CALCIUM HYDROXYAPATITE CERAMIC USED AS A DELIVERY SYSTEM FOR ANTIBIOTICS

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Porous blocks of calcium hydroxyapatite ceramic were evaluated as delivery systems for the sustained release of antibiotics. We tested gentamicin sulphate, cefoperazone sodium, and flomoxef sodium in powder form placed in a cylindrical cavity in calcium hydroxyapatite blocks, using in vitro studies of elution and in vivo studies in rats.

Gentamicin sulphate gave a maximum concentration within the first week, which gradually decreased but was still effective at 12 weeks, when 70% of the antibiotic had been released. Even at this stage the antibiotic concentration from a 75 mg dose was five times the minimum inhibitory concentration for staphylococci. In the in vivo studies the release of gentamicin sulphate into the normal bone of rats was at similar rates and levels. The bacteriocidal activity of the drugs was not affected by packing into calcium hydroxyapatite ceramic and the blocks were completely biocompatible on histology.

This new system overcomes the disadvantages of other drug delivery systems, avoiding thermal damage to the antibiotics and a second operation for the removal of the carrier. Some mechanical strength is provided by the ceramic and healing may be accelerated by bone ingrowth into its micropores.

A high concentration of antibiotics at the site of infection is useful in the treatment of chronic haematogenous or implant-associated osteomyelitis. The parenteral administration of drugs may not achieve effective local concentrations, as infected bone has an uncertain blood circulation. Several delivery systems have been used, including closed suction-irrigation (Kelly, Martin and Coventry 1970; Tong et al 1987), and materials which can retain antibiotics such as the patient's own blood (Zilch and Lambiris 1986), plaster pellets (Dahners and Funderburk 1987), fibrin (Zilch and Lambiris 1986), acrylic resin (Hanamura 1984), collagen (Ascherl et al 1986), and acrylic bone cement (Vécsei and Barquet 1981; Majid et al 1985; Salvati et al 1986).

Porous calcium hydroxyapatite (CHA) ceramic has excellent biocompatibility (Bhaskar et al 1971; Klawitter et al 1976; Uchida et al 1984, 1985, 1987; Eggli, Müller and Schenk 1988), can resist mechanical force (Lemons 1988) and is effective in filling bone defects after resection of benign tumours (Uchida et al 1990). We have already used porous CHA as a slow-release delivery system for an anticancer drug, taking advantage of its interconnecting pore structure, and animal experiments (Uchida et al 1992) have confirmed its sustained-release capacity.

We now report a modification of this delivery system applied to the sustained release of antibiotics for the treatment of chronic osteomyelitis.

MATERIALS AND METHODS

Preparation of antibiotic-CHA. Porous CHA ceramic blocks of 10 × 10 × 10 mm and 4 × 3 × 3 mm (Central Glass Laboratories, Japan) were sintered at 1200°C for two hours. The porosity of the blocks was 30% to 40%, with micropore diameters of from 40 to 150 μ and the interconnecting pore structure open on the surfaces of the blocks. One of three different antibiotic powders, gentamicin sulphate (Schering, USA), cefoperazone sodium (Pfizer, USA), and flomoxef sodium (Shionogi, Japan), was packed into a central cylindrical cavity in each porous block, and the cavity sealed with α-tricalcium phosphate glue (Fig. 1). There were 75 mg of antibiotic in the larger blocks for the in vitro studies and 5 mg in the smaller blocks for the in vivo studies.

In vitro studies. Six large blocks were prepared for each antibiotic. Each was placed in a Falcon tube containing 5 ml of phosphate-buffered saline (PBS) and incubated

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0301-620X/92/4382 $2.00
J Bone Joint Surg [Br] 1992; 74-B: 600–4.

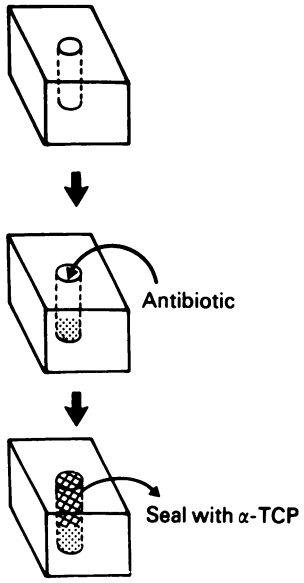
at 37°C. The PBS was replaced at 24-hour intervals for 90 days, and the removed elution fluids were preserved at −45°C for later determination of the antibiotic concentrations.

