

In Vitro Elution and Antibacterial Activity of Clindamycin, Amikacin, and Vancomycin from R-gel Polymer

Leslie A. Thomas¹, DVM, Tatiana Bizikova², MSc, and Anne C. Minihan¹, DVM, Diplomate ACVS

¹Chesapeake Veterinary Surgical Specialists, Annapolis, MD and ²Royer Animal Health, LLC, Frederick, MD

Corresponding Author

Dr. Leslie Thomas, DVM, 808 Bestgate Rd,
Annapolis, MD 21401
E-mail: gatorvet05@gmail.com

Submitted February 2010

Accepted October 2010

DOI:10.1111/j.1532-950X.2011.00861.x

Objective: To characterize the in vitro elution and bioactivity of 2 formulations of antibiotics in a novel, dissolvable, cross-linked dextran polymer matrix: Formulation 1—amikacin and clindamycin (AC); Formulation 2—amikacin, clindamycin, and vancomycin (ACV).

Study Design: Prospective, in vitro, experimental study.

Methods: Aliquots of the antibiotic impregnated polymer were incubated in PBS buffer for 10 days. PBS was changed every 24 hours and concentrations of the antibiotics eluted into saline were quantified. Antimicrobial activity of the eluent from each sampling period was tested for growth inhibition of *Staphylococcus aureus*.

Results: Both formulations of R-gelTM had a rapid initial release of antibiotics within the first 24 hours and then the concentrations decreased gradually over 10 days. The concentration of amikacin, clindamycin, and vancomycin remained above the breakpoint minimum inhibitory concentration of each drug for a minimum of 9 days. No significant difference ($P = .9938$, $P = .9843$) was present in the elution pattern or total amount of antibiotic eluted from clindamycin or amikacin, respectively. Eluent from both groups demonstrated bioactivity against *S. aureus* for the entire 10-day study period.

Conclusions: Amikacin and clindamycin together, or in combination with vancomycin, elute from R-gelTM effectively and at gradually decreasing concentrations for at least 10 days. The antibiotics maintained their bioactivity following polymerization and elution from the R-gelTM.

The traditional approach to treating bacterial infections is administration of systemic antibiotic therapy based on culture and susceptibility results, preferably taking into account minimum inhibitory concentration (MIC) values. However, standard culture and susceptibility methods may not appropriately estimate the antimicrobial concentrations needed for deep, localized infections especially with bacterial adherence to metallic implants.^{1,2} Local conditions such as biofilm production and bacterial adherence to metallic implants may lead to underestimation of the in vivo MIC values necessary for clinical resolution of infection.² Therefore local therapy is a useful adjunct or alternative to systemic antibiotic therapy as high local concentrations of antibiotic may be obtained with minimal risk of systemic toxicity.^{3,4}

Royer Animal Health provided the materials and laboratory equipment for the study. Sample analysis performed by the laboratory at the Department of Molecular Biomedical Sciences, North Carolina State University College of Veterinary Medicine.

Presented in part at VOS conference in Breckenridge, CO, February 2010.

Multiple methods have been devised to deliver antimicrobials directly to the source of the infection and previous studies have shown that using antibiotic impregnated implants can achieve antimicrobial tissue concentrations as much as 20 times the therapeutic level obtained in serum after systemic administration.^{5,6} The most familiar vehicle for local deposition is antibiotic impregnated polymethylmethacrylate cement (AIPMMA). AIPMMA is capable of significantly increasing local concentrations of antibiotics; however, its nonresorbable nature typically requires additional surgery for removal to avoid a foreign body reaction or bacterial colonization.^{7,8} Additionally, several human studies have demonstrated that polymethylmethacrylate (PMMA) may induce inflammatory and cellular immune responses and apoptosis of osteoblastic cells in vitro.⁹⁻¹¹ Whereas other forms of bioabsorbable polymers such as polylactide-glycolide, hydroxyapatite, collagen, and D-, L-lactic acid oligomer have been studied, lack of commercial availability and difficulty in preparation has limited clinical use.¹²⁻¹⁴ Research is needed to develop the ideal vehicle with the following characteristics: ability to be sterilized, stability in storage, biodegradable, biocompatible, and a consistent elution profile.

The cross-linked dextran polymer matrix (R-gelTM) developed and manufactured by Royer Animal Health

LLC is currently being used as a matrix to deliver amikacin and clindamycin in both large and small animal medicine. The elution properties and characteristics of this delivery system must be defined to justify or refute this clinical practice. The gelation process of the matrix occurs over a 2-minute period after the addition of a dihydrazide cross-linking reagent to solution of oxidized dextran. The chemical reaction involved is the addition of the hydrazide nitrogen to an aldehyde carbon. This adduct dehydrates to form a hydrazone. The residence time of the gel in vivo is 4–5 weeks with degradation via hydrolysis. The R-gelTM vehicle has many clinical benefits including injectability allowing the gel to conform to the specific wound contour, lack of migration following gelation, fully resorbable delivery matrix, ease of clinical application, and lack of susceptibility to proteolytic degradation.

