Antimicrobial Properties and Elution Kinetics of Linezolid From Polymethylmethacrylate

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Abstract

Polymethylmethacrylate (PMMA) impregnated with antibiotics is widely used in the treatment of osteomyelitis and infected arthroplasties. With the emergence of resistant bacterial strains, linezolid, which is active against gram-positive bacteria and toward which resistance has been scarce, has been suggested as an alternative. In the current in vitro study, the authors sought to determine and compare the efficacy and elution kinetics of linezolid from PMMA. Polymethylmethacrylate beads impregnated with linezolid, vancomycin, or gentamicin alone and in combinations were placed in suspensions of vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, and Staphylococcus epidermidis. The leaching out concentrations of antibiotics and growth inhibitory time in days were recorded. The mechanical strength of cement beads was evaluated in accordance with International Standard 5833. The growth inhibitory time of linezolid was significantly longer than that of vancomycin and gentamicin for methicillin-resistant S. aureus, vancomycin-resistant enterococci, and S. epidermidis. The combination of linezolid with gentamicin and vancomycin significantly increased the growth inhibitory time compared with either antibiotic used alone. Linezolid alone or in combination with vancomycin and gentamicin showed satisfactory elution kinetics and antimicrobial activity in vitro without compromising the mechanical strength of PMMA. Future research evaluating the in vivo profile of linezolid-loaded PMMA in experimental animals is needed before it can be considered for human use.

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n 1970, Buchholz and Engelbrecht\(^1\) introduced the concept of using polymethylmethacrylate (PMMA) with gentamicin to serve as a carrier for local antibiotic delivery. Since then, it has been used successfully for prophylaxis and the treatment of infected joint arthroplasties and osteomyelitis.\(^2\)\(^–\)\(^7\) Orthopedic spacers made with antibiotic-loaded PMMA deliver antimicrobial agents at the site of infection achieving high local concentrations with few systemic adverse effects and low serum and urine levels.\(^8\)\(^–\)\(^12\)

Polymethylmethacrylate can be impregnated with various antimicrobial agents. The antibiotic must be thermostable to endure the exothermic reaction of the polymerization of cement, must be water-soluble to permit diffusion into surrounding tissue, and must evoke minimal systemic and local allergic reactions. In addition, the antibiotic should target the spectrum of bacteria likely to cause infection, reach high local concentrations, conserve biomechanical properties of PMMA, and allow gradual release over time for sustained bactericidal effect. Aminoglycosides (eg, gentamicin and tobramycin) and vancomycin are the most commonly used antibiotics.\(^5\) However, with the emergence of multidrug-resistant bacterial strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE),\(^1\)\(^3\) a more effective antimicrobial agent is needed.

Recently, linezolid has been suggested as an alternative for the treatment of infections caused by MRSA and VRE. Linezolid is the first of a newer class of antibiotics termed the oxazolidinones. It inhibits bacterial protein synthesis by preventing the formation of the 70S initiation complex.\(^14\)\(^,\)\(^15\) Linezolid has shown in vitro and in vivo activity against a broad range of antibiotic-resistant gram-positive bacteria,\(^16\) including MRSA, *S. aureus* with intermediate resistance to glycopeptides,\(^17\)\(^,\)\(^18\) and VRE. Promising results were noted by a number of studies using oral or intravenous linezolid for the treatment of soft tissue, bone, and joint infections.\(^13\)\(^,\)\(^19\)\(^–\)\(^21\) These factors make it a potential alternative for local antibiotic therapy in orthopedics. However, few data are available regarding the elution and antimicrobial profile of linezolid when impregnated in PMMA. One study reported on release kinetics of linezolid-loaded PMMA beads against *Bacillus subtilis* in vitro,\(^22\) whereas another in vitro study reported on the elution characteristics and antimicrobial properties of linezolid-impregnated PMMA hip spacers against MRSA.\(^23\) However, to better evaluate the efficacy of linezolid when used with PMMA, a comparison with other commonly used antibiotics is needed.

The aim of this in vitro study was to determine and compare the elution kinetics and antimicrobial properties of linezolid when impregnated in PMMA either alone or in combination with aminoglycosides against bacteria commonly encountered in orthopedic surgery.

