Kasetsart J. (Nat. Sci.) 44 : 1115 - 1120 (2010)

Use of Enrofloxacin in Calcium Beads for Local Infection Therapy in Animals

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ABSTRACT

Antibiotic-loaded beads have been used widely for the treatment of local bacterial infections, but many bacterial pathogens have become resistant to antimicrobial agents. Various antibiotic beads were prepared and studied for their efficacy to treat bacterial infections, using the elution profiles of enrofloxacin from calcium sulfate beads *in vitro*. The beads were prepared by mixing 1.0 g enrofloxacin and 10.0 g calcium sulfate. The beads were placed in phosphate buffer (pH 7.4) for 20 d and eluted enrofloxacin concentrations were determined by agar diffusion microbioassay. The study showed that calcium sulfate beads released enrofloxacin at rates that were higher than the minimal inhibitory concentration (MIC) throughout the 20-day period, with the rate of enrofloxacin release being rapid early in the period. The cumulative eluted enrofloxacin after 20 d was 8.08±0.92% (mean±standard deviation). It was concluded that enrofloxacin-loaded calcium sulfate beads provided a possible treatment for local infections in veterinary medicine. In addition, the calcium beads were simple to prepare, were effective carriers and had antibacterial properties.

Keywords: calcium sulfate, enrofloxacin, bead, elution, local infection

INTRODUCTION

Antibiotic-impregnated beads have been used to treat bacterial infections, especially osteomyelitis and prosthesis infections (Holman *et al*., 1999; McKellar *et al*., 1999; Zilberman *et al*., 2008). The antibiotic bead is effective for antibiotic delivery to an infected tissue, in which tissue integrity and vascular supply is compromised and in addition, the bead does not cause antibiotic toxicity systemically (Wininger and Fass, 1996). For veterinary practice, antibiotic beads have been used to treat abscesses related with malocclusion in rabbits, local infection in horses and bumblefoot in raptors (Orsini *et al*., 2004; Remple, 2006).

Various kinds of material have been used to prepare antibiotic beads, such as polymethyl methacrylate (PMMA), calcium sulfate, calcium phosphate, chitosan, polyethylmethacrylate/n-butyl methacrylate and hydroxyapatite ceramic (Wininger and Fass, 1996; Santschi and McGarvey, 2003; Adriano *et al*., 2005; Anal and Stevens, 2005; Rauschmanna *et al*., 2005; Sanicola

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Received date : 03/03/10 Accepted date : 10/06/10

and Albert, 2005; Sasaki *et al*., 2005). Antibiotics that can be mixed with PMMA are limited, because it releases heat during polymerization. Calcium sulfate beads can be prepared more conveniently and release greater amounts of antibiotics than PMMA beads (Udomkusonsri *et al*., 2008; Bunmanee *et al*., 2009). Since calcium sulfate does not release heat during setting, various antibiotic agents could be incorporated with calcium sulfate to prepare the beads, without losing any antimicrobial properties. Gentamicin is one of the most common drugs used to prepare antibiotic bone cement, due to its wide antibacterial spectrum and heat stabilty. Bacterial resistance to antimicrobial activity is a problem in clinics. Therefore, to develop suitable antimicrobial beads, it is necessary to test the efficiency of antibiotics in bead form.

Enrofloxacin is a synthetic antimicrobial agent of the fluoroquinolone group, which is used extensively in veterinary medicine. It inhibits prokaryotic topoisomerase II (DNA gyrase), which is an important enzyme for bacterial replication (Vancutsem *et al*., 1990). It has broad spectrum antibacterial activity, especially against gram-negative bacteria, such as *Pseudomonas* spp. (Mitchell, 2006; Okewole *et al*., 2008). However, enrofloxacin-calcium sulfate beads are not available commercially.

The current study aimed to determine the *in vitro* release characteristics of enrofloxacin-calcium sulfate beads that had been produced in the laboratory. The concentration and percentage of enrofloxacin released from the calcium beads were determined.

