■ RESEARCH

The concentration of antibiotic in fresh-frozen bone graft

Y. Chang,
H.-N. Shih,
D. W. Chen,
M. S. Lee,
S. W. N. Ueng,
P.-H. Hsieh

From Chang Gung
University, Taoyuan,
Taiwan

We investigated the antibiotic concentration in fresh-frozen femoral head allografts harvested from two groups of living donors. Ten samples were collected from patients with osteoarthritis of the hip and ten from those with a fracture of the neck of the femur scheduled for primary arthroplasty. Cefazolin (1 g) was administered as a pre-operative prophylactic antibiotic. After storage at -80°C for two weeks the pattern of release of cefazolin from morsellised femoral heads was evaluated by an *in vitro* broth elution assay using high-performance liquid chromatography. The bioactivity of the bone was further determined with an agar disc diffusion and standardised tube dilution bioassay. The results indicated that the fresh-frozen femoral heads contained cefazolin. The morsellised bone released cefazolin for up to four days. The concentration of cefazolin was significantly higher in the heads from patients with osteoarthritis of the hip than in those with a fracture. Also, in bioassays the bone showed inhibitory effects against bacteria.

We concluded that allografts of morsellised bone from the femoral head harvested from patients undergoing arthroplasty of the hip contained cefazolin, which had been administered pre-operatively and they exhibited inhibitory effects against bacteria *in vitro*.

Deep-freezing and irradiation are the two most popular methods of processing allograft bone.¹ Proponents of fresh-frozen grafts cite their improved biological and biomechanical characteristics compared to irradiated material.¹ Femoral heads obtained from cadavers or living donors following arthroplasty of the hip are widely used as fresh-frozen bone allografts.² The use of allograft bone impregnated with antibiotics reduces the incidence of infection.³ Prophylactic administration of antibiotics is recommended for joint arthroplasty, and therapeutic levels can be achieved in most viable tissue, including bone.⁴ Therefore, femoral heads harvested from patients at joint arthroplasty may contain antibiotics. The cephalosporins are active against most strains of *Staphylococcus aureus* and *Staph. epidermis*, which are the most common organisms causing infection following orthopaedic surgery.⁵ Cefazolin, a first-generation cephalosporin, is commonly used for prophylaxis during joint replacement because of its broad spectrum, favourable pharmacokinetics, good safety profile and better bone penetration.⁴ In this study we measured the antibiotic concentration, the characteristics of antibiotic release and the ability of fresh-frozen femoral heads harvested during arthroplasty of the hip to eradicate bacteria.

Materials and Methods

The study was approved by the institutional review board, and written informed consent was provided by all participants. Between September and December 2009, patients with advanced arthritis of the hip or Garden grade III or IV fracture of the neck of the femur⁶ who were scheduled for primary total hip replacement (THR) or primary hemiarthroplasty were asked to participate in this study. Patients with significant comorbidity such as renal impairment, liver dysfunction, malignancy, or known allergy to cephalosporin were excluded.

As a prophylactic antibiotic 1 g of cefazolin (Chi Sheng, Taipei, Taiwan) was given intravenously 30 minutes before the operation. The femoral heads were excised and irrigated with normal saline to remove the surface blood clot in an aseptic manner. After removal of the cartilage, the heads were morsellised to fragments of 2 mm to 3 mm in diameter using a Bone Mill (Medicon, Tuttlingen, Germany). The bone fragments were stored at -80°C for two weeks before conducting the cefazolin broth elution assay or antibiotic bioassay.

Antibiotic broth elution assay. After thawing, 2 g of bone fragments were placed in polypropylene tubes with 5 ml phosphate-buffered saline (PBS, pH 7.3) and were shaken in a

■ Y. Chang, MD, PhD,
Orthopaedic Surgeon
■ H.-N. Shih, MD, Orthopaedic
Surgeon
■ D. W. Chen, MD, Orthopaedic
Surgeon
■ M. S. Lee, MD, PhD,
Orthopaedic Surgeon
■ S. W. N. Ueng, MD,
Orthopaedic Surgeon
■ P.-H. Hsieh, MD, Orthopaedic
Surgeon
Department of Orthopaedics
Chang Gung Memorial
Hospital, Taiwan, 5 Fu-Hsin
Street, Kweishan, Taoyuan,
Taiwan.

Correspondence should be sent
to Dr P.-H. Hsieh; e-mail:
hsiehph@adm.cgmh.org.tw

©2010 British Editorial Society
of Bone and Joint Surgery
doi:10.1302/0301-620X.92B10.
24704 \$2.00

J Bone Joint Surg [Br]
2010;92-B:1471-4.

