Drug Elution From High-Dose Antibiotic-Loaded Acrylic Cement: A Comparative, In Vitro Study

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Abstract

High-dose antibiotic-loaded acrylic cement (ALAC) is used for managing periprosthetic joint infections (PJIs). The marked increase in resistant high-virulence bacteria is drawing the attention of physicians toward alternative antimicrobial formulations loaded into acrylic bone cement. The aim of this in vitro study was to determine the elution kinetics of 14 different high-dose ALACs. All ALAC samples showed a burst release of antibiotics in the first hour, progressively decreasing over time, and elution curves strictly adhered to a nonlinear regression analysis formula. Among aminoglycosides, commonly seen as the most appropriate antibiotics to be loaded into the bone cement, the highest elution rate was that of tobramycin. Among the glycopeptides, a class of antibiotics that should be considered to treat PJIs because of the prevalence of aminoglycoside resistance, vancomycin showed better elution than teicoplanin. Clindamycin, which can be associated with aminoglycosides to prepare ALACs and represents a useful option against the most common pathogens responsible for PJIs, showed the highest absolute and relative elutions among all the tested formulations. A noticeable elution was also detected for colistin, an antibiotic of last resort for treating multidrug-resistant bacteria. The current study demonstrates theoretical advantages in the preparation of ALAC for some antibiotics not routinely used in the clinical setting for PJIs. The use of these antibiotics based on the infecting bacteria sensitivity may represent a useful option for physicians to eradicate PJIs. In vivo testing should be considered in the future to confirm the results of this study.
Deep infection remains one of the most challenging complications following total joint replacement, and septic mobilization is still a leading cause of implant failure. Faced with the increasing demand for joint arthroplasty surgery, intense efforts have been made to reduce the overall burden of periprosthetic joint infection (PJI), and the use of antibiotic-loaded acrylic cement (ALAC) has been introduced as a prophylactic therapy. Antibiotic-loaded acrylic cement can be useful for locally administering a high drug dose, which cannot be done through systemic administration without general complications and toxicities. A low-dose ALAC, defined as 1 g or less of powdered antibiotic per 40 g of bone cement, is generally used to prevent PJI. Higher antibiotic doses are recommended to treat PJIs; at least 3.6 g of antibiotic per 40 g of acrylic cement is desirable for effective elution kinetics and for sustaining the therapeutic levels of antibiotics.

Because of their broad spectrum and safety, aminoglycosides (eg, gentamicin) and glycopeptides (eg, vancomycin) are the antibiotic formulations most commonly loaded into acrylic bone cement. Particularly, vancomycin is currently recommended as an antimicrobial prophylaxis in arthroplasty when resistant organisms are of specific concern. The marked increase in resistant, high-virulence bacteria is drawing the attention of physicians toward alternative antimicrobial formulations loaded into acrylic bone cement. To date, few studies have used reliable and valid measures to investigate the elution properties of a broad range of antibiotics. The goal of the current study was to determine the elution kinetics of some polymethylmethacrylate (PMMA) cement antibiotic formulations that may be used in the future to treat PJIs resulting from multidrug-resistant bacteria. Some of these formulations have been poorly investigated in the past, and little is known about how most of their elution kinetics compare.

**Materials and Methods**

The specimens were prepared from polymerized cement (Cemex XL; Tecres SpA, Sommacampagna, Italy) with a mold under aseptic conditions. This cement is a radio-opaque, low-viscosity material with a powder-to-liquid ratio of 2.7:1. The antibiotic formulations shown in the Table were manually mixed with 40 g of PMMA copolymer powder with a plastic spatula for 120 seconds to achieve a uniform powder prior to adding the liquid monomer under laminar flow conditions. For each sample, the obtained resin weighed approximately 54.64 g. The specimens with evident defects were discarded. The mixing and test equipment were maintained at 23±1°C for at least 2 hours before beginning the mixing procedure or the test. The specimens were aged in air at 23±1°C and more than 40% relative humidity for 24 hours before being tested. All tests were performed at 23±1°C.

