FULL PAPER *Pharmacology*

Elution Profiles of Cefazolin from PMMA and Calcium Sulfate Beads Prepared from Commercial Cefazolin Formulations

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ABSTRACT. Antibiotic beads have become popular for the treatment of local bacterial infections. The preparation of antibiotic beads from commercial pharmaceutical antibiotics is a convenient method in clinic. The elution characteristics of cefazolin from polymethylmethacrylate (PMMA) (SmartSet HV, Depuy I and Cemfix 3) beads and calcium sulfate beads were studied. Commercial cefazolin formulation was incorporated in PMMA or calcium sulfate at 1 g cefazolin /10 g of matrix substances to form beads. The concentrations of eluted cefazolin during 15 days were greater than MIC for *Staphylococcus aureus* (ATCC 25923). The eluted cefazolin concentrations were in the range of 3.6 ± 1.2 to 4.6 ± 0.4 mg for PMMA beads and 15.4 ± 1.7 mg for calcium sulfate beads. The accumulated eluted cefazolin from PMMA beads and calcium sulfate beads for 15 days were 34.41 ± 3.93 to 38.67 ± 3.04% and 95.94 ± 3.93%, respectively. The various storage conditions; at room temperature or 4°C, with or without light-protection, for 6 months had little effects on the amounts of eluted cefazolin. The results showed both in-housed cefazolin-PMMA beads and cefazolin-calcium sulfate beads could be the effective tools for the treatment of local bacterial infections.
KEY WORDS: antibiotic bead, calcium sulfate, cefazolin, elution, PMMA.

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Antibiotic-impregnated beads or antibiotic beads have been used to treat bacterial infections, especially osteomyelitis and prosthesis infection [10, 15, 29]. The antibiotic bead is an effective route to deliver antibiotic to an infected tissue in which tissue integrity and vascular supply are compromised and does not cause antibiotic toxicity systemically [3, 28]. For veterinary practice, the antibiotic beads have been used to treat abscesses related with malocclusion in rabbit, local infection in horses and bumble foot in raptor [18, 21].

Polymethylmethacrylate (PMMA) is a non-biodegradable polymer material that is usually used to prepare antibiotic beads, spacers and other antibiotic-delivery devices. Various kinds of materials also had been used, such as calcium phosphate [25], calcium sulfate (plaster of Paris) [20, 23, 24], chitosan [1, 2], polyethylmethacrylate/n-butyl methacrylate [22] and hydroxyapatite ceramic [28]. In addition, various antibiotics had been employed to prepare antibiotic beads, such as gentamicin, tobramycin, cephalosporin, polymyxin and vancomycin [2, 9, 19, 25, 27, 28]. However, the numbers of antibiotics that used to mix with PMMA are limited since PMMA releases heat during polymerization. Besides PMMA, calcium sulfate is a biodegradable material that could elute high antibiotic levels [17, 24]. Since calcium sulfate does not release heat during setting, various antibiotics could be used to prepare the beads without losing their antibacterial efficacy.

This research studied the *in vitro* releasing characteristics of cefazolin from PMMA and calcium sulfate beads which used commercial cefazolin in the form of injecting powder. The efficacy of cefazolin beads was determined by the cefazolin elution that was greater than MIC for *Staphylococcus aureus.* In addition, efficacy of cefazolin-beads after various storage conditions for 6 months was studied.

MATERIALS AND METHODS

Preparation of cefazolin impregnated beads: Beads were prepared from Cefazillin™ (1 g cefazolin/vial, Injecting cefazolin, T.P. Drug Laboratories, Thailand) and matrices; 3 commercial brands of PMMA (SmartSet HV, Johnson & Johnson; Depuy I, Johnson & Johnson; and Cemfix 3, Teknimed S.A.) and calcium sulfate (Sigma). PMMA beads were formed by mixing 1 vial of Cefazillin™ with 10 g of PMMA and then adding 3.5 m*l* of monomer. For calcium sulfate beads, 1 vial of Cefazillin and 10 g of calcium sulfate were mixed thoroughly and then added 4.5 m*l* of 0.1 M phosphate buffer (pH 7.4). The PMMA and calcium sulfate mixtures were poured into mold. After beads were set, they were packed and then sterilized with ethylene oxide.