MATERIALS AND METHODS

Preparation of antibiotic beads

Calcium beads were prepared by mixing thoroughly 1 g of enrofloxacin powder (pharmaceutical grade) with 10 g calcium sulfate (Sigma) and then adding 4.0 mL of 0.1 M phosphate buffer (0.1 M PB, pH 7.4). The calcium sulfate mixture was poured into a mold and air-dried at room temperature.

In vitro drug release studies

An elution method was employed to determine the characteristics of enrofloxacin released from the calcium beads. A phosphate buffer (0.1 M PB, pH 7.4), was used as the dissolution medium. Two beads were placed in 1.5 mL of PB at 37°C for 24 h and the elution test was carried out with five replications. The dissolution PB was collected and 1.5 mL of fresh PB was added every 24 h for the 20 d of the experimental period. All dissolution media were kept at -20°C until analysis.

Determination of eluted enrofloxacin concentration

The released enrofloxacin levels were characterized by agar-well diffusion microbiological assay, in which *Bacillus subtilis* (ATCC 6633) was used as an indicator organism (Ficker *et al*., 1990).

In brief, molten Mueller-Hilton agar (Difco) was inoculated with *B. subtilis* spore. After cooling, 8-mm wells were cut into the solidified seeded agar. Standard enrofloxacin (Sigma) was prepared at concentrations of 0.25-4 μg/mL for a standard curve. Standard enrofloxacin and eluted samples were transferred by pipette into the wells. Plates were incubated at 37°C for 18-20 h and the diameter of inhibition zones was recorded. All eluted samples were tested in triplicate. A plot of the control-drug concentrations against the inhibition zones was used to produce a standard curve and the drug concentrations were extrapolated from the standard curve. The lower limit of sensitivity of the assay was 0.2 μg/mL.

Determination of minimal inhibitory concentration

MICs of enrofloxacin were determined

against two bacteria, *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25928) by a broth microdilution method (CLSI, 2006).

Statistical analysis

The comparison of eluted enrofloxacin concentrations from calcium sulfate beads between days was analyzed using ANOVA. Statistical significance was accepted at $P<0.05$.

RESULTS

The enrofloxacin-calcium sulfate beads were 14.08 ± 0.5 mg in weight and cylindrical in shape, with a diameter of 6.28 mm and average height of 4.26 mm. The total amount of enrofloxacin was 12.8 ± 0.05 mg/bead (mean ± standard deviation).

Enrofloxacin-calcium sulfate beads released enrofloxacin throughout the 20-day experimental period (Figure 1). The eluted enrofloxacin concentrations were higher than MICs, with the MICs of enrofloxacin against *P. aeruginosa* and *S. aureus* being 0.25 and 0.125 μg/mL, respectively.

A continuous release of enrofloxacin was observed throughout the entire period, with a high rate at the beginning that slowed down in the later period. Even on the last day of the sampling period, the drug was still being released from the beads. The accumulated enrofloxacin released over the 20 d was 2.07±0.24 mg/sample. The total enrofloxacin eluted from the beads was 8.08±0.92%. The weight of the calcium beads after elution was 13.95 ± 0.24 mg.

The enrofloxacin eluted on the first day was significantly ($p<0.05$) greater than on the remaining 19 d. The concentration of the drug eluted after day 9 was significantly ($p<0.05$) different from the initial four days of the experiment.