The R-gelTM product currently available contains a cumulative dose of 50 mg clindamycin HCl and 100 mg amikacin sulfate. Antibiotic combinations may demonstrate antagonism, additivity, or synergy when coeluting from a carrier vehicle. Addition of multiple antimicrobials to a polymer may affect the release kinetics, and therefore each combination of antibiotics must be tested individually to determine elution properties. A recent study of elution of a combination of amikacin and cefazolin from AIPMMA showed that combining these antibiotics resulted in a significantly shorter duration of elution than occurred with incorporation of the antibiotics separately.¹⁵ A separate study evaluating incorporation of liquid versus powder forms of amikacin within PMMA and hydroxyapatite cement (HAC) found that the powdered form demonstrated greater elution from PMMA.¹⁶ Therefore it is prudent to study the elution characteristic of each antibiotic combination and formulation in new drug delivery systems. The R-gel polymer matrix is commercially available in a pre-packaged, sterilized, kit as an animal device. Because of the classification as a device and not a drug, it has not undergone the rigorous kinetics testing for FDA drug approval.

Our first objective was to determine the elution properties of 2 formulations of antibiotics in a novel cross-linked dextran polymer matrix. Our second objective was to assess the in vitro bioactivity of the eluted antimicrobials against *Staphylococcus aureus* after entrapment and rerelease from the R-gelTM.

MATERIALS AND METHODS

The commercially available form of R-gelTM is supplied in a 2-syringe kit (<http://www.royerah.com> Royer Animal Health LLC, Frederick, MD). The first syringe contains 2 mL oxidized dextran solution and the second contains a cross-linking reagent plus 50 mg clindamycin HCl (Chemwerth Inc., Woodbridge, CT) and 100 mg amikacin sulfate (Chemwerth Inc.) in powder form. The 2 syringes are connected and the contents are mixed via reciprocation for 15 cycles until the powder is completely dissolved within the gel for a total volume of 2.1 mL. For the purpose of this

study a third syringe containing 50 mg vancomycin HCl (Tecoland Corporation, Edison, NJ) was added and mixed with the oxidized dextran solution via 15 reciprocation cycles before the addition of the syringe containing clindamycin, amikacin, and the cross-linking reagent. Therefore 2 sample groups were created. Group 1 contained amikacin and clindamycin (AC) and group 2 contained amikacin, clindamycin, and vancomycin (ACV).

The R-gelTM was prepared as described previously and four aliquots of 0.3 mL were pipetted into individual sterile 1.7 mL plastic tubes (VWR International, West Chester, PA) during the liquid phase to allow accuracy of measurement. Once the gelation process was complete the gel was covered with 1 mL sterile phosphate-buffered saline (PBS) with a pH of 7.4 (Spectrum Chemicals, New Brunswick, NJ) and the tubes were sealed with a plastic cap. The total amount of amikacin, clindamycin, and vancomycin contained within each aliquot was 150, 75, and 75 mg, respectively. The tubes were incubated in a 37°C water bath without agitation. The PBS was sampled every 24 hours for 10 days. The entire volume of PBS was removed at each sampling period and replaced with an additional 1 mL PBS. Samples were stored at 0–4°C until assayed. All samples were analyzed in triplicate by the clinical pharmacology laboratory at the Department of Molecular Biomedical Sciences, North Carolina State University College of Veterinary Medicine. The concentration of amikacin and vancomycin in the sample eluents was determined by the fluorescence polarization immunoassay (FPIA) (TDx, Abbott Laboratories, Abbott Park, IL). The sensitivity range of this assay was 0.8–50 µg/mL for amikacin and 2–100 µg/mL for vancomycin. The assay was validated by adding various known concentrations of amikacin or vancomycin to PBS, to generate a standard curve. The concentration in each sample was determined by a plot of polarization versus the standard curve. If sample concentrations were above the sensitivity test range, standard dilution methods were used to determine concentrations.

Clindamycin concentrations were determined by reverse phase high-pressure liquid chromatography (HPLC; Agilent Technologies, Wilmington, DE). Calibration and blank samples were prepared and the calibration curves generated were linear with a r^2 value of 0.99 or higher. Limit of quantification for each of the clindamycin samples was 0.05 µg/mL, which was determined from the lowest point on a linear calibration curve that produced an appropriate signal-to-noise ratio. The upper limit of quantification for the clindamycin assay was 20 µg/mL. The test samples in PBS were diluted appropriately so that the concentrations fell within the upper and lower limits of the calibration curve. Control samples were diluted in an identical manner to ensure test accuracy and the coefficient of variability was < 15%. The laboratory used guidelines and standards published by the United States Pharmacopeia.¹⁷

The remaining elution aliquot from each sampling period was used for bioactivity studies. To determine the antimicrobial activity of the eluent, the PBS from each

sampling period was tested for growth inhibition of *S. aureus*. Tryptic soy agar plates were prepared by mixing 20 g of agar (DIFCO, Sparks, MD) with 500 mL of sterile water and boiled for one minute. The mixture was autoclaved for 30 minutes at a temperature of 200°F. The agar was then poured into twenty-five 100 mm diameter polystyrene plates (VWR International) and stored in a laminar flow hood (model VBM/-/400; The Baker Company, Sanford, ME) with an ultra-violet light for 12 hours. After preparation, the plates were stored in a laboratory refrigerator at 0–4°C until use.