Antibacterial activities of cefazolin beads were determined by a modified Kirby-Bauer assay [13]. The cefazolin-PMMA and calcium sulfate beads were placed onto Mueller-Hinton agar seeded with *Bacillus subtilis* (ATCC 6633) at 37°C for 18 hr. The antibacterial properties presented with inhibitory clear zone around beads.

In vitro drug elution studies for 15 days: An elution

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method was employed to determine the elution characteristics of cefazolin from the cefazolin beads. A phosphate buffer (0.1 M PB, pH 7.4) was used as the dissolution medium. Each of the cefazolin beads (total n=5) was incubated in 1.5 m*l* of PB at 37°C for 24 hr. The dissolution PB was collected and 1.5 m*l* of fresh PB was added every 24 hr for 15 days of experimental period. All dissolution medium were kept at –20°C with light protection until analysis.

Storage cefazolin beads: Beads were kept at 4 various conditions: 1) non-light protection (clear plastic bags) at 4°C, 2) light protection (clear plastic bags wrapped with aluminum foil) at 4°C, 3) non-light protection at room temperature (25°C) and 4) light protection at room temperature. After 6 month-storage, the cefazolin elution from each group of cefazolin-beads was studied as described in *in vitro* drug elution studies. This cefazolin elution study was done for only 5 days.

Determination of cefazolin concentrations: The eluted cefazolin concentrations were characterized by agar-well diffusion microbiological assay using spores of *B. subtilis* (ATCC 6633) as an indicator organism [6]. Mueller-Hilton agar (MHA) was molten and inoculated with *B. subtilis* spore at the concentration of 0.5×10^5 cfu/m*l* of agar. The 20 m*l* of seeded MHA agar were poured into 90 mm petri dish. After cooling, 8-mm wells were cut into the solidified MHA. Standard cefazolin (Sigma) were diluted with 0.1 M phosphate buffer, pH 6.0, at concentrations of 2, 4, 6, 8 and 10 μg/m*l*. The average diameters of clear zones of 2, 4, 6, 8 and 10 μg/m*l* standard cefazolin were 12.9, 18.9, 19.6, 22.6 and 24.9 mm, respectively. Each eluted sample was performed in triplicate. The concentration of eluted antibiotic was determined by extrapolation from the standard curve. The lowest detectable concentration of cefazolin was 2.0 μg/m*l*.

Statistical Analysis: Results were expressed as mean± standard error of the mean. The statistics were calculated using SPSS. ANOVA was used for group comparison and P values less than 0.05 were considered significantly.

RESULTS

Bead preparation: Cefazolin sulfate beads were formed in the cylindrical shape and measured 6.28 ± 0.14 mm in diameter and 4.26 ± 0.08 mm in height. Weights of cefazolin-PMMA and calcium sulfate beads were 132.34 ± 2.67 mg for cefazolin-SmartSet HV, 122.84 ± 5.32 mg for cefazolin-Depuy I, 136.18 ± 2.32 mg for cefazolin-Cemfix 3 and 186.08 ± 6.87 mg for cefazolin-calcium sulfate beads.

Both cefazolin-impregnated PMMA beads and cefazolin-calcium sulfate beads had antibacterial activities which were determined by inhibitory zones around beads (Fig. 1), while control PMMA beads and calcium sulfate beads (no cefazolin) showed no antibacterial properties.

