Gentamicin-loaded bone cement with clindamycin or fusidic acid added: Biofilm formation and antibiotic release

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Abstract: The formation of staphylococcal biofilms on experimental bone cements, loaded with 0.5 or 1.0 g of active gentamicin and an additional equivalent amount of gentamicin, clindamycin, or fusidic acid was investigated. The biofilms were formed in a modified Robbins device over a 3-day time span and the influence of the additional antibiotics was quantified by expressing the number of colony forming units relative to the corresponding bone cement containing only gentamicin. Combinations of gentamicin with either fusidic acid or clindamycin reduced growth of clinical isolates of both gentamicin-sensitive Staphylococcus aureus and gentamicin-resistant coagulase-negative staphylococci to approximately 28%. To determine whether adding a second antibiotic has influence on the gentamicin release, cement blocks were placed in phosphate buffer and aliquots were taken at designated sampling intervals. The influence of the additional antibiotics was quantified by expressing the percentage released of the total amount of antibiotic incorporated in the different bone cements. After 3 days, all bone cements had released similar percentages of gentamicin, whereas more clindamycin and fusidic acid were released after doubling their concentration in the bone cements. In conclusion, bone cements loaded with combinations of gentamicin and clindamycin or fusidic acid are more effective in preventing biofilm formation than bone cements with gentamicin as a single drug. In addition, the presence of clindamycin or fusidic acid in gentamicin-loaded bone cement has no influence on the total gentamicin release. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res 73A: 165–170, 2005

Key words: gentamicin-loaded bone cement; fusidic acid; clindamycin; elution; biofilm

INTRODUCTION

In 1970, Buchholz and Engelbrecht1 introduced the use of acrylic bone cement as a release system for antibiotics in joint arthroplasties to achieve high local concentrations of antibiotics around an implant. The clinical efficacy of antibiotic-loaded bone cements has initially been considered quite good, and studies have reported lower rates of infection in total hip replacements when using antibiotic-loaded bone cement in contrast to when plain cement was used.2 Almost 90% of all orthopedic surgeons in the United States use prophylactic antibiotic-loaded bone cement for the fixation of implants.3 This is despite the fact that the release mechanisms of antibiotic from bone cements is poorly understood and controlled, with some reports claiming the release of antibiotic from bone cements for up to 5 years after implantation.3 This long-term release of low concentrations of antibiotics around an implant has been associated with the introduction of antibiotic-resistant bacteria.5

Gentamicin is in Europe the most commonly used antibiotic in bone cement because of its wide antibacterial spectrum and its ability to remain stable at high temperatures reached during polymerization of the polymethylmethacrylate. In a study of 33 infected hip joints, Weber and Lautenbach6 noted that 29% of bacteria isolated preoperatively were resistant to gentamicin. Unfortunately, after the use of gentamicin-loaded bone cement, resistance increased to 41% of bacteria isolated postoperatively. The increase in gentamicin resistance among bacterial strains responsible

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for orthopedic infections has stimulated the development of bone cements containing a combination of gentamicin and a second antibiotic.

Recently, Copal® bone cement, containing a combination of gentamicin and clindamycin, has been introduced on the European commercial market. The combination of gentamicin and clindamycin has a synergistic bactericidal effect on \( >90\% \) of the bacteria common to infected arthroplasty cases. Gonzalez Della Valle et al. found that the presence of tobramycin has a synergistic-like effect on the bactericidal activity of vancomycin. In contrast, the combination of gentamicin and clindamycin in bone cement inhibited growth of *Staphylococcus epidermidis*, but in an adhesion assay the combination was not significantly more effective than a monotherapy of gentamicin. There are also conflicting studies concerning the amount of antibiotics released when using a combination of drugs. Some reports show a synergistic effect, whereas others show an inhibitory effect.

Fusidic acid is an antibiotic that has been available since the 1960s and a small number of studies have examined the use of fusidic acid for the treatment of bone infections, yielding evidence of good efficacy. Moreover, fusidic acid remains stable when exposed to the high temperatures generated during the curing of the bone cement. For this reason, the properties of fusidic acid incorporated into bone cement have been examined. Beeching et al. showed large inhibitory effects of fusidic acid on the growth of *Staphylococcus aureus* for up to 7 weeks, with the largest inhibitory effect in the first week.

