EXPERIMENTAL IMPLANT-RELATED OSTEOMYELITIS TREATED BY ANTIBIOTIC-CALCIUM HYDROXYAPATITE CERAMIC COMPOSITES

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The efficacy of locally implanted antibiotic-calcium hydroxyapatite ceramic composites was investigated for the treatment of experimentally produced, implant-related osteomyelitis in rats. High concentrations of antibiotics were detected at the site of infection and bacteria were eradicated without removal of the metal implants. Parenteral antibiotics and surgical debridement, alone or in combination with antibiotic-impregnated acrylic bone cement, all failed to eradicate the infections.

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Despite the advances in prophylaxis against infection, chronic osteomyelitis after joint replacement surgery and internal fixation of fractures remains a considerable problem (Marks, Nelson and Lautenschlager 1976; Murray 1984; Wahlig et al 1984). Such infection is often resistant to the parenteral administration of antibiotics. Removal of the implant is often necessary for its control, and usually leads to severe functional disability.

A high concentration of antibiotics at the site of the infection would seem to be essential to achieve a cure and on this basis the impregnation of acrylic bone cement with antibiotics was developed, but was found to have some limitations (Buchholz, Elson and Heinert 1984; Murray 1984; Kirkpatrick et al 1985; Vaudaux et al 1985; Salvati et al 1986; Baker and Greenham 1988).

Porous calcium hydroxyapatite (CHA) ceramics, which have excellent biocompatibility (Uchida et al 1984, 1985, 1987) have been used for the sustained release and long-term delivery of various chemicals (Bajpai and Benghuzzi 1988; Gerhart et al 1988), anticancer drugs (Uchida et al 1992) and antibiotics (Shinto et al 1992). We have been able to maintain high concentrations of antibiotics for long periods both in vivo and in vitro with antibiotic-impregnated CHA composites (Shinto et al 1992).

We now describe the use, in rats, of locally implanted antibiotic-CHA composites to treat experimentally produced osteomyelitis around metal implants.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing approximately 500 g, were anaesthetised with 10 mg/kg intraperitoneal ketamine hydrochloride. The left hind leg was cleaned with 70% ethyl alcohol, the proximal part of the tibia exposed anteriorly and a hole drilled through the cortex into the medullary cavity using a 1.2 mm diameter dental burr. A stock strain of Staphylococcus aureus 209p (0.1 ml of 2.5 x 10^7 to 7.0 x 10^7 CFU/ml) was injected through this hole and stainless-steel implants (4 x 1 x 1 mm; 316L; Mathup Ltd, Bettlach, Switzerland) were inserted into the medullary cavity. The hole was covered with bone wax to prevent bacterial leakage into the soft tissues. The skin was closed by staples and the animals were then allowed free movement in their cages for seven weeks, before treatment began.

The 150 animals were divided into two groups of 75. The first group was used to compare the effect of antibiotic-CHA composites with that of parenteral antibiotic therapy. In 25 animals, antibiotic-CHA composites, 4 x 3 x 3 mm in size (Central Glass Co Ltd, Tokuyama, Japan), each containing 5 mg of gentamicin sulphate powder (Schering Co, Kenilworth, NJ, USA) were implanted near the site of infection; 25 animals were treated with intraperitoneal injection of gentamicin sulphate (1 mg/kg body-weight/day) for five weeks; and the remaining 25 rats received no treatment.
The second group was used to compare antibiotic-CHA composites with CHA only, and with surgical debridement with and without the implantation of antibiotic-impregnated acrylic bone cement. In 25 animals, surgical debridement only was performed; in another 25 animals surgical debridement was followed by the implantation of gentamicin-impregnated acrylic bone cement (5 mg/composite; Simplex; Howmedica, Rutherford, NJ, USA); and in the remaining 25 animals a CHA block without antibiotics was implanted into the infection site.

All treatments began seven weeks after bacterial inoculation. Thereafter five animals from each group were killed each week and the results were assessed by radiography, histopathology, bacteriology, and scanning electron microscopy.

**Bone antibiotic content.** The local concentration of the antibiotic in the bone was measured in those animals treated with antibiotic-CHA composites, antibiotic bone cement and intraperitoneal injections.

**Radiographic evaluation.** Xeroradiographs were taken with Softex film (Type C-SM: Fuji Film Co Ltd, Tokyo, Japan). Periosteal reaction and new bone formation, sclerotic and lytic changes, cortical thickening, and the radiolucent zone between the implant and the bone were all assessed independently by two of the authors.

**Bacteriology.** The soft tissues were removed from the bones under aseptic conditions. The bone sample was cut into small pieces with a rongeur, placed in a Falcon tube containing 20 ml of phosphate-buffered saline (PBS), and then homogenised under aseptic conditions for five minutes by a Polytron homogeniser (Kinematica, Switzerland). A 0.1 ml sample of the suspension was serially diluted in PBS and each dilution was placed in a trypticase soy agar plate for bacterial culture. Isolated bacteria were re-examined by tube dilution tests to measure their sensitivity to gentamicin, and the phage type was determined to exclude contamination.

**Histopathology.** The bone was dissected free of soft tissues and fixed in 10% formaldehyde after the removal of the metal implant. Decalcified sections were stained with haematoxylin and eosin.

**Scanning electron microscopy.** The samples were fixed with 2.5% glutaraldehyde in 0.086 M cacodylate buffer (pH 7.4) for three hours at room temperature. They were then rinsed in cacodylate buffer for 24 hours at 4°C, dehydrated in a graded series of ethanol, coated with gold and viewed by a T-800 Hitachi scanning electron microscope.