In vivo studies. Albino Sprague-Dawley rats weighing 500 to 600 g were anaesthetised with 10 mg/kg intraperitoneal ketamine hydrochloride (Sankyo, Japan). An ethylene-oxide sterilised small block, containing 5 mg of gentamicin sulphate, was then implanted into the proximal tibia of each rat under sterile conditions. The skin was closed by staples and the animals were then allowed free movement in their cages. The composite blocks were removed at regular intervals after killing the animals and specimens of local bone and muscle, and of plasma, kidney and liver were removed and preserved at −45°C. These were later homogenised with 10 ml of PBS and the suspension used for antibiotic assay.

Antibiotic assay. Microbiological assays were performed by an agar diffusion paper-disc method using *Bacillus subtilis* (ATCC 6633), and the results confirmed by fluorescence polarisation immunoassay using a TDX machine (Abbott Laboratories, USA). The lowest sensitivity level for the assay was 0.05 μg/ml.

Bacteriocidal activity. The elution fluids obtained from the in vitro study and the suspension obtained from homogenised local bone in the in vivo study were evaluated for the bacteriocidal activity of gentamicin sulphate. Two 0.1 ml samples of each specimen were placed in the central hole of a trypticase soy agar plate containing a stock strain of *Staphylococcus aureus 209p* (10 CFU/ml), and incubated at 37°C for 24 hours. The zones of bacterial inhibition were recorded and compared with the inhibition zones for similar concentrations of freshly made gentamicin sulphate solution.

Histology. Blocks containing gentamicin sulphate were implanted into the tibiae of seven Sprague-Dawley rats to assess the biocompatibility of the implants. The animals were killed at monthly intervals and a portion of the tibia containing the implant was removed with the surrounding bone marrow and muscles. The specimen was fixed in 10% buffered formal saline, decalcified in 10% formic acid and sections were stained with haematoxylin and eosin for light-microscopic examination.



Diagrams of an antibiotic-CHA ceramic block. The small cylindrical hole is made by ultrasound, powdered antibiotics are packed into the hole and sealed in with α-tricalcium phosphate (TCP) in polylactic acid.

Fig. 1

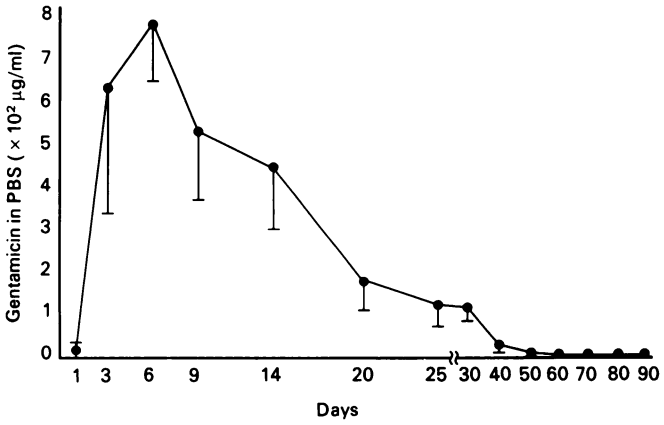


Fig. 2

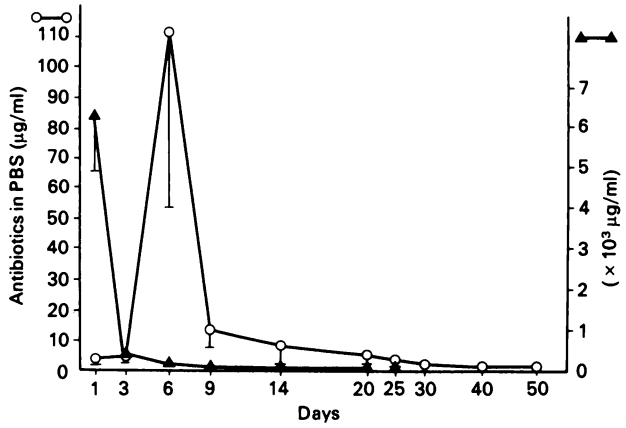


Fig. 3

Figure 2 – Gentamicin concentration in the elution fluid (mean ± SD for six samples). Figure 3 – Levels of cefoperazone sodium (circles) and flomoxef sodium (triangles) in elution fluid (mean ± SD for six samples).

RESULTS

In vitro study. Gentamicin sulphate elution from the composite increased to reach its peak during the second week, then gradually decreased until the end of the experiment at 90 days. The mean concentration of the drug at 90 days remained at least five times higher than the minimum inhibitory concentration for staphylococci (1.25 μg/ml), although the composite contained only

75 mg of gentamicin sulphate (Fig. 2). By 90 days, 70% of the drug had been released. Cefoperazone sodium showed a similar release pattern to gentamicin sulphate, reaching its peak on the sixth day, and gradually decreasing until 20 days, after which it was detected for 30 more days in low concentration (Fig. 3). Flomoxef sodium showed the most rapid release, with a very high concentration on the first day, but it was not detected after 25 days (Fig. 3).