The *S. aureus* culture was prepared from a Lyfocults® disk (ATCC 29213) dissolved in 20 mL sterile PBS. The culture of the *S. aureus* was applied to the tryptic soy agar plates with a sterile loop under the hood and incubated for one hour. Samples consisting of 5 µL of the eluent (undiluted and diluted 1:10) collected daily were placed on 6 mm diameter blank paper filter disks (BBL; Becton Dickinson Microbiology Systems, Cockeysville, MD). The higher concentration of antibiotics within the samples from the first 7 days, necessitated dilution by 1:10 to allow more precise measurement of the zone of inhibition. There were 3 disks per plate. Plates were incubated at 37°C for 24 hours and then the zones of inhibition were measured with a Vernier caliper. Three measurements were obtained from each disk and the average zone of inhibition was used for statistical analysis. Disks of known concentration of amikacin (30 µg), clindamycin (10 µg), and vancomycin (30 µg) were measured as controls.

The minimum inhibitory concentration (MIC) of the *S. aureus* isolate used is 0.1–1, 1, and 1–2 µg/mL for clindamycin, amikacin, and vancomycin, respectively. In considering the effectiveness of an antibiotic in elution, the MIC is a concentration specific to the organism cultured and the drug of interest, whereas the breakpoint MIC is specific to the host and the drug. For this reason, the breakpoint MIC of a drug is generally the same for any organism and is more applicable to broad statements of effective levels. The MIC breakpoints for amikacin, clindamycin, and vancomycin are ≤ 16 , ≤ 0.5 , ≤ 2 µg/mL, respectively.¹⁵

Concentrations of antibiotics at each sampling period were analyzed using a 1-way analysis of variances (ANOVA) for repeated measures for all treatment groups. Differences were considered significant at a $P < .05$. Data reported as mean \pm SD.

RESULTS

Elution of Clindamycin and Amikacin

Both preparations of R-gel™ had a rapid initial release of clindamycin in the first 24 hours (3537 µg [AC], 3418 µg [ACV]). Clindamycin concentration in the eluent decreased dramatically during the 2nd day and continued to decrease gradually over 10 days (Fig 1). The mean cumulative elution of clindamycin after 10 days was 6521 µg (AC) and 6481 µg (ACV), which represented $86.4 \pm .01\%$ and $86.9 \pm .02\%$ of the total amount of clindamycin incorporated into the R-gel™, respectively. ANOVA showed no significant difference in clindamycin concentration of the eluent with or without coelution of vancomycin, $P = .9938$. The concentration of clindamycin remained above the breakpoint MIC of clindamycin (0.5 µg/mL) for the entire 10 days in both samples.

Both preparations of R-gel™ had a rapid initial release of amikacin in the first 24 hours: (6040 µg [AC], 5450 µg [ACV]). Amikacin concentration in the eluent decreased dramatically during the second day and continued to decrease gradually over the 10 days (Fig 2). The mean cumulative elution of amikacin after 10 days was 10,945 µg (AC) and 11,110 µg (ACV), which represented $73.0 \pm .007\%$ and $74.0 \pm .04\%$ of the total amount of amikacin incorporated into the R-gel, respectively. ANOVA showed no significant difference in amikacin concentration of the eluent with or without incorporation of vancomycin, $P = .9843$. The concentration of amikacin remained above the breakpoint MIC of 16 µg/mL for 9 days in both samples, but fell below the breakpoint MIC for amikacin by day 10. The $C_{\max}:\text{MIC}_{\text{breakpoint}}$ of amikacin for Formulation 1 and Formulation 2 were 377.5 and 340.6, respectively.

