Cancellous bone as an antibiotic carrier

Eivind Witsø[1], Leif Persen[1], Kirsti Løseth[2], Pål Benum[1] and Kåre Bergh[2]

Departments of [1]Orthopaedic Surgery, [2]Microbiology, University Hospital, NO-7006 Trondheim, Norway.
Tel +47 73 86–74 78. Fax –87 22. Email: ewitsoe@online.no
Submitted 99-06-21. Accepted 99-10-20

ABSTRACT – We compared the release characteristics of antibiotics from in vivo and in vitro processed morselized cancellous bone. The bone was impregnated with 7 antibiotics and compressed into a wire-mesh cylinder. In vitro, the bone was processed by daily transfer of the cylinder with its contents into test tubes with broth. The amount of antibiotic eluted from the bone was measured after 1, 3 and 7 days. In vivo, the cylinder was implanted intramuscularly in the interscapular region in rats. After 1, 3 and 7 days, the cylinder was removed and the amount of antibiotic eluted in broth was measured. The results showed that morselized cancellous bone can act as a carrier of antibiotics in vitro and in vivo. The elution profiles of netilmicin-, vancomycin-, clindamycin- and rifampicin-impregnated cancellous bone processed in vitro and in vivo were similar.

■

In 1997, approximately one quarter of all revision total hip arthroplasties in Norway were done using impacted cancellous bone grafting (The Norwegian Arthroplasty Register 1998). This technique has been described in several experimental and clinical studies (Gie et al. 1993, Schreurs et al. 1994, Elting et al. 1995). Although deep infections develop in more than 2% after revision total hip arthroplasties (Murray 1990, Katz et al. 1997), no clinical studies have yet been published using morselized cancellous bone as an antibiotic carrier. We have previously shown that 8 antibiotics could be adsorbed onto and released from cancellous bone in vitro. In an in vitro elution model, the pattern of release of antibiotics from cancellous bone resembled the curve of a negative exponential function. However, the betalactams lost their antibacterial effect after a few days, while only rifampicin had an antibacterial effect in the elution broth for more than 3 weeks (Witsø el al. 1999). To explore the applicability of this in vitro model further, we studied the elution of antibiotics from morselized cancellous bone after in vitro and in vivo processing.

Material and methods

Preparation of cancellous bone

Human cancellous bone was morselized in a bone mill (Aesculap, Coarse, HARRIS, GB44) under sterile conditions, as described previously (Witsø et al. 1999). Briefly, 20 g of morselized bone was impregnated with antibiotics in 20 mL of antibiotic solution for 10 minutes at room temperature. We studied 7 antibiotics (Table). After the fluid was cleared from the bone, it was compressed into a wire mesh cylinder (diameter 11 mm, height 10 mm) made of stainless steel. The mean weight of bone in each cylinder was 1.00 (0.94–1.05) g. The antibiotic-impregnated bone was analyzed in triplicate, in vitro and in vivo. In addition, one cylinder containing bone impregnated with saline was used as a control.

Antibiotics, mg/mL, used for impregnation of cancellous bone (manufacturer, concentration)

Benzylpenicillin (Penicillin, A.L.)	100
Cefalotin (Keflin, Lilly)	100
Clindamycin (Dalacin, Upjohn)	150
Netilmicin (Netilyn,Schering-Plough)	100
Vancomycin (Vancocin,Lilly)	50
Ciprofloxacin (Ciproxin,Bayer)	2
Rifampicin (Rimactan,CIBA)	60

In vitro study

The cylinder with antibiotic-impregnated bone was put into an elution tube (15 × 100 mm sterile glass tube) containing 5 mL of cation-adjusted Mueller Hinton broth (Difco Laboratories, Detroit, MI, USA). In vitro, the bone was processed by daily transfer of the cylinder with its contents into a new elution tube after a standard washing procedure. The cylinders were kept at 37 °C. After 1, 3 and 7 days, the amount of antibiotics eluted from the antibiotic-impregnated bone after incubation at 37 °C for 24 h was determined by a bioassay technique.

In vivo study

The study was approved by the Norwegian Council for Animal Experimentation.

Male, albino, outbred Sprague Dawley rats (B&K Universal AB, Sollentuna, Sweden), 10 weeks old, 442 (390–504) g were given general anesthesia with a subcutaneous injection of 0.2 mL fentanyl-fluanisone + 0.2 mL midazolam. The animals were operated on under standard operating room conditions. In each animal, 1 cylinder with antibiotic-impregnated bone was implanted intramuscularly in the interscapular region. The muscular fascia was closed using a continuous 4-0 Vicryl suture and the skin was closed with metal clips.

