

In Vitro Elution Characteristics of Antibiotic Cement on MRSA Organism

Boonsin Buranapanitkit, M.D.* , Suntorn Wongsiri, M.D.* ,
Naticha Ingvaya, B.Sc.** , Alan Geater, Ph.D.† , Apiradee Lim, B.Sc.† ,
Sineenart Kalnouwakul, M.D.** , Kanyika Chamniprasas, M.D.*

*Department of Orthopaedic Surgery, **Microbiology Unit, Department of Pathology, †Epidemiology Unit,
Faculty of Medicine, Prince of Songkla University, Thailand

ABSTRACT

Antibiotic cements have been approved for their efficacy and effectiveness for treatment of orthopaedic infections, especially chronic osteomyelitis, which is caused mostly by gram positive bacteria such as staphylococcus aureus. However, the incidence of MRSA (methicillin-resistant staphylococcus aureas) infection has been increasing recently. This study aimed to compare in vitro elution characteristic of homemade antibiotic cement (fosfomycin, vancomycin and cefalexin) with commercial bead (Septopal) on MRSA organism by disc diffusion technique at various timepoints. The results showed that Septopal and cefalexin cannot inhibit growth of this organism, while fosfomycin and vancomycin had this effect after or for about one month. The inhibition effect was significantly higher in fosfomycin than in vancomycin during the first two weeks. The variability of vancomycin beads was more pronounced than that of fosfomycin beads. In conclusion, homemade fosfomycin and vancomycin cement can effectively inhibit growth of MRSA organisms in the in vitro study.

INTRODUCTION

Chronic osteomyelitis is a disabling disease. The etiology can involve the hematogenous route or trauma. The most common organism isolated from infected bone is staphylococcus aureus^{1,2,3}. However, bone infections from which MRSA (methicillin-resistant staphylococcus aureas) is isolated are now being reported increasingly^{3,4,5}. MRSA which is a common nosocomial infection can cause septic arthritis, epidural abscess, septicemia and osteomyelitis^{6,7,8,9}. The conventional treatment for MRSA osteomyelitis are prolonged intravenous vancomycin and surgical treatment^{3,9,10,11}. Prolonged systemic vancomycin can cause nephrotoxicity and ototoxicity^{3,11}. Klemm¹² found several distinct advantages of local antibiotic cement such as decreased systemic side effects compared to systemic antibiotics, lower costs, reduced hospitalization and prevention of infection recurrence. Currently, there is only one commercial local antibiotic cement available on the

market, Septopal, which contains gentamicin. Septopal is claimed to have efficacy against both gram positive and gram negative infections because of its high local concentration². However, no studies have yet proven its effect on MRSA organism. Other antibiotic cements such as fosfomycin and cefalexin may have an inhibitory effect on this organism, but to our knowledge, no previous studies have compared their effects on MRSA organism.

The objective of this study was to compare the in vitro inhibition effect of homemade fosfomycin, vancomycin and cefalexin cement with that of Septopal on MRSA organism.

MATERIALS AND METHODS

For this study, powdered vancomycin was obtained from Eli Lilly (Indianapolis, Indiana), fosfomycin was obtained from Thai Meiji (Bangkok, Thailand) and cefalexin was supplied from Glaxo Wellcome (Bangkok, Thailand). The cement used for preparation was Palacos-R (Merck, Darmstadt). Antibiotic cements were made as follows: Using the sterile technique, 4 grams of each powdered antibiotic were thoroughly admixed with methylmethacrylate-methacrylate copolymer powder. Methylmethacrylate monomer in N, N-dimethyl p-toluidine was added with thorough mixing, then liquid cement was poured into two halves of a silastic cement block to produce spherical beads. The diameter of each bead was 6.5 mm. The beads of Septopal were cut from the bead chain. We used 10 beads for each antibiotic cement sample.

A clinical isolate of MRSA was selected for study. It was susceptible to vancomycin and fosfomycin, but not to gentamicin or cefalexin.

