced periprosthetic fracture 14 months after acetabular reconstruction with vancomycin-supplemented impacted bone allografts in case 2.

67-year-old man in 2003. Two femoral heads were used to reconstruct the acetabulum, manually fragmented into 0.4 to 0.6 cm particles and mixed for 15 minutes with 1000 mg of powdered vancomycin (Lilly, Indianapolis, Indiana). Impaction grafting was performed as described by Slooff, Schimmel and Buma.⁹

The patient fell and sustained a Vancouver B1 displaced periprosthetic fracture 14 months later (Fig. 2).¹⁰ During a second revision and with the patient's approval, six biopsy specimens were taken with a 4-mm Yanshini needle from the acetabular reconstruction after stabilisation of the periprosthetic fracture by the same surgeon (FP) who had performed the previous revision surgery.

Pathological findings

The specimens belonging to both cases were fixed in 10% formalin and were transferred to xylene and graded ethanols and embedded in methylmethacrylate without decalcification. Bone polymethylmethacrylate was dissolved out, and sections were cut at 5 μ m and stained using the Goldner trichromic method and toluidin blue.

Low-magnification microscopy showed three areas on both biopsy specimens (Figs 3 and 4). Histologically normal laminar bone and periosteum were observed in the external area. Viable trabecular bone with polymethylmethacrylate was observed in the middle area of the specimen, as well as occasional macrophages, presumably in response to cement particles. Small islands of necrotic bone were observed in this zone. A variable amount of fibrotic tissue surrounding this bone was observed, as well as lymphocytes and plasma cells. The internal area showed fragments of necrotic and viable bone, interpreted as incorporated bone graft. The small fragments of necrotic trabecular bone were gradually replaced by viable new bone, described as creeping substitution, suggested that the necrotic bone was allograft. Acute inflammation or wear debris was not evident. There were also spaces containing polymethylmethacrylate.

Discussion

The pathological observations in these biopsy specimens support the hypothesis that high levels of vancomycin do not affect incorporation of bone graft in a clinical setting.

A decrease in osteoblast formation and cell death has been observed with high concentrations of this antibiotic.^{11,12} However, fragments of necrotic and viable bone were interpreted as incorporated bone graft in these two cases. Small fragments of necrotic trabecular bone were gradually replaced by viable new bone (creeping substitution), and are similar to those previously reported in allografts without vancomycin where blood vessels and fibrous tissue from the host invade the impacted graft in the first weeks after surgery.¹³⁻²³

Impacted cancellous bone allografts have demonstrated excellent clinical and radiological mid-term results in aseptic revision hip arthroplasties.²⁴⁻²⁶ The capacity of cancellous bone allograft to act as a vehicle for vancomycin makes it possible for local concentrations of this antibiotic to be 20 to 300 times higher than the 90% minimum inhibitory concentration for *Staphylococcus aureus*.

Our initial results with a two-stage treatment for a septic hip replacement with acetabular and femoral impaction grafting techniques and vancomycin-loaded allografts have

Fig. 3a

Fig. 3b

Histological slides of case 1 a) external area (LB, laminar bone; NB, necrotic bone) and b) internal area (O, osteoid; C, cement; LB, laminar bone; M, macrophage).

Fig. 4a

Fig. 4b

Fig. 4c

Histological slides of case 2 a) external area (P, periosteum; O, osteoid; LB, laminar bone); b) middle area (NB, necrotic bone; O, osteoid; F, fibrous tissue; C, cement; LB, laminar bone; M, macrophage) and c) internal area (NB, necrotic bone; LB, laminar bone).

been very promising. Thirty hips were treated by excision of implants, parenteral antibiotic therapy and second-stage reconstruction with vancomycin-supplemented impacted bone allografts and a plain cemented Charnley prosthesis. Control of infection was obtained in 29 cases at a mean follow-up of 32 months without evidence of progressive radiolucent lines, demarcation or resorption of graft.⁶

Vancomycin-supplemented bone allografts provide high local concentrations of antibiotic. The levels are higher than those reported with vancomycin-impregnated cement.²⁷⁻²⁹ A synergistic effect of the biological activity of vancomycin when combined with aminoglycosides in the cement has been observed.⁵

Although the presence of incorporated bone explains the underlying biology of vancomycin-supplemented allografts, new biopsies and autopsy retrievals of reconstructed hips are needed to confirm these findings.

The histological evidence in these cases encourages the continued use of vancomycin-supplemented bone allografts for second-stage acetabular and femoral reconstruction of an infected total hip arthroplasty.