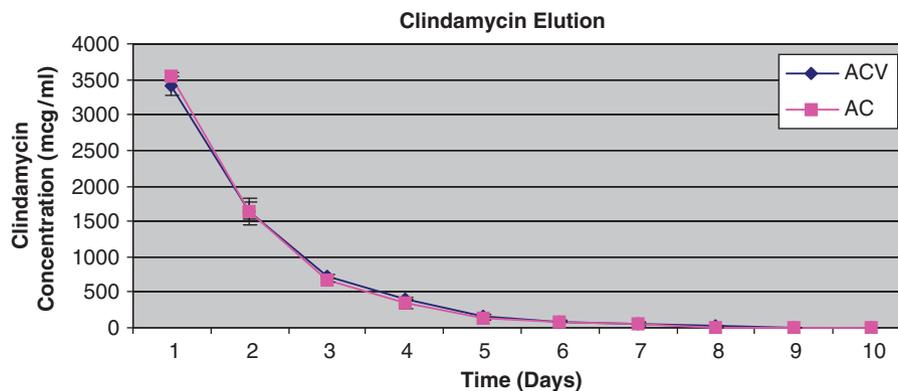


Figure 1 Clindamycin (C) elution concentration over time. Formulation 1 (AC) and Formulation 2 (ACV). There was no significant difference between the preparations. The concentration remained above the breakpoint minimum inhibitory concentration (MIC) for the entire study period. A, amikacin, V, vancomycin.

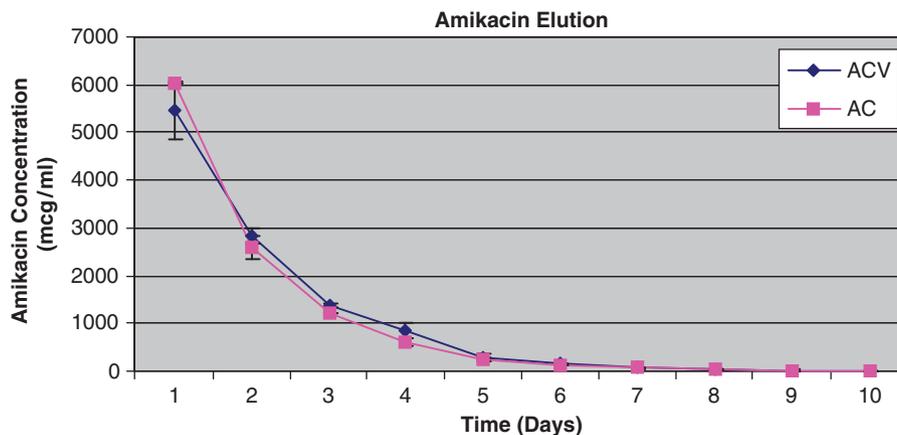


Figure 2 Amikacin (A) elution concentration over time. Formulation 1 (AC) and Formulation 2 (ACV). There was no significant difference between the preparations. C, clindamycin; V, vancomycin.

Elution of Vancomycin

The ACV preparation had a rapid initial release of vancomycin in the first 24 hours (2462 µg). Vancomycin concentration in the eluent decreased dramatically during the 2nd day and continued to decrease gradually over the 10-day study period (Fig 3). The mean cumulative elution of vancomycin after 10 days was 6336 µg, which represented 84.5 ± .04% of the total amount of vancomycin incorporated into the R-gel™. The concentration of vancomycin remained above the breakpoint MIC for vancomycin of 2 µg/mL for the entire 10 days.

Antimicrobial Activity

The daily eluents from both ACV and AC groups demonstrated bioactivity against *S. aureus* (Table 1). Bioactivity in this study was defined as inhibition of growth of *S. aureus* culture in vitro on agar medium. Significant differences were not observed ($P = .860$) in the mean zone of inhibition in test solutions from AC and ACV. Bioactivity was still present

on day 10. The effect of the individual antibiotics cannot be determined as they were not tested individually.

DISCUSSION

Our results indicate that amikacin, clindamycin, and vancomycin elute from R-gel™ polymer over a 10-day period. All 3 antibiotics had a high initial release followed by a steadily decreasing concentration curve. The average percentage release in the first 72 hours was 61.5% vancomycin, 64.8% clindamycin (ACV), 69.1% clindamycin (AC), 64.4% amikacin (ACV), 65.6% amikacin (AC). Copolymerization of amikacin and clindamycin with vancomycin did not have a significant effect on the elution profile.

Aminoglycosides remain the cornerstone of aerobic gram-negative therapy in many complicated or serious infections. Amikacin is a bactericidal aminoglycoside antibiotic that inhibits protein synthesis by irreversibly binding to both the 30S and 50S ribosomal subunit.¹⁵ Amikacin must be actively transported into bacterial cells, which is an