The method used to assay the elution of antibiotic from cement beads was a modification of the standard disk diffusion technique. An overnight culture of bacteria was isolated and diluted in phosphate buffered saline (pH 7.4) yielding about 2×10^7 colony-forming units per milliliter. Aliquots (0.1 ml) were pipetted onto blood agar and spread over the surface with a bent glass rod. A cement bead was placed over the surface and gently tapped down under sterile technique, and the plates were then incubated at 37°C for 24 hours. After incubation, the inhibition zone (the least diameter of clear zone around the bead) was measured using a vernier caliper, and the beads were transferred to a new plate that had been prepared by the same technique. Transfer to new plates was repeated every 24 hours until no inhibition zone appeared within 24 hours or until completion of the experiment after 28 days. The Palacos cement beads that did not contain antibiotic showed no inhibition zone.

Correspondence should be sent to:
Dr Boonsin Buranapanitkit
Dept of Orthopaedic Surgery
Prince of Songkla University
Haadyai, Songkla
Thailand 90110

Statistical analysis

Analysis was undertaken on the inhibition zone excess diameter which was defined as the total inhibition zone minimum diameter minus the diameter of the cement bead. Linear regression was used to compare mean excess diameters at various time points. Difference in excess diameter was considered statistically significant if $p < .05$.

RESULTS

Fosfomycin and vancomycin cements exhibited antimicrobial effect throughout the 28 days of experiment. Septopal retained its inhibition effect for less than 3 days, while cefalexin cement showed no inhibitory effect on MRSA (Figure 1). The antimicrobial effect was higher in fosfomycin than in vancomycin ($p < .05$) throughout the first week and both were more effective than Septopal (Table 1).

Table 1. Comparisons of inhibition effect (inhibition zone excess diameter) of antibiotic cements at various time points. Value having a different subscript within the same row are significantly different ($p < .05$)

Day	Antibiotic			
	Inhibition zone excess diameter(mm) Means (95% CI)			
	Fosfomycin	Vancomycin	Septopal	Cefalexin
1	a 17.8 (16.9, 18.7)	b 11.1 (10.2, 12.1)	c 5.2 (4.0, 6.3)	d 0
3	a 12.6 (11.4, 13.9)	b 9.8 (8.6, 11.1)	c 0	c 0
7	a 10.1 (8.8, 11.4)	b 7.4 (6.1, 8.7)	c 0	c 0
14	a 8.6 (6.2, 10.9)	a 6.7, (4.3, 9.1)	-	-
21	a 6.7, (4.7, 8.7)	b 3.8 (1.8, 5.8)	-	-
28	a 3.1 (1.2, 5.0)	a 2.8 (0.9, 4.7)	-	-

The antimicrobial effects of fosfomycin and vancomycin groups declined steadily but rather more rapidly in fosfomycin group than in vancomycin group. By 28 days, the inhibition zone excess diameter has decreased to between 15 and 25 percent of the day 1 value (Figure 1).

Variability among the 10 replicates in each treatment was somewhat greater in the vancomycin group than in the fosfomycin group (Figure 1).

DISCUSSION

This in vitro study showed that neither Septopal, which is the only commercial available antibiotic cement, nor cefalexin in a cement bead can inhibit growth of MRSA organism despite their high local concentrations, while both fosfomycin and vancomycin exhibit a strong inhibition, especially the first two weeks of the experiment with fosfomycin slightly superior to vancomycin. The inhibition effect declined gradually with little effect remaining at one month.

Fosfomycin is a broad spectrum antibiotic against both gram positive and gram negative organisms. It is water soluble and heat resistant. Ono et al¹³ undertook an experimental and clinical study of fosfomycin-impregnated bone cement and showed its dissolution was better than that of gentamicin and cefsolodin, but MRSA organism was not used in that study.

Vancomycin has an excellent effect against staphylococcus aureus, MRSA and enterococci, and is the antibiotic of choice for MRSA^{3,14,15}. Vancomycin-impregnated cement was found to elute from bone cement in Keuchle's study¹⁹. Ozaki¹⁶ reported 2 clinical cases of vancomycin-impregnated cement for MRSA infections with successful result. In this study, we found that the variability of homemade vancomycin beads was more pronounced than that of fosfomycin beads. This might be a result of the preparation technique because the vancomycin powder was not as homogeneous as fosfomycin or cefalexin.

Septopal, which contains gentamicin, has bacteriocidal effects against most gram negative organisms and also gram positive cocci such as staphylococcus aureus because of its high local concentration above the MIC level^{12,17,18}. However, the results of this experiment indicate that Septopal cannot be used in the case of MRSA infection. A similar conclusion is true regarding cefalexin.

Palacos was used in this experiment because it can release more antibiotic than other kinds of cement¹⁹ and can be compared with Septopal, which also contained palacos cement as a carrier system.