Each animal received a subcutaneous injection of 0.1mL (0.3 mg/mL) buprenorphin as postoperative sedation. 9 animals were operated on in each antibiotic group. Postoperatively, the animals were kept 3 and 3 in a cage and allowed free movement. They were given water ad lib and standard food ("Rat and Mouse diet" BKOO1E). The animals were kept in a room with 42% humidity, 20 °C and 12 hours light/12 hours dark cycles. After 1, 3 and 7 days, 3 animals in each antibiotic group were killed by CO_2 inhalation. The cylinder was removed under sterile conditions and placed in a test tube containing 5 mL of cation-adjusted Mueller Hinton broth. The test tube was incubated for 24 h at 37 °C , and the amount of antibiotics eluted in the broth was determined by a bioassay technique.

Bioassay for determination of antibiotic concentration

A suspension of *Staphylococcus aureus* ATCC 25923 was frozen in aliquots at –80 °C and thawed before use. In this study, we used a modification of broth microdilution for MIC determinations (Tamashiro 1994): 2 mL of the elution broth was diluted twofold, serially and a standard inoculum of *S. aureus* ATCC 25923, 5×10^5 CFU/mL (final concentration) was added to each tube. After incubation at 37 °C for 24 h, the highest dilution with no visible bacterial growth was noted. In this study, a minimum estimate of the antibiotic concentration in the elution broth is expressed as the dilution factor of the last tube with no visible growth, i.e., "Times above MIC (TAM)". We determined the susceptibility of *S. aureus* ATCC 25923 to the antibiotics studied by the E-test according to the instructions of the manufacturer (AB BIODISK, Solna, Sweden). The minimum inhibitory concentration (MIC) was read (benzylpenicillin: 0.047 mg/L, cefalotin: 0.25 mg/L, netilmicin: 0.25 mg/L, vancomycin: 1.5 mg/L, ciprofloxacin: 0.5 mg/L, clindamycin 0.094 mg/L, rifampicin: 0.016 mg/L).

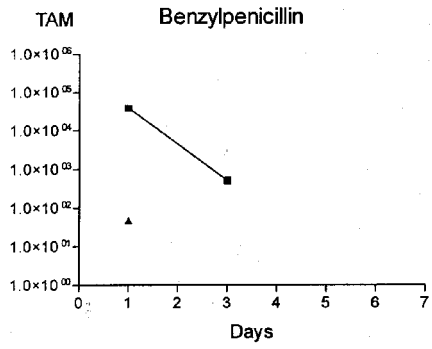
Results

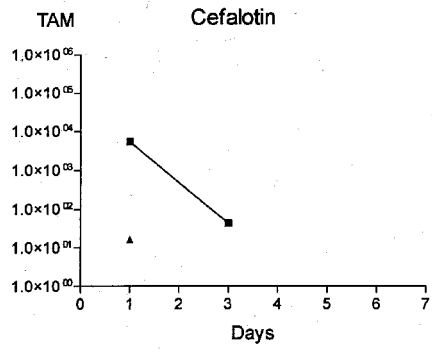
Elution of in vitro processed bone

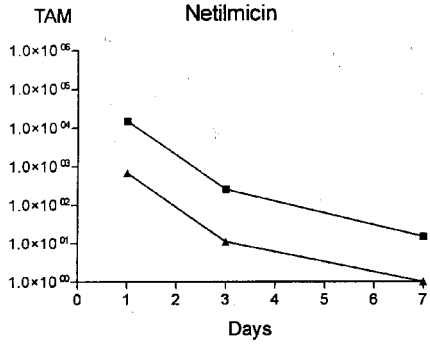
The control cylinder showed no inhibitory bacterial effect. Due to a technical failure, there were only 2 parallels containing ciprofloxacin-impregnated bone. Benzylpenicillin and cefalotin showed a rapid decline from very high values. Clindamycin, netilmicin, vancomycin, ciprofloxacin and rifampicin had a less steep curve and a bacteriostatic effect in the elution broth was noted for these antibiotics after 7 days (Figure).

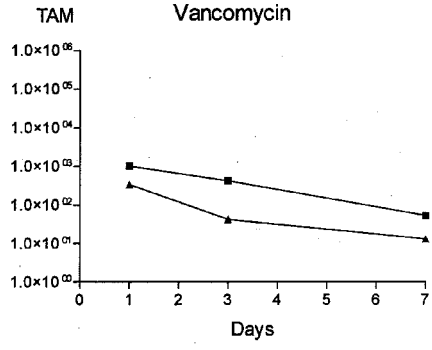
Elution of in vivo processed bone

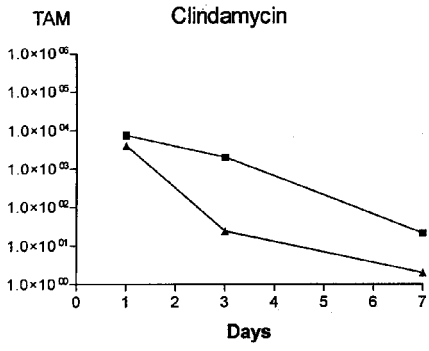
2 of the 63 rats died postoperatively: 1 was killed by the other rats and 1 died of unknown reasons. Apart from this, the rats tolerated the operation and the postoperative period well. The rats that were killed after 7 days had a mean weight increase of 21 g. In the benzylpenicillin group, one of the cylinders was contaminated by a gram negative rod. Of the 3 cylinders with rifampicin-im-