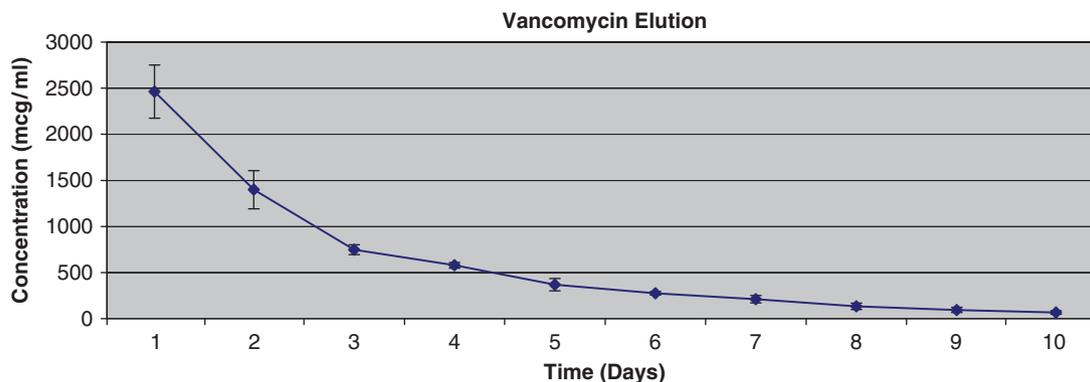


Figure 3 Vancomycin (V) elution concentration over time. Formulation 2 (ACV). Note the initial high release followed by gradual decline in concentration. The concentration remained above the breakpoint minimum inhibitory concentration (MIC) for the entire study period. A, amikacin, C, clindamycin.

Table 1 Zone of Inhibition of *Staphylococcus aureus* as Measured via Vernier Calipers

| | Bioactivity Versus <i>S. aureus</i> Zone of Inhibition (mm) | | |
|--------|--|-----|-----------------|
| | AC | ACV | Dilution Factor |
| Day 1 | 9.33 | 9.8 | 0.10 |
| Day 2 | 9.0 | 8.0 | 0.10 |
| Day 3 | 6.7 | 7.2 | 0.10 |
| Day 4 | 6.8 | 7.7 | 0.10 |
| Day 5 | 3.8 | 4.2 | 0.10 |
| Day 6 | 3.4 | 3.2 | 0.10 |
| Day 7 | 1.0 | 2.5 | 0.10 |
| Day 8 | 5.8 | 6.5 | Undiluted |
| Day 9 | 3.7 | 4.4 | Undiluted |
| Day 10 | 1.2 | 2.7 | Undiluted |

Eluents from each daily sample were plated and measured in triplicate. Average values are shown.

oxygen dependent process, thus anaerobes are inherently resistant. Aminoglycosides including amikacin exhibit concentration dependent killing of bacteria and therefore rely more on high peak concentrations to be effective, with a recommended $C_{max}:MIC$ ratio of $\geq 8-10$.¹⁵ The initial rapid elution of amikacin during the first 24 hours yielded $C_{max}:MIC$ ratios of 378 and 341 for AC and ACV, respectively. Having a C_{max} for amikacin of over 300 times greater than the breakpoint MIC for most organisms should be highly efficacious in vivo. However, recent research has demonstrated that gram negative organisms may develop adaptive resistance to aminoglycosides within the first 1–2 hours of initial antibiotic exposure.¹⁸ Adaptive resistance describes a reversible refractoriness to the bactericidal action of an antibacterial agent. The duration of adaptive resistance is related directly to the half-life of elimination of the aminoglycoside.¹⁹ Therefore amikacin impregnated implants may contribute to bacterial resistance by sustaining local concentrations after the initial high concentration.^{20,21}

Clindamycin is a semisynthetic antibiotic that acts by binding to the 50S ribosomal subunit of susceptible bacteria, thereby inhibiting peptide bond formation. Clindamycin is a time-dependent drug and is most effective when the drug concentration is maintained above the MIC throughout the dosing interval. Coverage is provided against most aerobic gram-positive cocci and anaerobic bacteria including *Staphylococcus*, *Bacteroides*, *Fusobacterium*, *Actinomyces*, and *Peptostreptococcus*. Previous studies evaluating the combination of amikacin and clindamycin have conflicting

Table 2 Measurement of Zone of Inhibition Versus *Staphylococcus aureus* of Controls of Known Concentration

| Bioactivity of Controls Versus <i>S. aureus</i> Zone of Inhibition (MM) | |
|--|----|
| Amikacin 30 mcg | 15 |
| Clindamycin 10 mcg | 14 |
| Vancomycin 30 mcg | 14 |
| Blank control | 0 |

Table 3 Pharmacodynamic Indices for Amikacin (A), Clindamycin (C), and Vancomycin (V) In Vitro Elution Samples from R-gel™ Polymer

| Variable | Treatment | Mean \pm SD |
|--------------------------------|-----------|-------------------|
| C_{max} ($\mu\text{g/mL}$) | A (AC) | 6040 \pm 24 |
| | A (ACV) | 5450 \pm 575 |
| | C (AC) | 3537 \pm 57 |
| | C (ACV) | 3339 \pm 133 |
| | V (ACV) | 2462 \pm 288 |
| $C_{max}:MIC$ | A (AC) | 377.5 \pm 1.86 |
| | A (ACV) | 340.6 \pm 35.95 |
| T > breakpoint MIC (days) | C (AC) | 10 |
| | C (ACV) | 10 |
| | V (ACV) | 10 |