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References

1. Witso E, Persen L, Loseth K, Bergh K. Adsorption and release of antibiotics from morselized cancellous bone: in vitro studies of 8 antibiotics. *Acta Orthop Scand* 1999; 70:298-304.
2. Witso E, Persen L, Loseth K, Bergh K. Cancellous bone as an antibiotic carrier. *Acta Orthop Scand* 2000;71:80-4.
3. Winkler H, Janata O, Berger C, Wein W, Geotgopoulos A. In vitro release of vancomycin and tobramycin from impregnated human and bovine bone grafts. *J Antimicrob Chemother* 2000;46:423-8.
4. Buttaro M, Gonzalez Della Valle A, Piñeiro A, et al. Vancomycin-supplemented bone allografts incorporation. *Acta Orthop Scand* 2003;74:505-13.
5. Buttaro M, Pusso R, Piccaluga F. Vancomycin-supplemented impacted bone allografts in infected hip arthroplasty. *J Bone Joint Surg [Br]* 2005;87-B:314-19.
6. Buttaro M, Gimenez MI, Greco G, Barcan L, Piccaluga F. High active local levels of vancomycin without nephrotoxicity released from impacted bone allografts in revision hip arthroplasty. *Acta Orthop Scandinavica* 2005;76:336-40.
7. Gie GA, Linder L, Ling RSM, et al. Impacted cancellous allografts and cement for revision total hip arthroplasty. *J Bone Joint Surg [Br]* 1993;75-B:14-21.
8. Gruen TA, McNiece GM, Amstutz HC. "Modes of failure" of cemented stem-type femoral components: a radiographic analysis of loosening. *Clin Orthop* 1979;141:17-27.
9. Slooff TJ, Schimmel JW, Buma P. Cemented fixation with bone grafts. *Orthop Clin North Am* 1993;24:667-77.
10. Brady OH, Garbuz DS, Masri BA, Duncan CP. Classification of the hip. *Orthop Clin North Am* 1999;30:215-20.

11. **Edin ML, Miclau TM, Lester GE, Lindsey RW, Dahners LE.** Effect of cefazolin and vancomycin on osteoblasts in vivo. *Clin Orthop* 1996;333:245-51.
12. **Miclau T, Dahners LE, Lindsey RW.** In vitro pharmacokinetics of antibiotic release from locally implantable materials. *J Orthop Res* 1993;11:627-32.
13. **Ling RS, Timperley AJ, Linder L.** Histology of cancellous impaction grafting in the femur: a case report. *J Bone Joint Surg [Br]* 1993;75-B:693-6.
14. **Schreurs BW, Buma P, Huiskes R, Slagter JL, Sloof TJ.** Morsellized allografts for fixation of the hip prosthesis femoral component: a mechanical and histological study in the goat. *Acta Orthop Scand* 1994;65:267-75.
15. **Heekin RD, Engh CA, Vinh T.** Morsellized allograft in acetabular reconstruction: a postmortem retrieval analysis. *Clin Orthop* 1995;319:184-90.
16. **Nelissen RG, Bauer TW, Weidenhielm LR, LeGovan DP, Mikhail WE.** Revision hip arthroplasty with the use of cement and impaction grafting: histological analysis of four cases. *J Bone Joint Surg [Am]* 1995;77-A:412-22.
17. **Buma P, Lamerigts N, Schreurs BW, et al.** Impacted graft incorporation after cemented acetabular revision: histological evaluation in 8 patients. *Acta Orthop Scand* 1996;67:536-40.
18. **Schimmel JW, Buma P, Versleyen D, Huiskes R, Slooff TJ.** Acetabular reconstruction with impacted morsellized cancellous allografts in cemented hip arthroplasty: a histological and biomechanical study on the goat. *J Arthroplasty* 1998;13:438-48.
19. **Musculo DL, Ayerza MA.** Allografts. In: Rosenberg AG, ed. *The adult hip*. Philadelphia: Lippincott-Raven, 1998:297-312.
20. **Ullmark G, Linder L.** Histology of the femur after cancellous impaction grafting using a Charnley prosthesis. *Arch Orthop Trauma Surg* 1998;117:170-2.
21. **Linder L.** Cancellous impaction grafting in the human femur: histological and radiographic observations in 6 autopsy femurs and 8 biopsies. *Acta Orthop Scand* 2000;71:543-52.
22. **Weidenhielm LR, Mikhail WE, Wretenberg P, et al.** Analysis of the retrieved hip after revision with impaction grafting. *Acta Orthop Scand* 2001;72:609-14.
23. **van der Donk S, Buma P, Slooff TJ, Gardeniers JW, Schreurs BW.** Incorporation of morsellized bone grafts: a study of 24 acetabular biopsy specimens. *Clin Orthop* 2002;396:131-41.
24. **Piccaluga F, González Della Valle A, Encinas JC, Pusso R.** Femoral stem revision with impaction grafting and a Charnley stem: a two- to twelve-year follow-up. *J Bone Joint Surg [Br]* 2002;84-B:544-9.
25. **Halliday BR, English HW, Timperley AJ, Gie GA, Ling RS.** Femoral impaction grafting with cement in revision total hip replacement: evolution of the technique and results. *J Bone Joint Surg [Br]* 2003;85-B:809-17.
26. **Schreurs BW, Bolder SB, Gardeniers JW, et al.** Acetabular revision with impacted morsellised cancellous bone grafting and a cemented cup: a 15- to 20-year follow-up. *J Bone Joint Surg [Br]* 2004;86-B:492-7.
27. **Brien WW, Salvati EA, Klein R, Brause B, Stern S.** Antibiotic impregnated bone cement in total hip arthroplasty: an in vivo comparison of the elution properties of tobramycin and vancomycin. *Clin Orthop* 1993;296:242-8.
28. **Klekamp J, Dawson JM, Haas DW, DeBoer D, Christie M.** The use of vancomycin and tobramycin in acrylic bone cement: biomechanical effects and elution kinetics for use in joint arthroplasty. *J Arthroplasty* 1999;14:339-46.
29. **Gonzalez Della Valle A, Bostrom M, Brause B, Harney C, Salvati E.** Effective bactericidal activity of tobramycin and vancomycin eluted from acrylic bone cement. *Acta Orthop Scand* 2001;72:237-40.