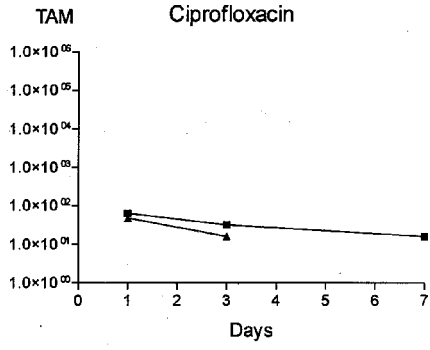


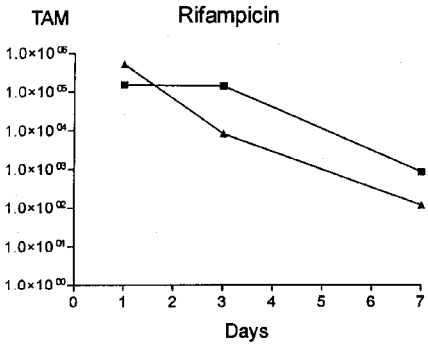












Elution profiles of various antibiotics from in vitro and in vivo processed bone. ■ in vitro, ▲ in vivo.

pregnated bone removed from the rats after 7 days, 1 diverged 2 dilution steps from the other 2 parallels when eluted in broth. None of the other parallels diverged more than ± 1 dilution step.

The cylinders with benzylpenicillin- and cefalotin-impregnated bone removed from the rats after 24 h and eluted in broth showed an antibiotic concentration in the broth equal to 16–48 TAM. The cylinders with benzylpenicillin- and cefalotin-impregnated bone that were removed from the rats after 3 and 7 days showed no bacteriostatic effect when eluted in broth. The cylinders with ciprofloxacin-impregnated bone removed from the rats after 1 and 3 days had a bacteriostatic effect when eluted in broth. The cylinders with clindamycin-, netilmicin-, vancomycin- and rifampicin-impregnated bone removed from the rats after 1, 3 and 7 days had a bacteriostatic effect when eluted in broth. Only rifampicin had a higher concentration in the broth than 100 TAM after 7 days in the rat (Figure). The elution profiles of the cylinders with clindamycin-, netilmicin-, vancomycin- and rifampicin- impregnated bone removed from the rats were very similar to the elution profiles of the respective antibiotic-impregnated bone processed in vitro. It was not possible to compare the in vitro and in vivo elution profiles of benzylpenicillin-, cefalotin- and ciprofloxacin-impregnated bone due to very small amounts of antibiotics eluted from the in vivo processed bone.

Discussion

We found that morselized cancellous bone can act as a carrier of benzylpenicillin, cefalotin, clindamycin, netilmicin, vancomycin, ciprofloxacin and rifampicin in vivo. The 7 antibiotics eluted from the in vivo processed bone showed a decline in antibacterial activity in the elution broth throughout the study. This could be due to: a) true elution of the antibiotics from bone, b) inactivation of the antibiotics by rat serum substances or c) development of a diffusion barrier. However, the elution profiles of bone processed in vitro and in vivo, impregnated with clindamycin, netilmicin, vancomycin and rifampicin, were very similar. This observation strongly indicates that the release kinetics for these antibiotics from cancellous bone in vivo is about the same as that in an in vitro elution model.

Benzylpenicillin and cefalotin were eluted in vitro from bone removed from the rats for a shorter time and to a considerably less degree than from antibiotic-impregnated bone processed in vitro. According to previous studies, the betalactams have less thermal stability than the other antibiotics (Witsø et al. 1999). This cannot explain the differences in the elution profiles. Elution in vitro occurred at the same temperature as the body temperature of the rats (37 °C). Hydrolysis of betalactams in vivo may explain the differences between the in vitro and in vivo results.