**Elution of Antibiotics**

For each formulation, 5 cylinders measuring 6 mm in diameter and 12 mm in height (weight, 400 mg) were separately immersed in glass tubes with a defined volume in an isotonic sterile saline solution (pH=7.5) at 37°C in an aerobic incubator. The saline solution was completely removed and replaced with the same volume of fresh solution after 1, 4, 24, 48, and 72 hours and 7, 14, 21, and 28 days of immersion, and multiple aliquots of the elution samples were frozen at -24°C until the final analysis. The elution samples were analyzed in duplicate in the same experiment. The antibiotic recovery was calculated as both the total eluted micrograms and the percentage with respect to the amount of the active pharmaceutical ingredient (API) of each sample. Standard antibiotic concentrations were prepared in saline and processed along with the samples.

**Antibiotic Dosing**

The antibiotic concentrations in the eluted samples were analyzed using the standard large-plate agar-well diffusion assay method, according to the Clinical and Laboratory Standards Institute. Bacillus subtilis spore suspension (Merck KGaA, Darmstadt, Germany), Escherichia coli ATCC 25922 (Microbiologies Inc, St Cloud, Minnesota), Staphylococcus aureus ATCC 6538 (Microbiologies Inc) and Staphylococcus epidermidis ATCC 51625 (Microbiologies Inc) with a final concentration of 0.02% seeded in Iso-Sensitest Agar (Oxoid Ltd, Basingstoke, United Kingdom) were used as test microorganisms, as appropriate and according to each agent’s susceptibility to the different antibiotics. Inoculated agar was poured into sterile, level, 245×245-mm plastic bioassay dishes. After solidification, 8-mm-diameter wells were punched out and filled with 50 µl of solution (samples and standards). After incubating overnight at 37°C, the diameters of the inhibition zones for each of the standard and eluted samples were measured. The unknown antibiotic concentrations in the samples were determined using standard curves created with known antibiotic concentrations and plotted on a logarithmic scale. All samples and standard concentrations were assayed in duplicate.

**Statistical Analysis**

Nonlinear regression analysis models were created to set the ideal elution rates of the antibiotics, and the $R^2$ was calculated as previously described. In the comparison between the different formulations, an analysis of variance (ANOVA) was used to evaluate the differences between the means. After the authors determined that differences existed among the multiple means, Bonferroni’s test was used to determine which means differed. A post-hoc analysis was performed to calculate the power (1-β error probability, 2-tailed) achieved by the authors’ statistical tests (alpha=.05), the sample size, and the effect size. GraphPad Prism version 5.0 statistical software (GraphPad, Inc, San Diego, California), SPSS
RESULTS

The cement samples showed a burst release of antibiotics in the first hour, followed by a lower elution rate. The elution curves showed a high-degree fit to the regression curves, with an $R^2$ ranging from 0.804 to 0.951 ($P<0.001$ for all). (Figures 1-3). All antibiotic formulations showed an elution throughout all the observation times. In detail, at a 0.6% formulation, colistin discontinued its elution after the first hour, tigecycline continued its elution after 24 hours, colistin at 2.4% formulation discontinued its elution after 7 days, and meropenem discontinued its elution after 21 days.

The absolute and relative (µg and %) total releases of the APIs from the PMMA cylinders are summarized in the Table. The absolute release of antibiotics from all the PMMA cylinders is summarized in the Table.

