Locally-administered antibiotics in wounds in a limb

We used a goat model of a contaminated musculoskeletal defect to determine the effectiveness of rapidly-resorbing calcium-sulphate pellets containing amikacin to reduce the local bacterial count. Our findings showed that this treatment eradicated the bacteria quickly, performed as well as standard polymethylmethacrylate mixed with an antibiotic and had many advantages over the latter. The pellets were prepared before surgery and absorbed completely. They released all of the antibiotic and did not require a subsequent operation for their removal. Our study indicated that locally administered antibiotics reduced bacteria within the wound rapidly. This method of treatment may have an important role in decreasing the rate of infection in contaminated wounds.

Open fractures and traumatic musculoskeletal wounds are often complicated by infection, the occurrence of which is determined by the severity of the injury, its location and local host factors.¹⁻³ In order to decrease the potential for infection current management involves debridement and irrigation of the wound and systemic antibiotic therapy.⁴ High concentrations of systemic antibiotics are usually required to attain sufficient levels in contaminated tissues with a compromised vascular supply. Antibiotic toxicity and systemic side-effects may then present serious problems.⁵⁻⁶

Local systems of delivery are attractive because they minimise problems with the systemic toxicity and maximise the local inhibitory concentration. In 1960, Charnley⁷ described the use of polymethylmethacrylate (PMMA) for the fixation of implants. Subsequently, Buchholz and Engelbrecht⁸ recognised the potential of antibiotic-loaded cement in the prevention and treatment of infection. Numerous other materials for local systems of drug delivery have been used including calcium sulphate,⁹ hydroxyapatite,¹⁰ demineralised bone matrix,¹¹ fibrin gel¹² and chitosan.¹³ One problem with delivery systems such as PMMA, is that the beads must be made by the theatre staff and they do not resorb in the body. Antibiotic is released from the surface of the cement and from cracks and voids. PMMA allows the ingress of physiologic fluids which permits elution of the antibiotic.¹⁴

Calcium sulphate has been tested as a delivery system for the treatment of osteomyelitis and is effective both in treating the infection and allowing the formation of new bone.¹⁵ In 1997, calcium sulphate impregnated with tobramycin (Osteoset-T; Wright Medical Technology Inc., Arlington, Tennessee) was introduced.⁷ This product was engineered to release antibiotic while slowly resorbing over a period of four to six weeks. It is an adequate vehicle delivering local antibiotic for the prevention or reduction of musculoskeletal infection. It is naturally bone-stimulating, readily degrades by dissolution and can be loaded with antimicrobial agents.¹⁶⁻¹⁸ Its dissolution rate can be tuned to match the intended application. Recently, calcium sulphate has been manufactured to resorb and elute antibiotics over a much shorter period of time.¹⁹ These rapidly-resorbing pellets dissolve completely within 12 hours and elute an effective concentration of antibiotic throughout their life.

Our aim was to compare the ability of rapidly-resorbing pellets of calcium sulphate with that of PMMA beads loaded with antibiotic to reduce bacteria in a contaminated musculoskeletal wound in an animal model.

Materials and Methods
Amikacin (amikacin sulphate; Bedford Laboratories, Bedford, Ohio) was chosen as the local antibiotic because the strain of *Pseudomonas aeruginosa*, which is the bacteria model, has good sensitivity to the drug and is heat-stable.
**Pellet manufacture.** The pellets of Amikacin-loaded calcium sulphate were made by mixing 10.0 g of calcium-sulphate dihydrate powder (Terra Alba, US Gypsum Corp, Chicago, Illinois), 0.40 g of sodium carboxymethylcellulose (Hercules Incorporated, Wilmington, Delaware), 0.42 g of amikacin, and 8.4 g of deionised water.\(^2\) The resulting 4% amikacin-loaded paste was cast into elastomer moulds containing 100 pellet-shaped cavities measuring 4.7 mm by 3.4 mm. The pellets were cured for 24 hours and then sterilised using low-dose gamma irradiation (25 KGY). A total of 50 pellets, weighing 2.6 g, comprised one dose for treatment (the amount that was placed within the wound after irrigation and debridement) and contained 104 mg of amikacin. Control pellets, combining calcium sulphate and carboxymethylcellulose only, were cast in a similar fashion to the antibiotic-loaded pellets.

The PMMA beads were made using radiopaque bone cement (Surgical Simplex P; Stryker, Mahwah, New Jersey). Powdered cement weighing 40.0 g was mixed with 2.4 g of amikacin and 20 ml of liquid monomer. The resulting paste containing 4% amikacin was formed into beads of a size similar to the calcium-sulphate models using a sterile bead mould. A total of 50 beads, weighing 3.1 g, contained 124 mg of amikacin and constituted one treatment dose.