Received 18 February 2010;
Accepted after revision 1 June
2010

rotator at 37°C. The study was continued for ten days, with daily transfer of the bone into a test tube with PBS after washing in saline. The cumulative and daily releases of cefazolin were measured, and 2 ml elution samples of PBS were collected at eight intervals on days 1 to 7 and 10 and stored at -80°C until analysis.

The concentrations of antibiotic were determined using high-performance liquid chromatography (HPLC) using a model ALC 717 chromatograph (Waters Associates, Milford, Massachusetts) with a stainless-steel column (300 mm × 3.9 mm, 10 µm particle size). The mobile phase consisted of 50% methanol in 50% d₂H₂O. The HPLC system had a sensitivity of 0.1 µg/ml for cefazolin. The concentrations of cefazolin in the bone samples were obtained from the HPLC analysis by comparison with daily prepared standard curves, relating the peak area to concentrations of cefazolin.

Antibiotic bioassay. The bioactivity of fresh-frozen allograft was determined using an agar disc diffusion bioassay with *Staph. aureus* (ATCC 25923, minimally inhibitory concentration to cefazolin: 1 µg/ml).⁷ The technique was based on the inhibitory activity of discs containing a standardised concentration of cefazolin (PDM Diagnostic-Discs, AB Biodisk, Solna, Sweden). The standard paper discs and the test allograft were placed on the *Staph. aureus*-seeded agar and incubated overnight at 37°C.

In order to determine the bioactivity of cefazolin in the broth elution fluid, aliquots from the same samples were analysed by a standardised bioassay (National Committee for Clinical Laboratory Standards, USA, 1993). The tube dilution bioassay was used with serial twofold dilutions. The same strain of *Staph. aureus* was chosen as the test organism.

Statistical analysis. The bone graft from each donor and the antimicrobial concentration at each time point were sampled and tested in triplicate. The results are reported as mean (SD). We used Student's *t*-test to determine statistical differences in the antibiotic concentrations between the osteoarthritis and fracture groups. A *p*-value of < 0.01 was considered significant.

Results

For the osteoarthritis group we enrolled ten patients (ten hips) who had undergone primary THR between September and December 2009, and who met the inclusion criteria for the study. There were four women and six men, with a mean age of 57 years (30 to 81). During the same period we collected a fracture group of ten patients (ten hips) with a displaced fracture of the neck of the femur who had a primary hemiarthroplasty. There were seven women and three men, with a mean age of 74 years (61 to 89).

After storage at -80°C for two weeks, all of the allografts from patients with osteoarthritis showed an inhibitory effect against bacteria in the agar disc diffusion bioassays (Fig. 1). However, this effect was only observed in some of the allografts from the patients with a fracture (Fig. 1). Cefazolin was detected in the broth elution fluid during the

Fig. 1

The fresh-frozen morsellised bone exhibited an inhibitory effect against bacteria, as determined by agar disc diffusion bioassay with *Staphylococcus aureus* (ATCC 25923) (OA, osteoarthritis group; Fx, femoral neck fracture group).

Fig. 2

Additional cefazolin released from fresh-frozen morsellised bone in broth elution assay. The data are presented as the amount of additional absolute release of cefazolin in 1 g of bone per day. Data are expressed as mean ± standard error of mean (SEM) for femoral heads from ten donors for each group (* significant difference (*p* < 0.01) between the two groups (OA, osteoarthritis group; Fx, femoral neck fracture group).

first four days following shaking in PBS (Fig. 2). The fresh-frozen bone showed an initial release during the first day, which was more than twice as high in the osteoarthritis group as in the fracture group (Fig. 2) (*p* < 0.01). The rate of release decreased rapidly during the next few days. On the first day, the cefazolin concentration was 69 (SD 10.6) µg/g of bone in the osteoarthritis group and 30 (SD 6.8) µg/g of bone in the fracture group. The concentration then gradually dropped to 2.4 (SD 0.9) µg/g bone and 2.1 (SD 0.8) µg/g bone in the osteoarthritis and fracture groups, respectively, on the fourth day. There was very little further release after the fifth day in both groups. The total

Fig. 3

Cumulative cefazolin released from fresh-frozen morsellised bone in broth elution assay. The data are expressed as the cumulative amount of cefazolin in 1 g of bone. Data are expressed as mean $\pm$ standard error of mean (SEM) for femoral head from ten donors from each group (* Significant difference ($p < 0.01$) between the two groups. OA, osteoarthritis group; Fx, femoral neck fracture group).

cumulative release after ten days was 98.4 (SD 14) $\mu\text{g/g}$ bone from the osteoarthritis and 46.6 (SD 10.5) $\mu\text{g/g}$ bone from the fracture group (Fig. 3). At all time points the cumulative release from the osteoarthritis group was significantly higher than from the fracture group (Fig. 3) ($p < 0.01$). The bioassay of the sample fluid confirmed the bioactivity of the released cefazolin against the test micro-organism (*Staph. aureus*) in the tube dilution bioassay.