C_{max} , maximum concentration reached; T > MIC, time concentration remained above breakpoint MIC; $C_{max}:MIC$, ratio of maximum concentration to breakpoint MIC.

results showing antagonism, no significant effect, or even synergism.^{22,23} A study evaluating clindamycin and aminoglycosides against 50 strains of *S. aureus* found synergistic effects at 24 and 48 hours.²⁴

An in vivo study in dogs comparing elution of antibiotics from AIPMMA found that pharmacologically, clindamycin was better at maintaining high concentrations in seroma fluid, granulation tissue and bone than cefazolin, ciprofloxacin, ticarcillin, tobramycin, or vancomycin.²⁵ Previous studies have also shown that Clindamycin may reduce glycocalyx formation.^{26,27} These attributes of clindamycin make it an appropriate choice for incorporation into an delivery matrix.

Vancomycin was included in this study as it is frequently used to treat methicillin-resistant *S. aureus* (MRSA) infections in human medicine and applications of R-gel™ are also being studied as topical wound therapy for chronic, nonhealing wounds in people. Although MRSA is becoming a more prevalent issue in veterinary medicine,^{28,29} we are not advocating use of vancomycin in animals if alternative treatments are available. There is currently no formulation of vancomycin that is approved for veterinary use. Use of vancomycin also raises concerns of increasing infection or colonization of vancomycin-resistant enterococci which has been reported in a variety of species.³⁰ Vancomycin appears to have some synergistic activity when combined with aminoglycosides,³¹ however the combination may increase the risk of aminoglycoside-related nephrotoxicity.³²

The amount and duration of elution of an antibiotic from different materials is affected by several factors. These include the type or combination of antibiotics, properties of the material, surface area, concentration of the antibiotic, and method of elution.^{5,33–37} It would have been ideal to study elution of each antibiotic separately before performing this coelution study, however, this was not possible as the gel is provided and marketed by the manufacturer in these specific combinations. Incorporation of antibiotics into other vehicles such as PMMA has often required evaluation of the effect of the antibiotic concentration on the mechanical strength of a construct. Biomechanical studies have suggested that < 10% of the weight

of AIPMMA should be attributable to the antibiotic component if the cement is to be used for implant fixation.³⁴ Since R-gelTM is a completely biodegradable device not intended to impart stability to any construct, the dosage of antibiotics may be increased without jeopardizing surgical results.

A potential concern with use of an absorbable delivery matrix is that changes in the surface area of absorbable materials can have an effect on the elution profile. Intuitively this effect should be minimal as it should not affect the total concentration released and the effect of surface area on elution should be of greater concern in nonresorbable materials. The surface area of the R-gelTM is inconsistent as it is designed to conform to the wound bed during application. This may have an effect on the in vivo elution during clinical application. Another potential flaw with this study is that the R-gelTM was not agitated during the study period. This likely led to drug elution from the matrix based on passive diffusion and may have inadvertently decreased the rate of elution. Further studies are necessary to determine pharmacodynamic indices for the delivery matrix in vivo.

The antibiotic concentration currently incorporated was arbitrary and based on published single dose parenteral recommendations for a 4–5 kg patient. Clinically, over dosage or toxicity should not occur even if complete elution of the antibiotics takes place immediately since the total antibiotic content is equivalent to the systemic dose typically administered to a 5 kg dog. Further studies of different dosing regimens are necessary to determine the optimal antibiotic concentration for clinical efficacy.

A potential advantage of the R-gelTM matrix over AIPMMA is that the gelation reaction is not exothermic in nature. The increased temperature during the mixing of AIPMMA can decrease the effectiveness of some antibiotics and limits therapeutic choices to antibiotics that are sufficiently heat stable.^{38–40} The R-gelTM matrix also has the advantage of being implanted immediately following reciprocation within the syringes without additional surgical time for polymerization and cooling.

S. aureus was selected for microbiology testing as it is the most frequent bacterium isolated from lesions of osteomyelitis in the dog^{41–43} and has been used by previous elution studies.^{44,45} The eluent from both formulations in our study remained biologically active for the entire duration of the study period. The addition of vancomycin did not significantly contribute to the bioactivity of the eluent against *S. aureus* and thus should be reserved for cases with multidrug resistant infections. Conclusions regarding significant differences between the 2 formulations are limited because of the small sample size and lack of sensitivity in measuring the zone of inhibition. However, the bioactivity study does demonstrate that antibiotics can be incorporated into the gel matrix, elute out of the matrix and retain efficacy versus a common pathogen.

