Elution characteristics of vancomycin, teicoplanin, gentamicin and clindamycin from calcium sulphate beads

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The in vitro release of vancomycin, teicoplanin, gentamicin and clindamycin from biodegradable calcium sulphate (CaSO₄) carrier beads is described. All antibiotics showed prolonged release from the carrier beads, which was elevated during the first 24 h, with peak levels exceeding 2500 µg/bead. Doubling the antibiotic load of the beads revealed a more prolonged elution and a two-fold increase in antibiotic release. Local carrier-associated antibiotic treatment with CaSO₄ beads may prove to be effective in the management of chronic bone infections.

Introduction

Given the increasing antibiotic resistance of Gram-positive pathogens involved in bone infection, the successful treatment of chronic bacterial osteomyelitis remains a serious and challenging problem. Besides techniques for adequate and thorough debridement of the wound, the administration of antibiotics plays a crucial role in therapy.

Systemic treatment is an inefficient method for achieving high local tissue concentrations of drug and therefore, local deposition of antimicrobial agents has become increasingly popular in the management of osteomyelitis or open fractures. Several biodegradable and non-biodegradable substances have been employed as the vehicle for delivery. Polymethylmethacrylate (PMMA) is a non-resorbable antibiotic-loaded bone cement that has been used clinically for more than 20 years, but with the use of commercially available PMMA additional surgery is needed for its removal, and individualized chemotherapy in terms of administration of different antibiotics is not possible. Absorbable carrier materials with a collagen basis stimulate the formation of seroma and do not guarantee a prolonged release of the antibiotic. The hemihydrate of calcium sulphate (CaSO₄), commonly known as plaster of Paris (POP), is a resorbable vehicle that does not require removal and releases its entire antibiotic load on resorption. The aim of this study was to evaluate in vitro the release kinetics of vancomycin, teicoplanin, gentamicin and clindamycin from biodegradable CaSO₄ carrier beads.

Materials and methods

Antimicrobial agents

Vancomycin 80, 160 and 320 g/L (Eli-Lilly, Bad Homburg, Germany), teicoplanin 80 g/L (Aventis, Frankfurt, Germany), gentamicin 80 g/L (Sigma, Deisenhofen, Germany) and clindamycin 80 g/L (Sigma) stock solutions were prepared according to the manufacturer’s recommendations and stored at –20°C.

Carrier beads

The CaSO₄ beads were manufactured by Coripharm (Dieburg, Germany) using a special vacuum technique. POP 50 g, Gips Special 40 (Heidelberger Cement AG, Heidelberg, Germany), was mixed with 30 mL of deionized water to yield optimal porosity. This liquid plaster was then poured into a mould that produced spherical beads of 6 or 4 mm in diameter. The beads were sterilized with γ radiation 25 kGy.

In vitro elution studies

Dry beads were initially soaked in 1 mL of the antibiotic solution for 3 min at room temperature and thereafter re-dried. The release of antibiotic by diffusion from a single bead was investigated by elution in 5 mL phosphate-buffered saline (PBS) at pH 7.4 and 37°C. After each 24 h,
the bead was removed and transferred to a tube containing 5 mL of fresh PBS. The eluates for each day were stored at –20°C and assayed within 10 days. Each elution series was replicated six times.

**Determination of the antibiotic concentration**

The elution samples of PBS were assayed by agar diffusion microbiological assay. Indicator organisms were *Bacillus subtilis* ATCC 6633 for vancomycin, teicoplanin and gentamicin, and *Micrococcus luteus* ATCC 9341 for clindamycin. Fifty millilitres of sterile nutrient agar (Antibiotika-Agar Nr.5; Merck, Germany) was seeded with the appropriate bacteria at a concentration of 10⁸ cfu/L and poured into 22 cm diameter round Petri dishes. The agar was allowed to set, and 14 wells (10 mm diameter) were punched into the agar and then filled with 100 μL of the elution samples and antibiotic calibrators. Calibrator concentrations were 0.625, 1.25, 2.5 and 5.0 mg/L, prepared in PBS. After incubation at 37°C for 18 h, the zones of inhibition were read using a micrometer. The antibiotic concentration of the eluates was determined by computer-assisted regression analysis. Each sample and calibrator was analysed on two separate agar plates, giving two readings per sample, with the mean being taken for calculation.