Discussion

We have confirmed that the administration of cefazolin before surgery results in its presence in fresh-frozen femoral heads. The morsellised fresh-frozen femoral heads from patients with a hip arthroplasty exhibited inhibitory effects against bacteria *in vitro* after two weeks of deep-frozen storage, with those from patients with osteoarthritis having a significantly higher content of cefazolin than those with fractures of the femoral neck. The fracture might disrupt the major blood supply to the femoral head,⁸ resulting in only a limited amount of cefazolin reaching the head.

The availability of the bone, reduction in the operation time and the absence of donor site morbidity are reasons for using an allograft. Typically, between 1% and 22% of the donated bone grafts are found to be contaminated and cannot be used,^{9,10} the higher percentages usually occurring in large cadaver allografts. Femoral heads from living donors show lower rates of contamination.¹¹ The concentration of antibiotics in the femoral heads retrieved from living donors might contribute to this.

Allograft bone may serve as a dead foreign body that is not protected by the local cellular defence mechanisms. Infection rates of 4% to 12% have been reported following implantation of allograft.¹² Tomford et al,¹³ in a retrospective study, found that all infections related to the use of allografts occurred in patients who received a large

allograft, such as an osteo-articular or diaphyseal graft, harvested from cadaver donors. No infection was seen when using femoral heads harvested from patients at THR.¹³ Similar results were described by Hart, Campbell and Kartub¹⁴ who found no infection after the use of frozen femoral head allografts in 101 patients. The reasons for the difference in the incidence of infection between large bone and a femoral head allograft were difficult to determine. Large bone allografts were always used in patients with failed joint arthroplasty or after resection for tumour. These were all long operations, involving extensive soft-tissue dissection and large loss of blood.¹³ These factors lead to a higher risk of infection.^{15,16}

In this study, the fresh-frozen femoral heads released cefazolin above the minimal inhibitory concentration of the test strain (*Staph. aureus*; MIC, 1 $\mu\text{g/ml}$) for up to four days. The concentration of cefazolin in the femoral head might contribute to the reduction in the incidence of infection after allograft implantation. However, the smaller amount released from the femoral head following fracture may give a local concentration of antibiotic which may be too low or not maintained long enough to prevent infection and it may induce antibiotic resistance.¹⁷

The stability of the antibiotic is another concern. In an *in vitro* study, Robinson et al¹⁸ observed that the degradation of a cefazolin solution did not exceed 10% and 20% after storage at 38°C for 48 hours and 96 hours, respectively. Cefazolin should therefore be stable in fresh-frozen femoral heads after implantation for up to four days. Based on the standardised single-disc susceptibility test,¹⁹ a 30 μg cefazolin disc should be interpreted to indicate whether the tested organism is likely to respond to treatment. The 30 μg cefazolin disc gave a zone diameter between 15 mm and 20 mm for the test strain of *Staph. aureus* used in this study. Based on the results of the broth elution assay, most of the allografts released cefazolin, which was $> 30 \mu\text{g/g}$ of bone (69 (SD 10) for osteoarthritis and 30 (SD 6.8) $\mu\text{g/g}$ of bone for the fracture group) on the first day of shaking. In addition, the broth elution samples showed compatible inhibition of the bacteria to the standard cefazolin solution in tube dilution bioassay. This showed that the HPLC method was capable of measuring cefazolin in the broth elution samples. However, every allograft from patients with osteoarthritis and some in the fracture group provided inhibitory effects against bacteria in the agar disc diffusion bioassay (Fig. 2), suggesting that limited amounts of antibiotic leaked out of the bone graft into the agar. Broth elution provided a higher efficiency of antibiotic release than did agar disc diffusion. Our preliminary studies have shown that pressing the bone graft deeper into the agar enlarges the zone of inhibition, indicating that more antibiotic is being released from the graft.

In this study, the cefazolin concentration in fresh-frozen femoral heads was much higher than the penetration characteristic of cefazolin in bone (2.0 $\mu\text{g/g}$ to 18.7 $\mu\text{g/g}$ bone), as reported in the literature.^{4,20} This may be because

our morsellised specimens of frozen bone were not free of blood, unlike those used in the bone penetration study.⁴ The blood in the vessels in our test bone might have contributed to the higher content of cefazolin.