**Antibiotic assay.** The bone surrounding the infection site was homogenised with 10 ml PBS, and the resulting suspension was used for antibiotic assay. Microbiological assays were performed by an agar diffusion paper-disc method using Bacillus subtilis (ATCC 6633), and the results confirmed by the fluorescence polarisation immunomassay method using a TDX machine (Abbott Laboratories, Chicago, USA). The lowest sensitivity level for the microbiological assay was 0.05 µg/g.

**Statistics.** The results were analysed by Student's t-test.

**RESULTS**

**Radiology.** The antibiotic-CHA composites when implanted into experimentally infected bone altered the radiographic findings, but it was not possible to compare the results of each group radiologically.

**Bacteriology.** In the untreated animals, bacterial colonies were cultured at every stage up to 12 weeks (the maximum

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**Fig. 1**

Comparison of the bactericidal effect of local implantation of an antibiotic-CHA composite (AB-CHA) and intraperitoneal antibiotic injections (IP-AB). The antibiotic-CHA composite was significantly more effective than no treatment (control) and intraperitoneal injections (p < 0.01, p < 0.05 respectively).

**Fig. 2**

Comparison of the bactericidal effect of four different treatments for experimental osteomyelitis: debridement, implantation of calcium hydroxyapatite (CHA); implantation of antibiotic-impregnated bone cement (AB-PMMA); and implantation of antibiotic-CHA composite (AB-CHA). All the treatments were significantly more effective (p < 0.05) than no treatment (control). AB-CHA was significantly more effective (p < 0.05) than the other treatments.

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Comparison of gentamicin concentrations in bone after administration by three different routes. The antibiotic-CHA composite (AB-CHA) gave significantly higher concentrations ($p < 0.01$) than did antibiotic-impregnated bone cement (AB-PMMA) or intraperitoneal antibiotic injections (IP-AB).

Fig. 3

Bone antibiotic content. In the animals treated by period of the experiment). One week after implantation the antibiotic-CHA composite had caused a decrease in the number of bacteria isolated and none was grown from any of the animals after the third week.

Compared with the control group, the bacteria colony count also decreased significantly in the animals treated with intraperitoneal antibiotics but some colonies were still grown up to the end of the experiment (Fig. 1).

Surgical debridement alone and the implantation of antibiotic-impregnated acrylic bone cement, both significantly decreased the number of bacteria cultured, but neither treatment eradicated the infection. The bacterial counts around the CHA composites without antibiotics were similar to those recorded after surgical debridement. These results are compared in Figure 2 with the effect of the antibiotic-CHA composite used in the first group of animals.

Scanning electron microscopy revealed: (a) no bacteria on the surface of the antibiotic-CHA composite ($\times 16800$), (b) Staphylococcus aureus on the surface of the stainless-steel implants, in some cases associated with the biofilm ($\times 20800$), and (c) bacteria on the surface of antibiotic-impregnated bone cement ($\times 16800$).
intraperitoneal injections the maximum concentration of antibiotic in the bone reached 4.6 ± 1.0µg/g one hour after injection, then gradually declined to an undetectable level after 24 hours. The maximum concentration reached in the animals treated with antibiotic-impregnated bone cement was 23.6 ± 0.7 µg/g and antibiotic release continued for 90 days. In those with the antibiotic-CHA composite, the antibiotic concentration in bone reached 60 ± 12 µg/g after 8 days and declined gradually for 90 days (Fig. 3).

**Scanning electron microscopy.** Bacteria were not seen on the surface or in the micropores of the antibiotic-CHA composite, but were observed on the surface of the antibiotic-impregnated bone cement and on the stainless-steel implants (Fig. 4).

**DISCUSSION**

Postoperative infection of an orthopaedic implant is often a catastrophe, and usually requires removal of the prosthesis. The formation of a biofilm on the surface of the implant increases the resistance of the bacteria to the host’s defences and to antibiotics (Gristina and Costerton 1984, 1985; Buxton et al 1987). Unless the implant is removed, such infections are therefore difficult to eradicate because the antibiotics must penetrate the biofilm. To this end it is essential to maintain a high concentration of an appropriate antibiotic at the infected site for a sufficiently long time. In this study, gentamicin-impregnated CHA produced twenty times higher concentrations than did intraperitoneal injections, and 2.5 times higher concentrations, for 1.2 times longer, than did the acrylic bone-cement drug-delivery system.

In our experiments the antibiotic-CHA composites eradicated infection without removal of the metal implants. Neither parenterally administered antibiotics nor surgical debridement, alone or in combination with the implantation of antibiotic-impregnated acrylic bone-cement, were as successful. These results support our view that a high concentration of antibiotics maintained for a long period at the site of the infection can cure implant-related infection without removal of the implant.

Although the CHA-composite is biocompatible, the implantation of another non-absorbable foreign substance at the site of an infected implant may cause problems. If the infection were to persist or recur then the CHA may make the situation worse by acting as another foreign body. The long duration of release of the antibiotic may induce the development of resistant strains of bacteria.

If this method were to be applied in the clinical situation, surgical techniques for implanting the antibiotic-CHA composite would have to be developed to ensure continued stability of the prosthetic implant while allowing high concentrations of antibiotic to reach the site of infection. We believe that the method warrants further investigation.

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**REFERENCES**


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