In vitro drug elution studies for 15 days: Cefazolin-PMMA beads and -calcium sulfate beads eluted cefazolin continuously during the 15-days experimental period (Fig. 2). The concentrations of eluted cefazolin were higher than

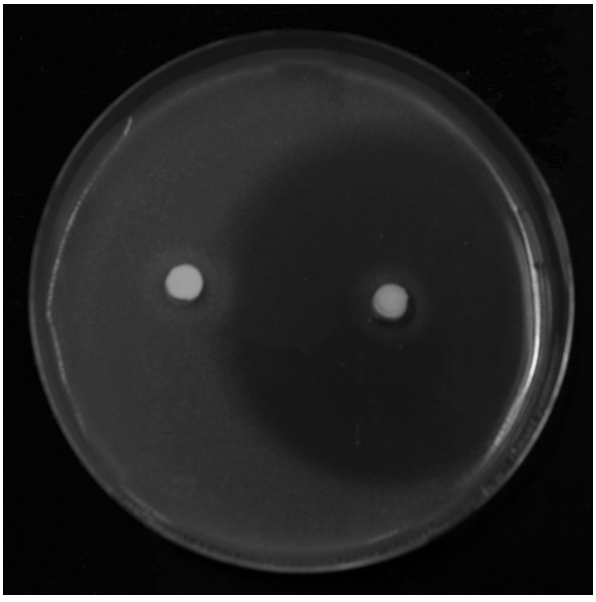

Fig. 1. Antibacterial activity of cefazolin-calcium sulfate beads on Mueller Hinton agar seeded with *Bacillus subtilis* (ATCC 6633). *Left*: Control calcium sulfate bead (no cefazolin). *Right*: Cefazolin-calcium sulfate bead showed inhibitory zone around bead.

MIC for *S. aureus* (ATCC 25923) which MIC was 0.125 μg/m*l* using broth microdilution method. The maximum levels of eluted cefazolin were on the first day and their levels rapidly declined thereafter.

The eluted cefazolin from calcium sulfate beads were significantly higher than from the other 3 PMMA beads on Day 1–11 ($P<0.05$). The eluted cefazolin from calcium sulfate beads decreased rapidly after 10 days. The eluted cefazolin from 3 matrices of PMMA beads did not differ significantly ($P<0.05$). In addition, the eluted cefazolin for calcium sulfate beads was significantly lower than from PMMA beads on day 14 and 15, but still greater than MIC throughout the study.

Total eluted cefazolin from calcium sulfate beads (95.94 ± 3.93%) were greater than PMMA beads in the 15 days experimental period (Table 1). For PMMA bead samples, the total eluted cefazolin from 3 different PMMA formulations; SmartSet HV, Depuy I and Cemfix 3; were 38.67 ± 3.04, 36.63 ± 10.37 and 34.41 ± 3.93%, respectively.

Storage antibiotic beads: Cefazolin-PMMA beads and cefazolin-calcium sulfate beads had still antibacterial activity even after 6 month storage in different conditions, and the eluted cefazolin concentrations were greater than MIC for *S. aureus* (ATCC 25923). The results also showed calcium sulfate eluted cefazolin greater than other 3 PMMA beads for 5 days of study period (Fig. 3). The various storage conditions had no effects significantly on cefazolin elution from SmartSet HV, Depuy I and Cemfix 3 beads. The PMMA beads and calcium sulfate beads stored at 4°C without light protection could elute cefazolin lower than the