The disc diffusion technique that was used in this experiment is the standard method for susceptibility testing of causative organisms. Although it is a semiquantitative method, the inhibition effect has been reported to correlate directly with the local concentration of antibiotic^{19,20}.

A limitation of the study is that in vitro study condition cannot totally simulate the clinical setting, because there are some factors that can affect the result such as pH, temperature, and blood flow. The effects of these factors need further investigation.

In conclusion, we have demonstrated that homemade fosfomycin and vancomycin cement can inhibit MRSA, while Septopal and cefalexin do not have this effect in vitro study. The effect of fosfomycin is stronger than that of vancomycin.

ACKNOWLEDGEMENT

The authors would like to thank Jongdee Somthai for her secretarial work. This project was supported by grants from the Faculty of Medicine, Prince of Songkla University, Thailand.

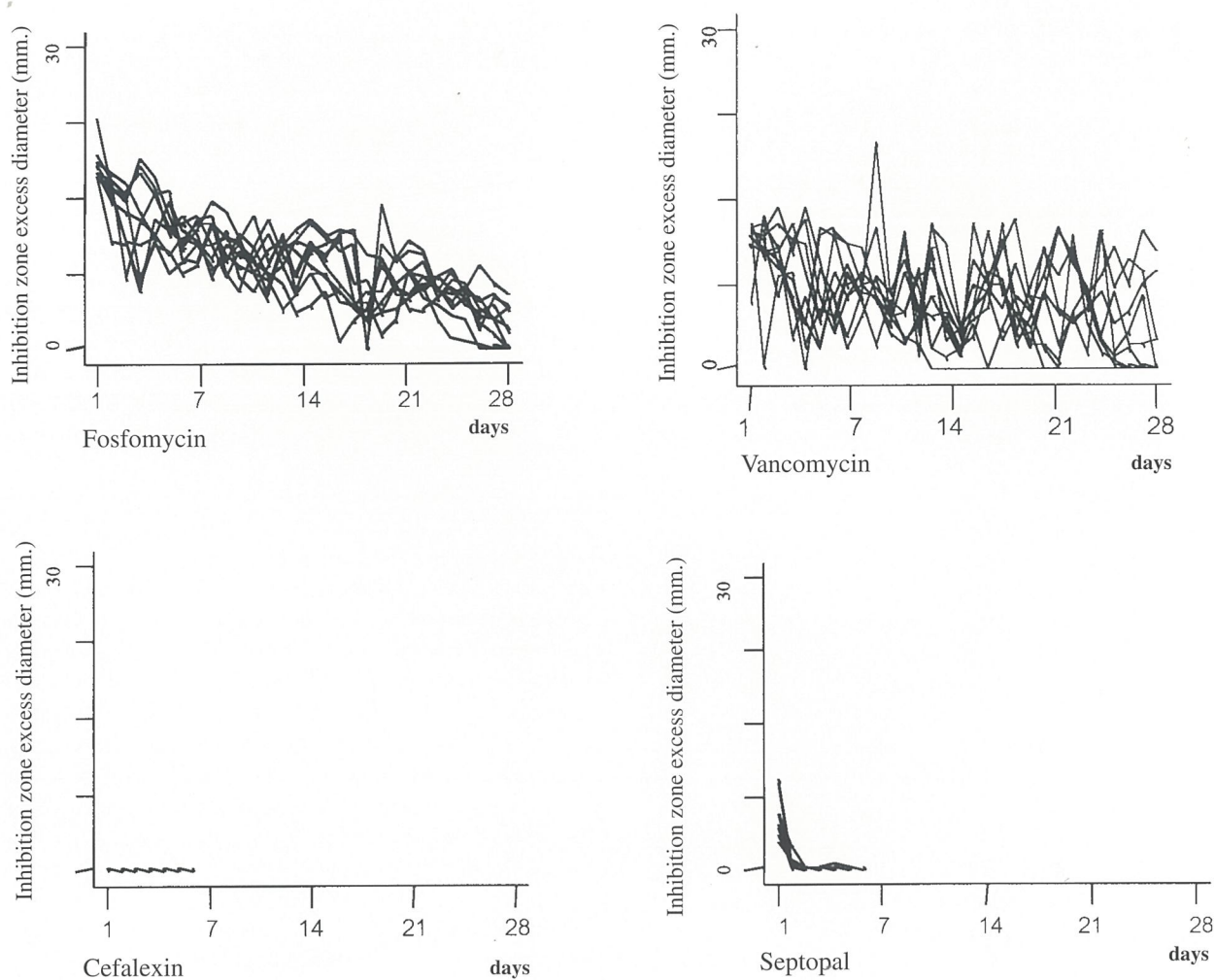


Fig. 1 Inhibition zone excess diameter in each replicate of each antibiotic cement on each day of the experiment.