### Table

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Formulation</th>
<th>Dose per 40 g of PMMA Powder</th>
<th>API per 40 g of PMMA Powder, g</th>
<th>API per 400 mg ALAC Cylinder, µg</th>
<th>API Absolute Elution, µg</th>
<th>API Relative Elution, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin sulfate (CT Laboratories, Sanremo, Italy)</td>
<td>Liquid</td>
<td>4 mL</td>
<td>1</td>
<td>0.73</td>
<td>5344</td>
<td>488.96±104.26</td>
</tr>
<tr>
<td>Gentamicin sulfate (Fujiian Fukang Pharmaceutical Co, Fuzhou, China)</td>
<td>Powder</td>
<td>4 g</td>
<td>2.4</td>
<td>1.76</td>
<td>12884</td>
<td>956.79±43.28</td>
</tr>
<tr>
<td>Streptomycin sulfate (Laboratorios Nomron SA, Madrid, Spain)</td>
<td>Powder</td>
<td>4 g</td>
<td>3</td>
<td>2.2</td>
<td>16105</td>
<td>2292.08±137</td>
</tr>
<tr>
<td>Tobramycin sulfate (Ibi Giovanni Lorenzini SpA, Aprilia, Italy)</td>
<td>Powder</td>
<td>4 g</td>
<td>2.8</td>
<td>2.05</td>
<td>15007</td>
<td>2613.82±491.06</td>
</tr>
<tr>
<td>Teicoplanin (Sanofi Aventis, Paris, France)</td>
<td>Powder</td>
<td>4 g</td>
<td>3.3</td>
<td>2.42</td>
<td>17716</td>
<td>2002.57±487.13</td>
</tr>
<tr>
<td>Vancomycin hydrochloride (Xellia Pharmaceuticals AS, Oslo, Norway)</td>
<td>Crystallized</td>
<td>Powder</td>
<td>4 g</td>
<td>4</td>
<td>2.93</td>
<td>21450</td>
</tr>
<tr>
<td>Lyophilized</td>
<td>Powder</td>
<td>4 g</td>
<td>4</td>
<td>2.93</td>
<td>21450</td>
<td>2356.81±241.83</td>
</tr>
<tr>
<td>Ceftriaxone sodium (Fidia Pharmaceutical S.p.A., Abano Terme, Italy)</td>
<td>Powder</td>
<td>4 g</td>
<td>3.3</td>
<td>2.42</td>
<td>17716</td>
<td>2423.44±310.37</td>
</tr>
<tr>
<td>Clindamycin phosphate (Pfizer Inc, New York, New York)</td>
<td>Powder</td>
<td>4 g</td>
<td>3.5</td>
<td>2.56</td>
<td>18741</td>
<td>10729.36±281.69</td>
</tr>
<tr>
<td>Colistin sulfate (UCB S.A., Brussels, Belgium)</td>
<td>0.6% Powder</td>
<td>3,000,000 IU</td>
<td>0.24</td>
<td>0.18</td>
<td>1318</td>
<td>45.38±2.7</td>
</tr>
<tr>
<td></td>
<td>2.4% Powder</td>
<td>12,000,000 IU</td>
<td>0.96</td>
<td>0.7</td>
<td>5124</td>
<td>578.96±12.33</td>
</tr>
<tr>
<td>Meropenem (Astra Zeneca, London, United Kingdom)</td>
<td>Powder</td>
<td>4 g</td>
<td>3</td>
<td>2.2</td>
<td>16106</td>
<td>3060.71±257.72</td>
</tr>
<tr>
<td>Rifampicin (Sanofi Aventis)</td>
<td>Powder</td>
<td>4 g</td>
<td>4</td>
<td>2.93</td>
<td>21450</td>
<td>4267.29±273.96</td>
</tr>
<tr>
<td>Tigecycline (Pfizer Inc)</td>
<td>Powder</td>
<td>1.5 g</td>
<td>0.5</td>
<td>0.37</td>
<td>2709</td>
<td>156.47±14.15</td>
</tr>
</tbody>
</table>

Abbreviations: ALAC, antibiotic-loaded acrylic cement; API, active pharmacological ingredient; PMMA, polymethylmethacrylate.
leased greater absolute and relative antibiotic quantities (10729.2±281.69 µg and 57.25±1.5%) compared with the other cements (P<.001 for both). The post-hoc analyses detected a power of 100% for both the absolute and relative means comparisons.