**Materials and Methods**

**Bacterial Strains and Media**

Methicillin-resistant *Staphylococcus aureus*, VRE, *Staphylococcus epidermidis*, *Escherichia coli*, and *Klebsiella pneumoniae* were grown on slants of brain-heart infusion agar (Becton, Dickinson and Company, Sparks, Maryland) and kept at 4°C for several weeks. Cultures of fresh bacteria were obtained every 3 to 4 days by inoculation from slants into Isotonic Sensitest Broth (ISB) (Oxoid Limited, Hampshire, United Kingdom) and overnight incubation at 37°C. The identity and purity of bacterial cultures were ascertained by plating culture samples on brain-heart infusion agar plates and on several differential agar plates (MacConkey agar; Difco Laboratories, Detroit, Michigan; CHROMagar MRSA and CHROMagar VRE; CHROMagar, Paris, France).

**Antibiotics, PMMA, and Bead Preparation**

Linezolid, vancomycin hydrochloride, and gentamicin were used in this study either alone or in various combinations. Two different commercially available and commonly used PMMA cements were included: Smart Set GHV (DePuy Orthopaedics Inc, Warsaw, Indiana) and CMW 1 (CMW Laboratories Ltd, Devon, United Kingdom). Cylindrical beads of 7-mm height, 5-mm diameter, and 260-mg weight were prepared following the manufacturers’ instructions in a sterile environment using silicon blocks with wells to accommodate appropriate bead sizes. Linezolid, vancomycin, or gentamicin were mixed with each type of PMMA to obtain beads containing 2.5% antibiotic weight/weight (1 g antibiotic per packet of PMMA). Antibiotic combinations in the form of linezolid with gentamicin and linezolid with vancomycin were used at a concentration of 2.5% for each antibiotic weight/weight to yield a total antibiotic concentration of 5% weight/weight. Both cements were used with each antibiotic and antibiotic combination. Polymethylmethacrylate beads with no antibiotic (blank beads) were prepared to serve as controls.

**Determination of Minimal Inhibitory Concentration of Antibiotics**

A 96-well microtiter plate was used. Each well was filled with 100 µL of serial 2-fold dilution of the test antibiotic in ISB. One hundred µL of 2 × 10\(^4\) CFU/mL bacterial suspension (culture in ISB) was added to each of these wells containing serially diluted antibiotic. Bacterial growth was evaluated under light microscopy. A cloudy medium was considered as positive for bacterial growth, and a clear medium indicated no bacterial growth. Each test was performed at least 3 times. The minimum inhibitory concentration (MIC) of each antibiotic was determined as the minimal concentration at which no visible growth existed.
Determination of Growth Inhibitory Time

In each well, 1 antibiotic-PMMA bead was placed and 600 µL of bacterial suspension (10^4 colony-forming unit [CFU]/mL) in ISB was added. Plates were incubated overnight at 37°C. The next day, the beads were transferred to new wells containing 600 µL of fresh bacterial suspension (10^4 CFU/mL) and incubated overnight at 37°C. This was continued until positive growth of bacteria was recorded and the medium became opaque. A sample of the opaque medium was plated to ascertain the identity of the growing bacteria as described previously. The number of days elapsed until positive growth was recorded and designated as the growth inhibitory time (GIT). As positive controls for bacterial growth, each bacterial suspension was incubated in 600 µL medium alone and in the presence of blank beads. As negative controls, antibiotic beads and blank beads were incubated each day in medium without bacteria.

Determination of Antibiotic Elution From PMMA Beads

Samples from the medium incubated with control antibiotic beads were diluted in ISB by making serial 2-fold dilutions in microtiter plates to a final volume of 100 µL/well. One hundred µL of 2×10^4 CFU/mL bacterial suspension was added to each well, and the plates were incubated overnight at 37°C. The highest dilution required to inhibit the growth of bacteria was recorded as the MIC. The concentration of the linezolid and gentamicin mixture was calculated using the average of the results obtained by the use of MRSA and K pneumoniae suspensions.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Linezolid</th>
<th>Vancomycin</th>
<th>Gentamicin</th>
<th>Linezolid+Vancomycin</th>
<th>Linezolid+Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>0.625</td>
<td>1.25</td>
<td>.1000</td>
<td>0.625</td>
<td>0.625</td>
</tr>
<tr>
<td>S epidermidis</td>
<td>0.312</td>
<td>1.25</td>
<td>7.81</td>
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<tr>
<td>VRE</td>
<td>1</td>
<td>.400</td>
<td>23.43</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E coli</td>
<td>250</td>
<td>125</td>
<td>0.625</td>
<td>Not tested</td>
<td>1</td>
</tr>
<tr>
<td>K pneumoniae</td>
<td>250</td>
<td>125</td>
<td>Not tested</td>
<td>0.625</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MRSA, methicillin-resistant Staphylococcus aureus; VRE, vancomycin-resistant enterococci.