REFERENCES

1. Cole WG. The management of chronic osteomyelitis. *Clin Orthop* 1991; 264: 84-89.
2. Blaha JD, Nelson CL, Frevert LF, et al. The use of Septopal (polymethylmethacrylate beads with gentamicin) in the treatment of chronic osteomyelitis. *Instruct Course Lect. AAOS* 1990; 509-514.
3. Mader JT, Shirtliff ME, Bergquist SC, Calhoun J. Antimicrobial treatment of chronic osteomyelitis. *Clin Orthop* 1999; 360: 47-65.
4. Musher DM. The gram-positive cocci : III, Resistance to antibiotics. *Hosp Pract* 1988; 23: 105.
5. Barrett FF, McGehee ER Jr, Findland M. Methicillin-resistant staphylococcus aureus at Boston city hospital: Bacteriologic and epidemiologic observation. *N Engl J Med* 1965; 279: 475.
6. Ho NK, Low YP, See HF. Septic arthritis in the new born, a 17 years clinical experience. *Singapore Med J* 1989; 39(4): 356-8.
7. Sugimoto M, Tamaki M, Nagashima T, et al. Cervical spinal epidural abscess caused by methicillin-resistant staphylococcus aureus (MRSA). *No shinkei Geka* 1994; 22(10): 973-6.
8. Wong H, Isaacs D, Howman GR, Uren R. Clinical and diagnostic features of osteomyelitis occurring in the first three months of life. *Pediatr Infect Dis J* 1995; 14(12): 1047-53.
9. Thomas GS, Mader JT, Pennick JJ, Cierny GIII. Methicillin-resistant staphylococcus aureus osteomyelitis. *Clin Orthop* 1985; 198: 231-239.
10. Watanakuakorn C. Treatment of infections due to Methicillin-resistant staphylococcus aureus. *Ann Intern Med* 1982; 97: 306.
11. Mader JT, Landon GS, Calhoun J. Antimicrobial treatment of osteomyelitis. *Clin Orthop* 1993; 295: 87-95.
12. Klemm KW. Antibiotic bead chain. *Clin Orthop* 1993; 295: 63-76.
13. Ono H, Yamaguchi T, Nakada K, et al. Experimental and clinical studies on fosfomycin-impregnated bone cement. *Clin Orthop Surg* 1988; 22 (9): 1073.
14. Sorrell TC, Packham DR, Shanker S, et al. Vancomycin therapy for methicillin-resistant staphylococcus aureus. *Ann Intern Med* 1982; 97: 344.
15. Peacock JE Jr, Moorman PR, Wenzel RP, Mandell GL. Methicillin-resistant staphylococcus aureus. Microbiologic characteristic, antimicrobial susceptibilities and assessment or virulence of an epidemic strain. *J Infect Dis* 1979; 139: 273.
16. Ozaki T, Yoshitaka T, Kunisada T, et al. Vancomycin-impregnated polymethylmethacrylate beads for methicillin-resistant staphylococcus aureus (MRSA) infection: report of two cases. *J Orthop Sci* 1998; 3 (3): 163-8.
17. Patizakis MJ, Mazur K, Wilkins J, et al. Septopal beads and autogenous bone grafting for bone defects in patients with chronic osteomyelitis. *Clin Orthop* 1993; 295: 112-118.
18. Bucholz HW, Elson RA, Heinert K. Antibiotic-loaded acrylic cement : Current concepts. *Clin Orthop* 1984; 190: 96.
19. Keuchle DK, Landon GC, Musher DM, et al. Elution of vancomycin, daptomycin and amikacin from acrylic bone cement. *Clin Orthop* 1991; 264: 302-310.
20. Adams K, Couch L, Cierny G, et al. In vitro and in vivo evaluation of antibiotic diffusion from antibiotic-impregnated polymethylmethacrylate beads. *Clin Orthop* 1992; 278: 244-252.