Among the aminoglycosides, tobramycin showed greater absolute and relative elutions compared with gentamicin and amikacin (P<.001 for each), and no significant differences were noted in the comparison with streptomycin. Regarding the glycopeptides, the crystallized formulation of powder vancomycin showed higher absolute elutions than the lyophilized formulation (P<.001 for both absolute and relative elution). The absolute elution of the crystallized formulation of vancomycin was also found to be greater than that of teicoplanin (P<.001). The 2.4% formulation of colistin showed a relative elution rate higher than the 0.6% formulation (P<.001). When different antibiotic classes were compared, the crystallized formulation of vancomycin showed a higher absolute elution than tobramycin (P<.001), but no significant differences were noted between the relative elutions. Both the absolute and relative elutions of clindamycin were found to be significantly higher than those of colistin at 2.4%, rifampicin, ceftriaxone, tigecycline, and meropenem (P<.001 for each).

**DISCUSSION**

Different antibiotic formulations must be considered in the conception of a high-dose ALAC to eradicate an infection, even more so if an increase in PIs from multidrug-resistant bacteria is observed. To the best of the authors’ knowledge, this report is the first in vitro study to compare the elution rates of such a wide number of high-dose ALACs. Few studies to date have examined the release of therapeutic doses of antibiotics from bone cement; most studies have compared a limited number of formulations. One previous work by Beeching et al. evaluated the elution of 9 different antibiotics from PMMA; however, no statistical comparisons among the formulations have been provided, thus limiting firm conclusions that can be drawn from this work.

The current authors used 4 g/mL of antibiotic per 40 g of acrylic cement powder to prepare all but 2 ALACs. This concentration is slightly higher than the minimal concentration used to obtain a therapeutic

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**Figure 1:** Elution curves of aminoglycosides from the cement over time: amikacin (A), gentamicin (B), streptomycin (C), and tobramycin (D). The interpolation line through the data points, the model of best fit with the nonlinear regression analysis formula y=b0+(b1/t) for the antibiotics tested, and the respective R² values are shown.

**Figure 2:** Elution curves of glycopeptides from the cement over time: teicoplanin (A), crystallized vancomycin (B), and lyophilized vancomycin (C). The interpolation line through the data points, the model of best fit with the nonlinear regression analysis formula y=b0+(b1/t) for the antibiotics tested, and the respective R² values are shown.
effect. Notably, antibiotic doses as high as 8 g loaded into cement beads or spacers have been previously shown to be generally clinically safe. They calculated that 4 g of antibiotic in the cement corresponds to an API ranging from 25% to 500% of the maximum intravenous daily dose (according to the manufacturer’s instructions) for all but 2 of the antibiotics (ie, tigecycline and colistin) that the authors evaluated. The use of 4 g of tigecycline and colistin to prepare these formulations would have provided APIs corresponding to 4000% and 1400% of the maximum intravenous daily dose, which the authors considered to be clinically unsafe. Therefore, the authors chose to use 1.5 g of tigecycline (ie, 0.5 mg of API) and 12,000,000 IU of colistin (ie, 0.96 g of API) per 40 g of PMMA to obtain concentrations of these antibiotics that were slightly lower than 500% of the maximum intravenous daily dose. A lower dose of colistin (ie, 3,000,000 IU; 0.24 g of API) was also evaluated because of the previously published data for this antibiotic.

The elution rates the authors detected were high in the early observation period and progressively decreased over time, in agreement with previous observations. Interestingly, they previously identified a nonlinear regression analysis formula to predict the elution of gentamicin and vancomycin over time. In the current study, all tested antibiotic elution curves strictly adhered to this formula, thus confirming its usefulness in future research and for physicians performing joint arthroplasty surgery.

The authors observed a 28-day elution rate that was remarkably higher than those reported by a previous study that evaluated vancomycin and meropenem after a similar observation time. However, note that any comparison of their results with previous studies is difficult because of some methodological differences. Indeed, different factors, such as the cement characteristics, contact/exchange surface, compound conditions, and antibiotic type and amount, have been previously thought to determine the antibiotic release from the acrylic cement. Aminoglycosides are commonly addressed as the most appropriate antibiotics to be loaded into PMMA cement because of their high heat stability and bactericidal action at low concentrations. Although gentamicin is widely used in the pharmacological management of PJJIs, it is not an ideal antimicrobial agent. The authors’ results show that the absolute elution of tobramycin is significantly higher compared with that of gentamicin, suggesting that the former should be used to treat the aminoglycoside-sensitive bacteria in PJJIs. It has been previously demonstrated that tobramycin exerts a noticeable broad spectrum activity and was effective against most gentamicin-resistant species and Pseudomonas aeruginosa.