Comparing the R-gelTM matrix to previously studied delivery systems is difficult without direct comparison because of the broad range of antibiotics and methodologies used in previous studies. One study comparing elution of

amikacin from HAC and PMMA found that cumulative release of the total antibiotic dose over 30 days was greater for HAC than AIPMMA ($40.8 \pm 8.4\%$ and $2.2 \pm 0.3\%$).¹⁶ A study by Phillips et al³³ reported only $5.62 \pm 0.27\%$ of total incorporated amikacin within AIPMMA eluted over a 30-day study period. The cumulative release of amikacin in our study was 73–74% over 10 days. This appears to indicate that amikacin elutes more rapidly and more completely from the R-gelTM matrix than AIPMMA or HAC. This is clinically relevant as the initial high concentration should be bactericidal and the rapid release may avoid bacterial adaptive resistance that may occur with continuous exposure to aminoglycosides.¹⁷

We conclude that amikacin and clindamycin together or in combination with vancomycin, elute from R-gelTM effectively and at gradually decreasing rates for at least 10 days. The addition of vancomycin did not significantly alter the elution profile for amikacin and clindamycin under the conditions that we evaluated. The antibiotics maintained their bioactivity following polymerization and elution from the R-gelTM. Further studies are warranted to determine the effects of R-gelTM impregnated with amikacin and clindamycin in vivo.

ACKNOWLEDGMENTS

The authors have no financial interest in this product and received no royalties or payments. The product and laboratory facility was supplied by Royer Animal Health.

Disclosure: Ms. Bizikova is an employee of Royer Animal Health.

REFERENCES

- Habash M, Reid G: Microbial biofilms: their development and significance for medical device-related infections. *J Clin Pharmacol* 1999;39:887–898
- Naylor PT, Myrvik QN, Gristina A: Antibiotic resistance of biomaterial-adherent coagulase-negative and coagulase-positive staphylococci. *Clin Orthop Relat Res* 1990;261:126–133
- Johnson KA: Osteomyelitis in dogs and cats. *J Am Vet Med Assoc* 1994;204:1882–1887
- Wininger DA: Antibiotic-impregnated cement and beads for orthopedic infections. *Antimicrob Agents Chemother* 1996;40:2675–2679
- Henry SL, Hood GA, Seligson D: Long-term implantation of gentamicin-polymethylmethacrylate antibiotic beads. *Clin Orthop* 1993;295:47–53
- Henry SL, Seligson D, Mangino P, et al: Antibiotic-impregnated beads, Part 1: bead implantation versus systemic therapy. *Orthop Rev* 1991;20:242–247
- Neut D, van de Belt H, Stokroos I, et al: Biomaterial-associated infection of gentamicin-loaded PMMA beads in orthopaedic revision surgery. *J Antimicrob Chemother* 2001;47:885–891
- Kendall RW, Duncan CP, Smith JA, et al: Persistence of bacteria on antibiotic loaded acrylic depots. *Clin Orthop* 1996;329:273–280