In vivo study. For the in vivo experiments, we used only gentamicin sulphate since it had shown the longest release duration in the in vitro study. The release pattern from the composite into normal bone of rats was similar to that found in the in vitro study, the antibiotic concentration reaching a peak eight days after implantation, and gradually decreasing until 90 days. The concentration of gentamicin sulphate in the kidney, liver, and plasma remained extremely low in comparison with that in bone (Fig. 4).

Bacteriocidal activity. The elution fluid from the in vitro study showed a strong bacterial inhibitory effect for a long period, and was directly related to the measured concentration of the drug released at each stage (Fig. 5). This demonstrates that the antibiotic packed into the ceramic remains chemically stable and fully active after release from the composite block.

Histology. At one month after implantation there was some bone ingrowth into the micropores of the antibiotic-

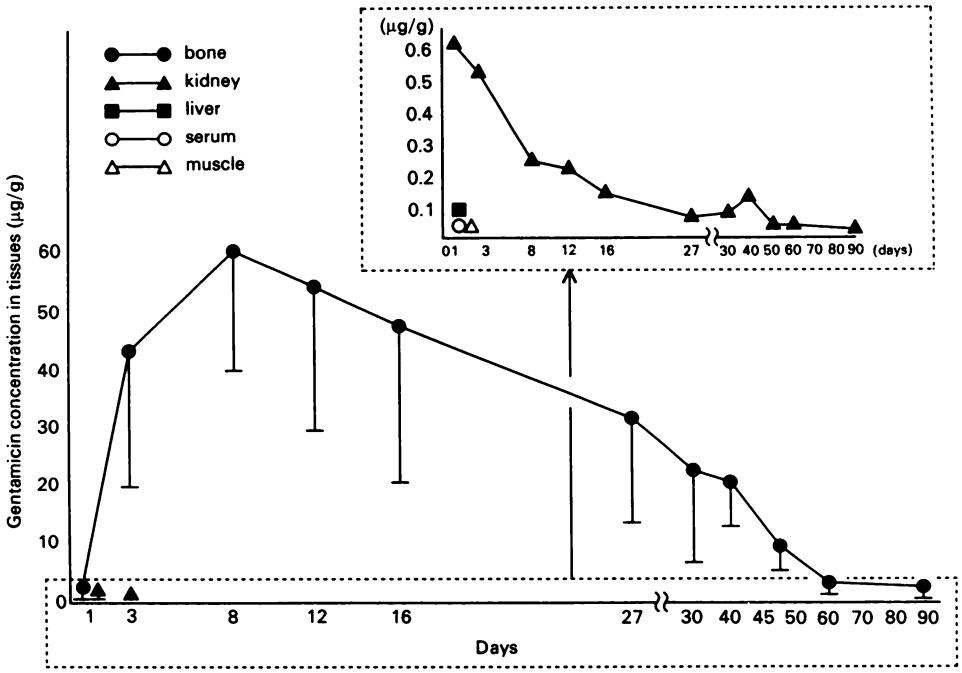


Fig. 4

Tissue and plasma concentrations of gentamicin after the implantation of a gentamicin-CHA slow-release block into rat tibiae (mean ± SD for six samples).

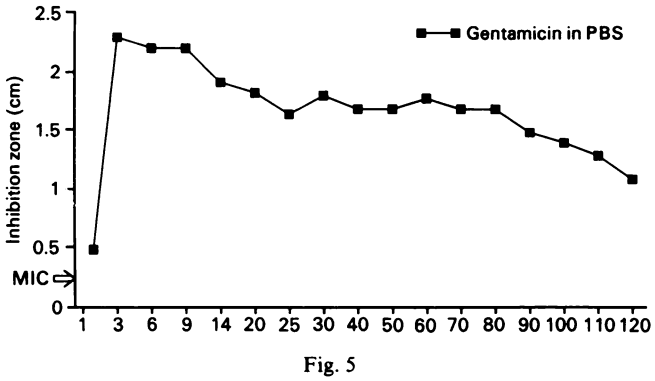


Fig. 5

Bacteriocidal activity of the gentamicin released from an antibiotic-CHA block into the solution corresponded to the released drug concentration shown in Figure 2.

CHA block in decalcified sections. The pores were not filled with new bone, however, until after six months, and no difference was detected between antibiotic-CHA blocks and CHA blocks, showing that packing gentamicin sulphate into a central opening did not alter the biocompatibility of the ceramic (Fig. 6).