Following primary arthroplasties, for revisions in which gentamicin- or tobramycin-loaded bone cement has been used, the use of glycopeptides (eg, vancomycin) should be considered because of the prevalence of gentamicin resistance. Among glycopeptides, vancomycin showed better elution compared with teicoplanin. Two different vancomycin formulations have been tested. The authors’ results showed that the crystallized vancomycin eluted from the PMMA better than its lyophilized counterpart. Accordingly, it has been previously demonstrated that lyophilized vancomycin does not evenly distribute throughout the cement and reduces its fatigue life.

The use of vancomycin in a clinical setting is further supported by the concentration achievable within the cortical bone. In a previous work by Adams et
al, vancomycin and clindamycin were the only 2 antibiotics that achieved high concentrations in cortical bone. The current authors next observed a 57% elution rate of clindamycin, which was the highest relative amount of antibiotic that was eluted in their experiments. On the basis of this observation and the capability of clindamycin to reduce the glycosylalx formation, this antibiotic represents an useful option against coagulase-negative staphylococci and Staphylococcus aureus, the most common pathogens responsible for PJI. Notably, the addition of clindamycin to an aminoglycoside-loaded cement yields higher antibiotic release and results in a stronger and more prolonged inhibition of bacterial growth.

Colistin continues to be an antibiotic of last resort for treating multidrug-resistant agents, primarily Pseudomonas aeruginosa, Klebsiella pneumoniae, and Acinetobacter baumannii. To the best of the current authors’ knowledge, few studies have evaluated the use of colistin to prepare ALAC, and no data are available for the pharmacological properties of PMMA loaded with a 2.4% colistin formulation. It has been previously demonstrated that a 0.6% colistin formulation can diffuse from PMMA with a concentration consistently above the minimum inhibitory concentration, thereby exerting a broad spectrum activity. The current authors observed that an elution rate of 2.4% colistin was higher than that of 0.6% colistin, and these data confirm that the antibiotic release is positively correlated to the quantity added to the cement. Moreover, the current authors’ elution data suggested that the colistin-loaded PMMA may be useful in treating cases of PJI that result from resistant bacteria.

Certain limitations of this study should be considered. First, the authors tested PMMA loaded with single formulations rather than combinations of antibiotics, which may represent an additional useful option for treating PJI. The study also lacked information about the physical and mechanical properties of ALAC, and the authors acknowledge that adding antibiotics decreases the bending strength of the cement compared with the plain formulations. However, this issue is negligible when an antibiotic-loaded cement spacer is temporarily used to treat a septic joint after removing the prosthetic implants. Finally, the data are from an in vitro study, and caution should be exercised when using the findings in a clinical context.

Despite these limitations, this study provides useful information on the pharmacological properties of different ALACs, some of which had been poorly studied previously. The important methodological strengths of the study, such as the wide number of tested antibiotic formulations, the use of reliable and valid measures, and the strict statistical analysis, must be considered. The authors decided to determine the elution rate of the effective API rather than the initial dose and, as far as they know, no similar experiment has been used in the studies available in the current literature. The decision to use the API as the reference affords an advantage when comparing the commercially available antibiotics from different manufacturers. The API determines the drug’s effective potency, which then determines the antimicrobial activity, apart from the amount and type of excipients used.

**CONCLUSION**

This in vitro study provides useful data for preparing ALACs with antibiotics not routinely used in the clinical setting for PJI. Some of these formulations can be considered in the future to treat PJIs from resistant bacteria. ALACs are effective in maintaining local high concentrations of antibiotic formulations, with a mathematically predictable elution kinetic, even if only for the short term. Surgeons should consider these results when evaluating the loading of antibiotics into PMMA bone cement during orthopedic procedures. Future studies should evaluate the use of a combination of antibiotics when ALACs are prepared, and in vivo testing is needed to confirm the results of this study.

**REFERENCES**