9. Wooley PNS, Fitzgerald RH: The immune response to implant materials in humans. *Clin Orthop Relat Res* 1996;325:63–70
10. Wimhurst JA, Brooks RA, Rushton N: Inflammatory responses of human primary macrophages to particulate bone cements in vitro. *J Bone Jt Surg [Br]* 2001;83:278–282
11. Chada HS, Wooley PH, Sud D, et al: Cellular proliferation and cytokine responses to polymethylmethacrylate particles in patients with cemented total joint arthroplasty. *Inflamm Res* 1995;44:145–151
12. Cutright DE, Perez B, Beasley JD, et al: Degradation rates of polymers and copolymers of polylactic and polyglycolic acids. *Oral Surg* 1974;37:142–152
13. Ikada Y, Hyon SH, Jamshidi K, et al: Release of antibiotic from composites of hydroxyapatite and polylactic acid. *J Control Rel* 1985;2:179–186
14. Wei G, Kotoura Y, Oka M, et al: A bioabsorbable drug delivery system for antibiotic treatment of osteomyelitis. *J Bone Jt Surg* 1991;73:246–252
15. Boothe DM: *Small animal clinical pharmacology and therapeutics*. Philadelphia, PA, Saunders, 2001
16. Ethell MT, Bennett RA, Brown MP, et al: In vitro elution of gentamicin, amikacin, and ceftiofur from polymethylmethacrylate and hydroxyapatite cement. *Vet Surg* 2000; 29:375–382
17. United States Pharmacopeia *United States Pharmacopeia and National Formulary (USP33-NF28)*, (621) *Chromatography*. Rockville, MD, United States Pharmacopeia, 2010
18. Barclay ML, Begg EJ, Hickling KG: What is the evidence for once-daily aminoglycoside therapy? *Clin Pharmacokin* 1994;27:32–48
19. Barclay ML, Begg EJ: Aminoglycoside adaptive resistance: importance for effective dosage regimens. *Drugs* 2001; 61:713–721
20. Daikos GL, Jackson GG, Lolans VT, et al: Adaptive resistance to aminoglycoside antibiotics from first-exposure down-regulation. *J Infect Dis* 1990;162:414–420
21. Barclay ML, Begg EJ, Chambers ST: Adaptive resistance following single doses of gentamicin in a dynamic in vitro model. *Antimicrob Agents Chemother* 1992;36:1951–1957
22. Zinner SH, Provonchee RB, Elias KS, et al: Effect of clindamycin on the in vitro activity of amikacin and gentamicin against gram-negative bacilli. *Antimicrob Agents Chemother* 1976;9:661–664
23. Ekwo E, Peter G: Effect of clindamycin on aminoglycoside activity in a murine model of invasive *Escherichia coli* infection. *Antimicrob Agents Chemother* 1976;10:893–898
24. Watanakunakorn C, Glotzbecker C: Effects of combinations of clindamycin with gentamicin, tobramycin, and amikacin against *Staphylococcus aureus*. *J Antimicrob Chemother* 1980;6:785–91
25. 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
26. Mayberry-Carson KJ, Mayberry WR, Tober-Meyer BK, et al: An electron microscopic study of the effect of clindamycin on adherence of *Staphylococcus aureus* to bone surfaces. *Microbios* 1986;45:21–32
27. Mayberry-Carson KJ, Tober-Meyer KB, Lambe DW, et al: An electron microscopic study of the effect of clindamycin therapy on bacterial adherence and glycocalyx formation in experimental *Staphylococcus aureus* osteomyelitis. *Microbios* 1986;48:189–206
28. Jones RD, Kania SA, Rohrbach BW, et al: Prevalence of oxacillin- and multidrug-resistant staphylococci in clinical samples from dogs: 1,722 samples (2001–2005). *J Am Vet Med Assoc* 2007;230:221–227
29. Weese JS, Faires M, Rousseau J, et al: Cluster of methicillin-resistant *Staphylococcus aureus* colonization in a small animal intensive care unit. *J Am Vet Med Assoc* 2007;231:1361–1364
30. van Belkun A, van den Braak N, Thomassen R, et al: Vancomycin-resistant enterococci in cats and dogs. *Lancet* 1996;348:1038–1039
31. Cottagnoud P, Cottagnoud M, Tauber MG: Vancomycin acts synergistically with gentamicin against penicillin-resistant pneumococci by increasing the intracellular penetration of gentamicin. *Antimicrob Agents Chemother* 2003;47:144–147
32. King DW, Smith MA: Proliferative responses observed following vancomycin treatment in renal proximal tubule epithelial cells. *Toxicol In Vitro* 2004;18:797–803
33. Phillips H, Boothe DM, Shofer F, et al: In vitro elution studies of amikacin and cefazolin from polymethylmethacrylate. *Vet Surg* 2007;36:272–278
34. Calhoun JH, Mader JT: Antibiotic beads in the management of surgical infections. *Am J Surg* 1989;157:443–449
35. Ramos JR, Howard RD, Pleasant RS, et al: Elution of metronidazole and gentamicin from polymethylmethacrylate beads. *Vet Surg* 2003;32:251–261
36. Marks KE, Nelson CL, Lautenschlager EP: Antibiotic-impregnated acrylic bone cement. *J Bone Joint Surg [Am]* 1976;58:358–364
37. Tobias KM, Schneider RK, Besser TE: Use of antimicrobial impregnated polymethylmethacrylate. *J Am Vet Med Assoc* 1996;208:841–845
38. Jeffries C, Lee A, Ling R: Thermal aspects of self-curing polymethylmethacrylate. *J Bone Joint Surg [Br]* 1975;57:511–518
39. Flick AB, Herbert JC, Goodell J, et al: Noncommercial fabrication of antibiotic-impregnated polymethylmethacrylate beads. Technical note. *Clin Orthop* 1987;223:282–286
40. Cunningham A, Demarest G, Rosen P, et al: Antibiotic bead production. *Iowa Orthop J* 2000;20:31–35
41. Hirsh DC, Smith TM: Osteomyelitis in the dog: microorganisms isolated and susceptibility to antimicrobial agents. *J Small Anim Pract* 1978;19:679–687
42. Smith CW, Schiller AG, Smith AR, et al: Osteomyelitis in the dog: a retrospective study. *J Am Anim Hosp Assoc* 1978; 14:589–592
43. Caywood DD, Wallace LJ, Braden TD: Osteomyelitis in the dog: a review of 67 cases. *J Am Vet Med Assoc* 1978;172:943–946
44. Cook VL, Bertone AL, Kowalski JJ, et al: Biodegradable drug delivery systems for gentamicin release and treatment of synovial membrane infection. *Vet Surg* 1999;28:233–241
45. Weisman DL, Olmstead ML, Kowalski JJ: In vitro evaluation of antibiotic elution from polymethylmethacrylate (PMMA) and mechanical assessment of antibiotic-PMMA composites. *Vet Surg* 2000;29:245–251