There are several limitations to our study. First, this is an *in vitro* study that does not necessarily reflect clinical circumstances. The quantity and flow of blood, limb mobility, host response and the stability of antibiotic *in vivo* were not taken into account. Second, all patients who had abnormal liver and renal function before operation were excluded. We do not know the antibiotic content in bones of patients with abnormal hepatic or renal function. Cefazolin is mostly excreted in urine. Third, morsellised bone was used. We do not know the characteristics of antibiotic release from a femoral head that has not been morsellised. Fourth, the morsellised bone was stored at -80°C for two weeks. We do not know whether a longer storage time would make any difference to the characteristics of antibiotic release or the ability to eradicate bacteria from fresh-frozen femoral heads. However, because the specimens in our study were all prepared and tested in a uniform and reproducible manner, these results should provide useful information.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

1. Costain DJ, Crawford RW. Fresh-frozen vs. irradiated allograft bone in orthopaedic reconstructive surgery. *Injury* 2009;40:1260-4.
2. Ivory JP, Thomas IH. Audit of a bone bank. *J Bone Joint Surg [Br]* 1993;75-B:355-7.
3. Borkhuu B, Borowski A, Shah SA, et al. Antibiotic-loaded allograft decreases the rate of acute deep wound infection after spinal fusion surgery in cerebral palsy. *Spine* 2008;33:2300-4.
4. Cunha BA, Gossling HR, Pasternak HS, Nightingale CH, Quintiliani R. The penetration characteristics of cefazolin, cephalothin, and cephadrine into bone in patients undergoing total hip replacement. *J Bone Joint Surg [Am]* 1977;59-A:856-9.
5. Coventry MB. Treatment of infections occurring in total hip surgery. *Orthop Clin North Am* 1975;6:991-1003.
6. Garden RS. Low-angle fixation in fractures of the femoral neck. *J Bone Joint Surg [Br]* 1961;43-B:647-63.
7. Anguita-Alonso P, Rouse MS, Piper KE, et al. Comparative study of antimicrobial release kinetics from polymethylmethacrylate. *Clin Orthop* 2006;445:239-44.
8. Gregor PJ. The effect of femoral neck fractures on femoral head blood flow. *Orthopedics* 1996;19:1031-6.
9. James LA, Ibrahim T, Esler CN. Microbiological culture results for the femoral head: are they important to the donor? *J Bone Joint Surg [Br]* 2004;86-B:797-800.
10. Liu JW, Chao LH, Su LH, Wang JW, Wang CJ. Experience with a bone bank operation and allograft bone infection in recipients at a medical centre in southern Taiwan. *J Hosp Infect* 2002;50:293-7.
11. van de Pol GJ, Sturm PD, van Loon CJ, Verhagen C, Schreurs BW. Microbiological cultures of allografts of the femoral head just before transplantation. *J Bone Joint Surg [Br]* 2007;89-B:1225-8.
12. Lord CF, Gebhardt MC, Tomford WW, Mankin HJ. Infection in bone allografts: incidence, nature and treatment. *J Bone Joint Surg [Am]* 1988;70-A:369-76.
13. Tomford WW, Thongphasuk J, Mankin HJ, Ferraro MJ. Frozen musculoskeletal allografts: a study of the clinical incidence and causes of infection associated with their use. *J Bone Joint Surg [Am]* 1990;72-A:1137-43.
14. Hart MM, Campbell ED Jr, Kartub MG. Bone banking: a cost effective method for establishing a community hospital bone bank. *Clin Orthop* 1986;206:295-300.
15. Gross AE, Lavoie MV, McDermott P, Marks P. The use of allograft bone in revision of total hip arthroplasty. *Clin Orthop* 1985;197:115-22.
16. Fitzgerald RH Jr, Nolan DR, Ilstrup DM, et al. Deep wound sepsis following total hip arthroplasty. *J Bone Joint Surg [Am]* 1977;59-A:847-55.
17. Thomes B, Murray P, Bouchier-Hayes D. Development of resistant strains of *Staphylococcus epidermidis* on gentamicin-loaded bone cement in vivo. *J Bone Joint Surg [Br]* 2002;84-B:758-60.
18. Robinson RF, Morosco RS, Smith CV, Mahan JD. Stability of cefazolin sodium in four heparinized and non-heparinized dialysate solutions at 38 degrees C. *Perit Dial Int* 2006;26:593-7.
19. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45:493-6.
20. Schurman DJ, Hirshman HP, Kajiyama G, Moser K, Burton DS. Cefazolin concentrations in bone and synovial fluid. *J Bone Joint Surg [Am]* 1978;60-A:359-62.