DISCUSSION

Several biomaterials have been used as vehicles for the transport and sustained release of antibiotics. Antibiotic-impregnated acrylic bone cement has been studied in detail (Chapman and Hadley 1976; Rosenthal, Rovell and Girard 1976; Whaling et al 1978; Bayston and Milner 1982; Kirkpatrick et al 1985; Baker and Green-

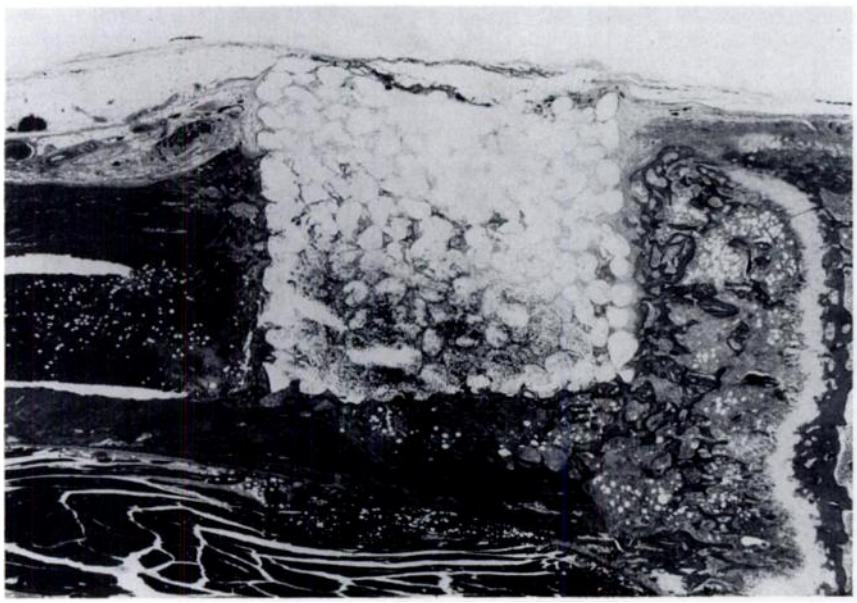


Fig. 6a

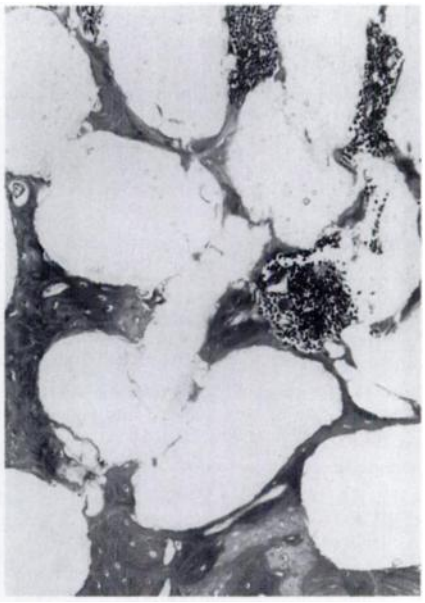


Fig. 6b

Histology six months after implantation of an antibiotic-CHA block, showing biocompatibility. Bone and bone-marrow cells have grown into the pore structure but some gentamicin particles are visible as small black granules (a, × 15, b, × 300).

ham 1988) as has its application for the treatment of chronic or implant-associated osteomyelitis. The reported disadvantages include:

1) reduced biocompatibility with bone;
2) short duration of release;
3) very low release rate; and
4) thermal damage to the antibiotics.

Recently, a biodegradable bone cement polypropylene fumarate-methylmethacrylate (PPF-MMA) has been proposed as a local drug delivery system, which overcomes some of these disadvantages (Gerhart et al 1988), although thermal damage to the antibiotics and a foreign-body reaction do still occur.

Our delivery system is superior to acrylic bone cement. Any antibiotic can be placed in CHA because there is no thermal damage to the drug. All the implanted antibiotic is released over a long period, none being trapped in the composite. CHA ceramics are fully biocompatible; bone and bone-marrow cells are seen to grow into the micropores and probably provide mechanical stability. These findings make it possible that filling the defects caused by the resection of infected bone with these composites can control infection and avoid the need for a second operation.

The different release patterns of the three antibiotics studied may be related to their solubility. Cefoperazone and flomoxef are more soluble in saline than gentamicin sulphate, but it showed the slowest release; this therefore indicates a means of controlling release rates.

In the treatment of chronic osteomyelitis, systemic administration of antibiotics for a long period may give rise to side-effects such as myelosuppression, nephrotoxicity and drug-induced hepatitis, while the side-effects of aminoglycosides are well known (Esterhai, Bednar and Kimmelman 1986). The plasma and liver concentrations of gentamicin sulphate reached after local administration with our system were low enough to avoid these serious side-effects.

We suggest that antibiotic-CHA composites may be a useful method for the treatment of chronic haematogenous or implant-associated osteomyelitis. We propose to test its effectiveness and clinical feasibility in the treatment of implant-associated chronic osteomyelitis in an animal model.

The authors would like to thank Professor Sydney Nade, University of Sydney, Australia for valuable comments and suggestions.

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.

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