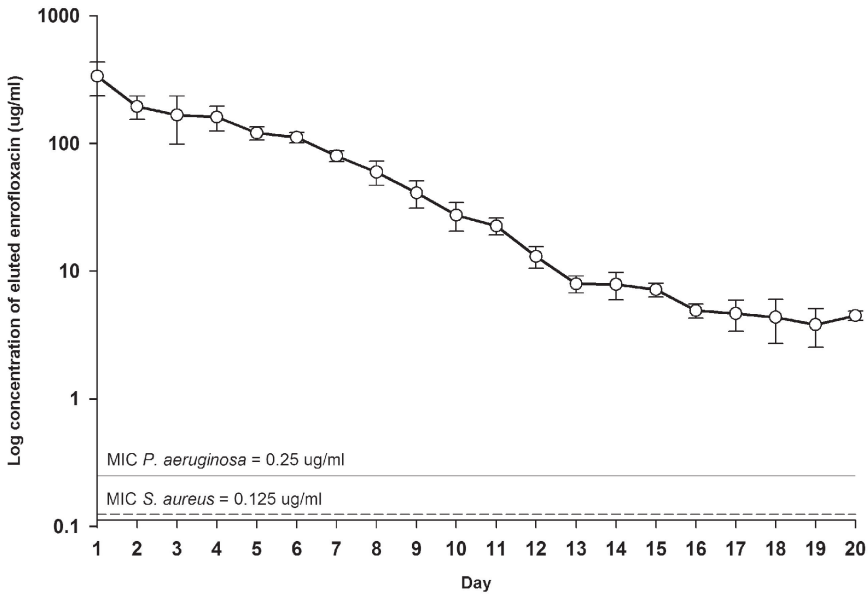

Figure 1 Daily concentrations of eluted enrofloxacin (μg/mL, mean ± standard deviation) from calcium sulfate beads. The enrofloxacin concentrations were characterized by agar-well diffusion microbioassay. The MIC levels for *Pseudomonas aeruginosa* (ATCC 27853) (shown as —) and *Staphylococcus aureus* (ATCC 25928) (shown as ----) were determined by a broth microdilution method.

DISCUSSION

In a previous study, enrofloxacin-calcium beads made from a 2-mL enrofloxacin injection solution (200 mg/2mL) and 20 g calcium sulfate were dissolved completely after being placed in the buffer and water (Bunmanee *et al*., 2009). It may be possible that the mixtures in the enrofloxacin injecting solution, such as n-butyl alcohol or other factors, could have caused the dissolution of the beads. In the current study, it was possible to make enrofloxacin-calcium sulfate beads that were not only stable in phosphate buffer and water, but also could release the drug at amounts greater than the MIC for a long period. The high amount of eluted enrofloxacin at the beginning of the experiment was associated with the dissolution of the drug adsorbed to the bead surface or the diffusion of drug close to the surface. After the initial high elution, the antibiotic in the matrix was able to dissolve in the tissue fluid and was eluted via pores and cracks within bead matrices (Díez-Peña *et al*., 2002; Frutos *et al*., 2002).

Enrofloxacin-PMMA bead in the previous study could release 5.18% enrofloxacin within 15 d, which was lower than in the current study and could be explained by the calcium sulfate beads having greater porosity than the PMMA beads, in which the antibiotic in the matrix could dissolve in the fluid and then be released via the pores (Díez-Peña *et al*., 2002; Frutos *et al*., 2002). Since calcium sulfate is a biodegradable material, antibiotic-calcium beads could be placed in the tissue and provide prolonged antibiotic release without secondary surgery for bead removal (Mader *et al*., 1997; Nelson *et al*., 2002; Ginebra *et al*., 2006). The fact that calcium sulfate does not release heat during bead setting, makes it a suitable candidate for antibiotic bead preparation with heat sensitive antibiotics.

In a previous study, cefazolin and gentamicin beads were prepared using calcium sulfate and PMMA and the results showed that calcium beads could release more antibiotic than PMMA beads and the concentrations of eluted antibiotics were above MICs (Udomkusonsri *et al*., 2008; Bunmanee *et al*., 2009).

CONCLUSION

The study demonstrated that enrofloxacin-impregnated calcium sulfate beads could be prepared for use in hospitals or clinics as a slow-release means for the treatment of local bacterial infection. The beads contained an antimicrobial activity greater than the MIC of *P. aeruginosa* and *S. aureus* for at least 20 d.

ACKNOWLEDGEMENTS

This study was financially supported in 2009 by the Kasetsart University Research and Development Institute (KURDI), Thailand.

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