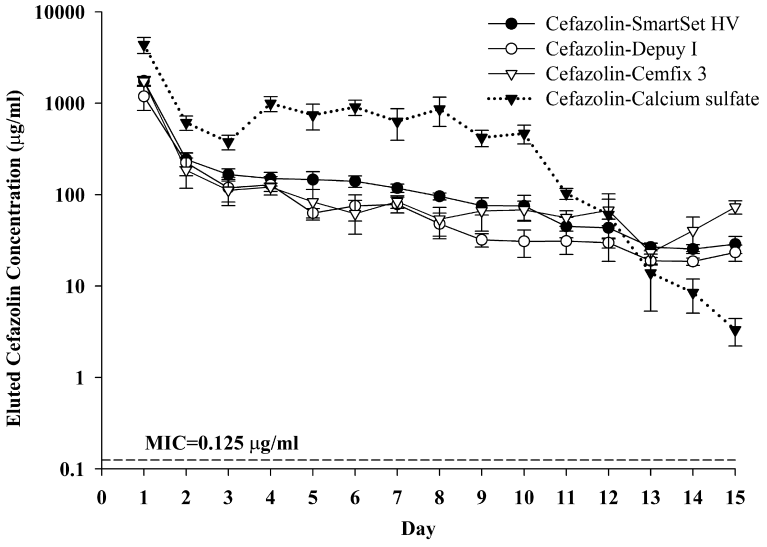

Fig. 2. Daily concentrations of eluted cefazolin (μg/m*l*) from PMMA (SmartSet HV, Depuy I and Cemfix 3) and calcium sulfate beads during 15 days of experiment period. The MIC for *Staphylococcus aureus* (ATCC 25928) was 0.125 μg/m*l* which is shown in a dash line (·····).

Table 1. Total and percentage of eluted cefazolin from 4 types of PMMA and calcium sulfate beads during 15 days of experiment period

Material	Cefazolin-SmartSet HV	Cefazolin-Depuy I	Cefazolin-Cemfix 3	Cefazolin-Calcium sulfate
Total eluted cefazolin (mg)	4.65 ± 0.36	3.60 ± 1.16	4.26 ± 0.49	15.38 ± 1.72*
% eluted cefazolin	38.67 ± 3.04	36.63 ± 10.37	34.41 ± 3.93	95.94 ± 3.93*

Note: * Means significant difference at the 0.05 level.

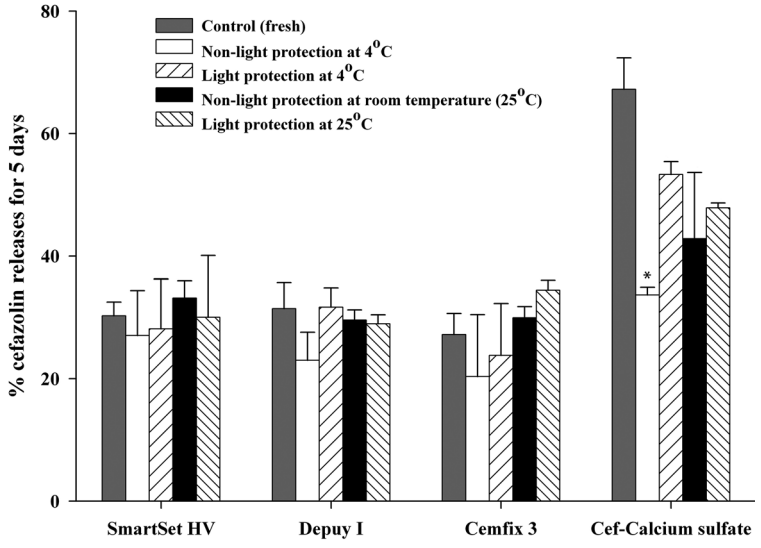

Fig. 3. Percentage of cefazolin eluted from PMMA (SmartSet HV, Depuy I and Cemfix 3) and calcium sulfate beads for 5 days after 6-month storage at various conditions:

A: Control fresh prepared beads, B: Non-light protection at 4°C, C: Light protection at 4°C, D: Non-light protection at room temperature, 25°C, E: Light protection at 25°C.

Note: * means significant difference at the 0.05 level.

other storage samples. For calcium sulfate beads, fresh prepared beads eluted cefazolin greater than stored beads. In addition, calcium sulfate beads stored at 4°C without light protection released cefazolin significantly lower than fresh prepared and stored beads in other conditions ($P<0.05$) (Fig. 3).