**Animal procedures.** We used a contaminated musculoskeletal wound model in the goat which has been previously described.\(^2\) All the procedures were performed in an Association for Assessment and Accreditation of Laboratory Animal Care accredited laboratory after obtaining approval from the Institutional Animal Care and Use Committee at the US Army Institute of Surgical Research. After the animal had been anaesthetised, the musculoskeletal wound was created on the left hindlimb. An 8 cm skin incision was made parallel to the crest of the tibia in the mid-portion of its medial aspect. Electrocautery was utilised to incise the periosteum at the level of the tibial crest for 5 cm. A parallel incision was made medially through the periosteum, leaving an intact strip of 6 mm on the anterior aspect of the tibia. The periosteum was elevated by electrocautery to expose the medial side of the tibia as far as the posterior medial ridge and the attachment of the posterior compartments medially. The fascia was elevated from the anterior compartment exposing the anterior muscle compartment. A 3 mm drill bit on a twist drill and a small osteotome were used to create a cortical defect 1.2 cm in diameter, avoiding breaching the cortical wall. Three Kelly clamps were spaced evenly over 5 cm of the exposed muscle of the anterior compartment and left in place for three minutes to induce a crush injury. Concurrently, electrocautery was used to create thermal damage to the intervening exposed muscle, overlying fascia, and the retracted medial periosteum. This resulted in a consistent complex musculoskeletal wound involving injury to muscle, fascia, periosteum and bone.

The wound was then inoculated with 1 ml *Pseudomonas aeruginosa* (lux) of > 10\(^8\) colony-forming units/ml. This strain of bacteria has been genetically-modified to emit light.\(^2\) Two 5 mm Schantz pins were placed in the proximal tibia to secure the limb to the camera system for subsequent imaging. The wound was closed and a dressing applied.

Six hours later the animal was anaesthetised again. The left lower limb was fixed to the camera by an external fixation frame and the previously placed Schantz pins. The limb was then aseptically prepared and draped. A photon-counting camera (Charge Couple Device Imaging System Model C2400; Hamamatsu Inc, Hamamatsu-City, Japan) was used to capture the quantitative and spatial distribution of the bacteria in the wound.

This procedure was undertaken in 20 castrated male goats weighing between 45 kg and 55 kg. They were divided into three groups: 1) a control group (n = 7) which received 50 rapidly-resorbing calcium-sulphate pellets without antibiotics; 2) an experimental group (n = 7) which received 50 rapidly-resorbing calcium-sulphate pellets with 4% by weight of amikacin; and 3) a clinical control group (n = 6) which was given 50 PMMA beads with 4% by weight of amikacin.

All the wounds were debrided by an orthopaedic surgeon (JGB) and irrigated by a manual bulb syringe (Kendall Cp, Mansfield, Maryland) with 9 l of saline. The wound edges were closed by staples and a sterile dressing applied. Two days after inoculation of the wound the animals were killed and imaging was performed again on the re-opened wounds.

In addition, we had historical data from two different groups of animals in which the same procedures had been carried out, but which had not received any antibiotics (n = 20).\(^2\)

Blood was taken from goats receiving local amikacin to assess their systemic levels of antibiotic at 6, 12, 24 and 42 hours after placement of the antibiotic beads. These plasma levels were assessed using a fluorescence polarisation immunoassay instrument (TDxFLx; Abbott Laboratories, Abbott Park, Illinois).

**Statistical analysis.** Aquacosmos imaging software (Hamamatsu Photonics, Hamamatsu, Japan) was used to quantify the photons emitted by the bacteria within the wound. We compared the ratios of photon counts for each animal at each time point with the baseline photon counts. All the ratios were analysed for the treatment groups using a hierarchical mixed-model analysis of variance allowing for treatment, time and the interactions between treatment and time as fixed effects, and a replicate study as a random effect. Pre-planned orthogonal contrasts between the treatments at each time point were carried out. Statistical significance was set at a p-value ≤ 0.05. All the values were reported as the mean ± SEM.