Results were presented as the mean±SD of 2 to 3 experiments performed in triplicate or quadruplet. Statistical significance was assessed using the 1-tailed Student’s t test. P<.05 was considered significant. Analysis was performed using SPSS statistical software (SPSS Inc, Chicago, Illinois).

Results

No differences existed in the antimicrobial properties and elution characteristics of antibiotics between the 2 types of cements used. The MIC of antibiotics used in this study against test bacteria is shown in Table 1. It ranged from 0.312 to 1.25 µg/mL for linezolid and vancomycin against gram-positive bacteria (ie, MRSA, VRE, and S epidermidis), which were relatively resistant to gentamicin (MIC, 125-250 µg/mL). Similarly, gram-negative bacteria (E coli and K pneumoniae) were sensitive only to linezolid with a GIT of 15±5 days. When linezolid and vancomycin were combined, the GIT for MRSA and S epidermidis increased by approximately 1.5 compared with linezolid alone and 2.5 to 4 times compared with vancomycin alone. This increase in GIT was significant compared with that of either drug when used alone (P<.01). No significant improvement in GIT was shown for VRE. When linezolid was combined with gentamicin, the GIT improved for all 3 gram-positive bacteria and was better compared with linezolid, vancomycin...
cin, or gentamicin used alone (P ≤ .01). The combination of linezolid with gentamicin showed the longest GIT for MRSA and VRE, whereas linezolid with vancomycin had the longest GIT for S epidermidis of all antibiotics and antibiotic combinations tested. Although gram-negative bacteria were resistant to linezolid when used alone, the addition of linezolid to gentamicin increased the GIT of gentamicin by a magnitude of 2.5 to 4.5 times that of gentamicin alone (P ≤ 10^-4).

The elution profile of antibiotics from cement showed a typical pattern (Figure). It consisted of a rapid phase, which was similar for all antibiotics used alone or in combination and lasted for up to 3 days. This phase was characterized by a massive outflow of antibiotics from PMMA, reaching peak concentrations during the first 2 hours. This was followed by a plateau phase, which was characterized by the sustained release of antibiotics and lasted for varying periods of time (15-40 days) for different antibiotics. Throughout this phase, the concentration of antibiotics in the surrounding medium was higher than their MIC. The combination of linezolid and gentamicin showed a significantly longer plateau phase compared with each antibiotic alone as well as the linezolid and vancomycin combination (P ≤ 10^-4).

Axial compression testing was performed to verify if the mechanical strength of PMMA was compromised because of the addition of antibiotics. The results are summarized in Table 3 and revealed no reduction in the mechanical strength of PMMA beads (P > .2) with the concentration of antibiotics used in this study (maximum 2 g antibiotic per PMMA packet or 5% weight/weight). Both types of cements maintained similar mechanical properties.

**DISCUSSION**

Antibiotic-loaded PMMA is used extensively in orthopedic surgery for the prevention and treatment of infections. The efficacy of antibiotics used in PMMA is influenced by its elution kinetics and sensitivity profile of infectious bacteria. Elution kinetics depend largely on the chemical structure of the antibiotic and the type of cement.29 Numerous studies have evaluated the elution kinetics of gentamicin and vancomycin.5,24,27,28 Because of the emergence of antibiotic-resistant bacteria like MRSA and VRE, linezolid has been suggested as an alternative.13,29