In the study of PMMA bone cement using a mouse model, antibiotic-impregnated cement plugs were implanted subcutaneously and removed after 6 hours and eluted in aqueous solution (Picknell et al. 1977). Other in vivo experiments have used the technique of seroma aspiration, comparing the elution profile in vitro and in vivo (Adams et al. 1992). Studies of antibiotic release from degradable products have eluted specimens of muscle and bone in vitro to estimate the amount of antibiotics eluted in vivo (Shinto et al. 1992). Our method, which is also a combined in vitro/in vivo method, seems suitable for studying the ability of morselized cancellous bone to act as an antibiotic carrier in vivo. In this study, we used a xenograft, which induces a marked inflammatory response (Goldberg and Stevenson 1992). This could increase the postoperative edema and thereby increase the amount of antibiotic that is eluted in vivo. The concentrations of antibiotics that were subsequently eluted in vitro from antibiotic-impregnated xenograft implanted in the rats might therefore be minimum values. A xenograft is slowly incorporated, if at all. This should hardly have any effect on the antibiotics eluted only during 7 days. In this study, we placed the bone graft intramuscularly. However, we intended to study whether cancellous bone could be used as a carrier of various antibiotics in vivo and to compare the elution profiles of bone impregnated with different antibiotics processed in vitro and in vivo. There probably would have been no differences in elution profiles between, for instance, vancomycin- and cefalotin-impregnated bone if the bone had been placed in a bone defect instead of intramuscularly.

Hardly any studies have been done on the release of antibiotics from cancellous bone in vivo and only a few antibiotics have been used (McLaren and Miniaci 1986, McLaren 1988). Little is known about basic pharmacokinetics concerning the release of antibiotics from cancellous bone in vivo. The influence of various antibiotic concentrations, the time allowed for antibiotic impregnation of the bone before implantation, different antibiotic combinations and the degree of bone morselizing remain unknown.

Obviously, cancellous bone can act as a carrier of many antibiotics in vivo. Furthermore, clindamycin, netilmicin, vancomycin, and rifampicin adsorbed to cancellous bone graft seem to be eluted in vivo in a similar manner to that observed in an in vitro elution model.

Adams K, Couch L, Cierny G, Calhoun J, Mader J T. In vitro and in vivo evaluation of antibiotic diffusion from antibiotic-impregnated polymethylmethacrylate beads. Clin Orthop 1992; 278: 244-52.

Elting J J, Mikhail W E M, Zicat B A, Hubbel J C, Lane LE, House B. Preliminary report of impaction grafting for exchange femoral arthroplasty. Clin Orthop 1995; 319: 159-67.

Gie G A, Linder L, Ling R S M, Simon J-P, Sloof T J J H, Timperley A J. Impacted cancellous allografts and cement for revision total arthroplasty. J Bone Joint Surg (Br) 1993; 75 (1): 14 -21.

Goldberg V M, Stevenson S. Biology of bone and cartilage allografts. In: Allografts in orthopaedic practice (Eds. Czitron and Gross). Williams & Wilkins, Baltimore, USA 1992: 1-13.

Katz R P, Callaghan J J, Sullivan P M, Johnston R C. Long-term results of revision total hip arthroplasty with improved cementing technique. J Bone Joint Surg (Br) 1997; 79 (2): 322-6.

McLaren A. Antibiotic-impregnated bone graft: Postop levels of vancomycin and tobramycin. Orthopaedic Trauma Assoc Annual Meeting 1988; 758-9.

McLaren A C, Miniaci A. In vivo study to determine the efficacy of cancellous bone graft as a delivery vehicle for antibiotics. The 12th Annual Meeting of the Society for Biomaterials. Minneapolis-St.Paul, Minnesota, USA 1986: 102.

Murray W R. Acetabular salvage in revision hip arthoplasty using the bipolar prosthesis. Clin Orthop 1990; 251: 92-9.

Picknell B, Mizen L, Sutherland R. Antibacterial activity of antibiotics in acrylic bone cement. J Bone Joint Surg (Br) 1977; 59 (3): 302-7.

Schreurs B W, Burma P, Huiskes R, Slagter J L M, Sloof T J J. Morzellized allografts for fixation of the hip prosthesis femoral component. A mechanical and histological study in the goat. Acta Orthop Scand 1994; 65 (3): 267-75.

Shinto Y, Uchida A, Korkusiz F, Araki N, Ono K. Calcium hydroxyapatite ceramic used as a delivery system for antibiotics. J Bone Joint Surg (Br) 1992; 74 (4): 600-4.

Tamashiro L. Broth microdilution MIC testing. In: Clinical microbiology procedures handbook (Ed. Isenberg H D) American Society for Microbiology, Washington, D.C. 1994: 5.2.1-29.

The Norwegian Arthroplasty Register 1998 (in Norwegian). ISBN: 82-91847-01-0.

Witsø E, Persen L, Løseth K, Bergh K. Adsorption and release of antibiotics from morselized cancellous bone. In vitro studies of 8 antibiotics. Acta Orthop Scand 1999; 70 (3): 298-304.