**Statistical analysis**

Statistical analysis was performed with Student’s t-test.

**Results and discussion**

The elution characteristics of the antibiotics are shown in Figure 1. All antibiotics showed a sustained release from the beads, 6 mm in diameter, that was elevated during the first 24 h and decreased over the course of the following days. The initial release was especially high for gentamicin and clindamycin, being >2500 μg/bead within the first 24 h.

The influence of the antibiotic concentration in a single 4 mm bead on the elution kinetics is shown in Figure 2. Beads that were initially soaked in 320 g/L vancomycin revealed significantly higher elution rates than beads soaked in 160 g/L. The influence of the antibiotic concentration within the bead on the elution characteristics was most apparent for the 10th elution day where there was a 10-fold greater antibiotic release from the more highly loaded beads.

Antimicrobial management of osteomyelitis generally involves pre- and post-operative systemic antibiotic therapy in conjunction with intra-operative anti-infective irrigation. With parenteral therapy, however, only a small fraction of the antibiotic will ultimately act at the infected site and high dosages may be required to reach effectively the poorly vascularized area of bone infection. These problems can be avoided via the controlled local release of antibiotics into the infected region, a technique that achieves high local concentrations while maintaining low systemic levels.¹

CaSO₄ as a filler for bone defects was first described by Dreesmann in 1892.² After local treatment of bone infection was established as an alternative to systemic treatment, CaSO₄ was also loaded with antibiotics.³⁴⁵ The antibiotic, however, was usually added before hardening of the CaSO₄.³⁴ Here, we report for the first time the possibility as well as advantage of loading hardened CaSO₄ beads with various antibiotic solutions. This means, first, that selecting appropriate antibiotics facilitates individualized therapy and secondly, that no degradation of the antibiotic owing to the sterilization process or thermostability reduces the activity of the antibiotic.² With regard to the total amount of antibiotic eluted within 10 days the kinetics demonstrated that in an initial phase the beads released approximately 45% of the glycopeptide antibiotic and about 80% of gentamicin and clindamycin within the first 24 h (Figure 1). In a second phase, a more gradual release...
Antibiotic release from CaSO₄ carrier beads

over a period of 10 days could be demonstrated for all of the antibiotics. This study further revealed a direct relationship between the antibiotic load in the beads and the amount released daily. As shown in Figure 2, doubling the antibiotic load of the bead gave a more prolonged elution and a two-fold increment in antibiotic release. Therefore, it may be expected that the MIC for relevant bacteria might be exceeded locally during treatment and that, if need be, the antibiotic concentration of the solution used to soak the carrier material initially is capable of being increased. Sufficiently high antibiotic concentration in the infected tissue especially during the first days of therapy is of major importance in preventing infection. Local carrier systems based on CaSO₄, PMMA or high molecular weight lactic acid polymers demonstrate adequate high peak concentrations on the first day of release. With elution kinetics as shown in this study revealing antibiotic release over at least 10 days, the CaSO₄ carrier system offers characteristics that, with regard to the duration of release, fall between those of collagen carriers, i.e. 2 days, on the one hand and PMMA or polylactate carriers, i.e. 30–350 days, on the other.

In conclusion, CaSO₄ as a vehicle for local antibiotic therapy displays several advantages. First, prolonged release of the antibiotic at the site of infection achieves elevated local concentrations while minimizing any risk of systemic toxicity. Secondly, a variety of antibiotics can be selected to load the beads, thereby facilitating individualized chemotherapy. Thirdly, the biodegradability of the carrier beads eliminates the need for a second surgical procedure for their removal.

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References


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