To the current authors’ knowledge,
only 2 studies have reported the elution profile of linezolid from PMMA.\textsuperscript{22,23} Anguita-Alonso et al\textsuperscript{22} found that linezolid used in 3 different concentrations (2.5%, 5%, and 7.5% weight/weight) maintained excellent stability and elution after PMMA polymerization in vitro. The PMMA used was Simplex P (Stryker Orthopedics, Mahwah, New Jersey) in the form of beads, and the indicator organism was \textit{Bacillus subtilis}. They also reported that compared with other antibiotics (ie, cefazolin, ciprofloxacin, gentamycin, levofloxacin, and rifampicin), the elution of linezolid from PMMA was least affected by an impregnated antibiotic concentration. Anagnostakos et al\textsuperscript{23} reported on in vitro elution characteristics of linezolid alone and in combination with gentamicin when used in PMMA hip spacers made out of Palacos cement (Merck, Whitehouse Station, New Jersey). Linezolid consistently showed elution concentrations above the susceptibility breakpoint for MRSA, thereby inhibiting MRSA growth for 8 days. Moreover, the study also reported an increased volume of released linezolid and duration of MRSA growth inhibition when gentamicin was added to linezolid.\textsuperscript{23}

The elution kinetics and antimicrobial properties of linezolid with vancomycin have not been previously described. The current study compared in vitro elution kinetics of linezolid from PMMA with those of gentamicin and vancomycin either alone or in combination. The authors showed that the GIT of linezolid was significantly longer than that of vancomycin for MRSA and \textit{S epidermidis} and longer than gentamicin for VRE. When gentamicin was added to linezolid, the GIT of linezolid for all 3 gram-positive bacteria was significantly increased. Increased GIT against MRSA when gentamicin was added to linezolid correlated with the findings of Anagnostakos et al.\textsuperscript{23} The GIT for MRSA and \textit{S epidermis} also significantly increased when vancomycin was added to linezolid. Although \textit{E coli} and \textit{K pneumoniae} were resistant to linezolid, the addition of linezolid to gentamicin significantly increased the GIT of gentamicin. This is new information that has not been previously reported. The typical pattern of elution of antimicrobials used in this study (alone and in combination) is consistent with previous studies.\textsuperscript{22} The prolonged GIT of linezolid when combined with gentamicin is not unique to linezolid alone. One study found that the elution of tobramycin increased by 68% and that of vancomycin by 103% when these antibiotics were combined.\textsuperscript{30} Because the MIC of antibiotics was not affected when combined, it is possible that this may be because of the alteration in the type of interaction between antibiotics and PMMA or the alteration in PMMA porosity. Further studies are needed to explain this phenomenon.

This study has some limitations. The study was performed in vitro, and, therefore, complementary studies using in vivo models are required before clinical implementation. This is especially important because in vitro studies do not provide physiological environment available in vivo and therefore may not account accurately for all possible variables related to antibiotic elution. Antibiotic concentrations and bioactivity were determined by microbiological assays. However, high-performance liquid chromatography, which may allow accurate determination of the antibiotic concentration, was not performed. Although no reduction was found in the compression strength of antibiotic-impregnated PMMA, the authors could not assess its longevity or other mechanical properties. These properties are important when antibiotic-impregnated PMMA is used in primary or revision arthroplasty but probably less so for the staged treatment of joint infections by spacers or beads. Another potential concern with the implantation of linezolid-loaded PMMA for a certain length of time is the possibility of systemic side effects. The use of intravenous and oral linezolid has been known to cause bone marrow depression and neuropathy if administered beyond 4 weeks.\textsuperscript{31,32} It is not known if similar side effects occur with linezolid PMMA spacers.

Regardless of the molecular mechanism, the current study suggests that PMMA impregnated with either linezolid alone or in combination with gentamicin or vancomycin has the potential to be efficacious in the prevention and treatment of bone and joint infections. It may also provide longer periods of protection against susceptible organisms. Further research should investigate the in vivo pro-

<table>
<thead>
<tr>
<th>Result</th>
<th>No Antibiotics</th>
<th>Linezolid</th>
<th>Gentamycin</th>
<th>Vancomycin</th>
<th>Linezolid + Vancomycin</th>
<th>Linezolid + Gentamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cement axial compression force, N</td>
<td>2285 ± 50\textsuperscript{b}</td>
<td>2552 ± 120\textsuperscript{b}</td>
<td>2301 ± 158\textsuperscript{b}</td>
<td>2344 ± 111\textsuperscript{b}</td>
<td>2480 ± 80\textsuperscript{b}</td>
<td>2513 ± 79\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Results are given as an average of at least 5 measurements.

\textsuperscript{b}The addition of antibiotics did not reduce the biomechanical properties of the bone cement (P > .2).
file and safety of linezolid-loaded PMMA in an animal model before it can be considered for human use.

REFERENCES