DISCUSSION

Cefazolin is the 1st generation of cephalosporin which normally used to treat bacterial infection successfully and cefazolin injecting powder is an available bactericidal agent in clinic. The preparation of cefazolin beads is not complicated. We used 3.5 m*l* monomer or 4.5 m*l* phosphate buffer mixed with PMMA or calcium sulfate, respectively, which could make the mixture in the suitable consistency before pouring into the molds. From the study, PMMA beads and calcium sulfate beads could elute cefazolin continuously for 15 days and eluted cefazolin levels were higher than MIC for *S. aureus* (ATCC 25923). The characteristic of cefazolin elution is biphasic with great eluted antibiotic at the beginning period and rapid diminishing thereafter. Philips *et al.* showed that cefazolin-PMMA beads eluted antibiotic continuously for 30 days and cefazolin levels were greater than MIC for common bacteria of dog and cat wounds [19]. These authors added more cefazolin than our study, in which mixed 1 g cefazolin with 6 g PMMA (Surgical Simplex P Radiopaque Bone Cement), while we used 1 g cefazolin with 10 g PMMA.

The mechanism of antibiotic elution was hypothesized that antibiotics in the matrix could be dissolved in the tissue fluid and eluted via pores and cracks within bead matrices [5, 7]. According to our elution test, we found that calcium sulfate beads eluted more cefazolin than PMMA beads. It might be explained that calcium sulfate beads had more porosity than PMMA beads [7, 11]. Calcium sulfate is a biodegradable material that is degraded in the tissues so antibiotic-calcium sulfate beads could be placed in the tissue for prolonged antibiotic elution without secondary surgery for bead removal [8, 14, 16]. Since calcium sulfate does not release heat during bead setting process, it becomes an advantageous candidate for preparing heat sensitive antibiotic beads.

Our study showed there was little difference of total cefazolin elution among 3 types of PMMA beads. It may be related with composition percentages of PMMA in those 3 manufacturing products which are SmartSet HV, Depuy I and Cemfix 3 that contain 84.0, 88.9 and 87.6% of PMMA, respectively. The lower amount of PMMA in cefazolin-SmartSet HV beads was related with higher eluted cefazolin. Nevertheless, eluted antibiotic is influenced with various factors, such as antibiotics quantity, surface area, permeability and molecular weight of materials [12, 26]. Wang *et al.* showed that beads prepared with Poly (DL-lactide): co-glycolide (PLA/PGA) and cefazolin could elute antibiotic continuously which was higher than MIC for *S. aureus* (ATCC 25923) for 4 weeks [27]. Then, it is possible that cefazolin beads in our study could elute antibiotic longer than 15 days. Nevertheless, the cefazolin-calcium sulfate beads and cefazolin-PMMA beads may have no effect on resistant Enterobacteriaceae which MIC is greater than 32 μg/m*l* [4]. Then, we need more studies to develop various antibiotic beads which are more effective on resistant bacteria.

Storage condition at room temperature (25°C) and 4°C with or without light protection had no changes in cefazolin elution from SmartSet HV and Depuy I beads after 6 months storage. In addition, there was significant difference of cefazolin releasing from fresh and 6 months storage-PMMA beads. Calcium sulfate beads decreased cefazolin elution after 6 month storage and it showed significantly reduction of cefazolin elution when calcium sulfate beads stored at 4°C without light protection.

In our study, cefazolin-impregnated calcium sulfate and cefazolin-PMMA beads could be used as the slow-release antibiotic materials for the treatment of bacterial infection, especially abscess, open wound and osteomyelitis. The cefazolin-calcium sulfate and PMMA beads can be made easily and could be stored for 6 months; at room temperature and 4°C, with or without light-protection, with no deleterious effect on elution characteristics. For the future, various antibiotic beads should be developed for treatment of resistant bacteria which now becomes a serious problem in clinical practice.

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