**Results**

The photon counts for the three groups were similar at the assessment before debridement six hours after bacterial inoculation (p = 0.85, Tukey’s test). Debridement and irrigation reduced the number of bacteria to between 12% and 17% of the baseline value in the three groups (p = 0.74,
Tukey’s test) (Fig. 1). At 48 hours after wounding and inoculation, the control group had bacterial rebound to 159% of the baseline value. This was statistically higher (p < 0.004, Tukey’s test) than the two groups receiving antibiotics. In the group with calcium sulphate and amikacin the bacterial photon counts were reduced to 0.4% of the initial pre-irrigation counts, and in the group with PMMA beads and amikacin the counts were decreased to 3%. These two groups were not statistically different (p = 0.99, Tukey’s test) than the two groups receiving antibiotics. In the combined therapy were considerably lower at 3.7 than those with systemic treatment alone (12.0%). Moehring et al27 compared antibiotic beads as the only method of antibiotic therapy with systemic antibiotics in a randomised, controlled trial of open fractures and observed similar infection rates of 8.3% and 5.3%, respectively.

Previous animal studies and human clinical trials have shown tobramycin-impregnated calcium-sulphate pellets to be effective in the treatment of osteomyelitis.9,17,18,28-30 Since biodegradable antibiotic drug-delivery systems are effective against osteomyelitis, they may also be used as prophylaxis and in reducing bacterial counts in wounds. They would eliminate the need for re-operation and removal and allow a wider variety of antimicrobials, including thermolabile agents, to be incorporated into the vehicle depending on the manufacturing process.

This study has its limitations. The goat model was chosen since its lower limb is closest in size to the human leg. Our use of engineered luminescent bacteria and photon imaging allows complete quantification of bacteria in the entire wound and is neither tissue-consumptive nor susceptible to sampling error, as are tissue biopsies. Unfortunately, this model limits us to the use of Pseudomonas aeruginosa; other bacteria may not produce the same results. The development of a clinical infection is dependent on a complex interplay of bacterial load, bacterial virulence and host factors. Our model evaluates bacterial counts, but this is only one area of a clinical infection. The immune system of the goat differs from that of the human and the bacterial counts and the response to local antibiotics may be different. However, both the current clinical and experimental methods of delivery of antibiotics showed a significant and impressive decrease in the local levels of bacteria without high systemic levels.

The amikacin-impregnated calcium-sulphate pellets used in our experiment were fast resorbing, dissolved completely in 12 hours and produced maximal elution, well above the minimum inhibitory concentration for the bacteria, in four to eight hours.19 These rates are much faster than those using the same wound model we observed that 48 hours after similar debridement and irrigation the bacterial levels rebounded to 70% above baseline.20-22 This local delivery of antibiotic is the first treatment that we have tested which has significantly decreased bacterial levels after debridement and irrigation.20-22

Local delivery of antibiotic increases the local concentration while simultaneously minimising the risk of systemic toxicity. Buchholz et al24 first observed in joint replacement that antibiotics can be incorporated into PMMA cement, elute into the surrounding tissues, achieve high local concentrations and reduce early post-operative infection. Local antibiotic therapy in the form of antibiotic-impregnated PMMA beads has been introduced for the treatment of osteomyelitis and open fractures.25 In clinical trials on open fractures, Ostermann, Seligson and Henry26 compared systemic antibiotics alone with combined treatment with systemic and local antibiotics. The infection rates for the combined therapy were considerably lower at 3.7 than those with systemic treatment alone (12.0%).

Discussion
Our study has shown that local delivery of antibiotic, both with antibiotic-impregnated calcium sulphate and antibiotic-impregnated cement, can effectively decrease the bacteria in a contaminated musculoskeletal wound model without significantly increasing the systemic levels of antibiotic. The levels of amikacin were lower than 1 mcg/ml/g at all time points and the systemic toxicity level was 2.5 mcg/ml/g. Plain calcium sulphate acted as a foreign body in the contaminated wound and caused the bacterial concentration to rebound. This observation was similar to that noted by Yarboro, Baum and Dahners23 in a surgical wound in a rat model in which gentamicin-loaded calcium sulphate reduced the bacterial counts and mortality. Animals treated with plain calcium sulphate had high bacterial counts and a high rate of mortality. In previous studies
with the 4% antibiotic-loaded conventional pellets cast from alpha-hemihydrate, which dissolve in de-ionised water in approximately six days and elute antibiotic over 28 days in phosphate-buffered saline. This quick burst of antibiotic in the wound shortly after contamination could reduce bacterial colonisation and proliferation. The real value may be as an adjuvant in complex contaminated wounds in which they can be used throughout treatment. Like PMMA beads, the pellets can be used during initial and subsequent debridements and because they are resorbable, they may also be used during the final closure of the wound and for prophylaxis in elective surgery.

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References