The aim of this study was to determine the effect of adding clindamycin or fusidic acid to a gentamicin-loaded bone cement on the formation of a staphylococcal biofilm on acrylic bone cement and on the release of the antibiotics. For completeness, commercially available Copal® bone cement, containing a combination of gentamicin and clindamycin, was also evaluated. Because gentamicin resistance is the main rationale for loading bone cements with combinations of different antibiotics, both a gentamicin-sensitive and a resistant strain were used.

**MATERIALS AND METHODS**

**Bone cement disc preparation**

Six of the polymethylmethacrylate bone cements evaluated were manufactured by DePuy International Ltd. (Blackpool, Lancashire FY4 4QG, UK). SmartSet® HV bone cements were loaded with 0.5 or 1.0 g of active gentamicin (sulfate) per package of 40.0 g of cement powder and an additional, equivalent amount of clindamycin (hydrochloride), fusidic acid (sodium fusidate), or gentamicin (sulfate). For completeness, Copal® (Merck Biomaterial GmbH, D-64271 Darmstadt, Germany) was used as well, which contains 1.0 g of active gentamicin (sulfate) and 1.0 g of active clindamycin (hydrochloride) per package of 42.6 g of cement powder.

The cements were prepared by adding the liquid monomer to the methacrylate powder. After mixing for 30 s with a spatula, the liquid cements were poured into 3.2-mm-thick Teflon molds containing holes with a diameter of 6 mm. This filled mold was manually pressed between two glass plates covered with copier overhead film (Océ, MC 110, ’s Hertogenbosch, The Netherlands) to facilitate removal after hardening, up to the time specified for final hardening. After 24 h, the cement discs were pulled out of the mold and stored in the dark at room temperature until further use. The total surface area of each disc was 1.2 cm\(^2\) and one disc weighed about 100 mg. All procedures were performed under sterile conditions.

**Bacterial strains and biofilm formation**

The bacterial strains were obtained from hip replacement infections in patients from the Department of Orthopedic Surgery at the University Medical Center Groningen, The Netherlands. A gentamicin-sensitive *S. aureus* 2580 [minimal inhibitory concentration (MIC) value of 0.75 \( \mu \)g/mL] and a gentamicin-resistant coagulase-negative *Staphylococcus 2577* (MIC value >256 \( \mu \)g/mL) were used as representatives of the most common pathogens in orthopedic infections.

The bacteria were aerobically cultured from cryopreservative beads (Protect Technical Service Consultants Ltd., UK) onto blood agar plates at 37\(^{\circ}\) C for 24 h. One colony from this plate was used to create a preculture in 10 mL of Tryptone Soya Broth (TSB) (Oxoid, Basingstoke, Great Britain) under the same incubating conditions. This preculture was used to inoculate a second culture (400 mL), which was grown overnight and used to inoculate a modified Robbins device (MRD), essentially a hollow rectangular cubicle (620 \( \times \) 20 \( \times \) 20 mm) with 10 holes in which cement discs can be plugged (see Fig. 1). The cement discs were glued onto
the sample holders with silicone paste under aseptic conditions. The silicone paste did not have any antibacterial properties. The bone cement discs were distributed over the 10 sample positions as indicated in Table I. Care was taken to avoid cross influences between samples, by placing cements with only gentamicin upstream (positions 1–4), whereas placing samples with higher antibiotics downstream of the low concentrations. The positions 5–8 were always filled with a bone cement containing gentamicin in combination with either fusidic acid or clindamycin. Positions 9 and 10 were reserved for Copal®.

The MRD was inoculated with the second culture and left for 5 h, allowing adherence of the bacteria to the different bone cement samples. Hereafter, the device was perfused for 72 h with TSB growth medium at a flow rate of 63 mL/h. Three separate runs were performed with each antibiotic combination. For each run, new cement discs were made and placed in the device. The temperature of the MRD was maintained around 37°C during the experiment.

**Biofilm evaluation**

The cement discs were removed from the MRD, put in 2 mL 0.9% NaCl, vortexed for 10 s, and finally sonicated for 60 s for microbiological evaluation. Serial dilutions were poured onto TSB agar plates. After incubation overnight, the numbers of colony forming units (CFU) were counted, and expressed relative to the surface area of the cement discs (CFU/cm²).

**Antibiotic release**

For the measurement of antibiotic release, sterile cement blocks were placed in 10 mL of phosphate buffer saline at pH 7.0 and stored in a dark environment at 37°C. At designated time intervals (1, 6, 24, and 72 h), 500-μL samples were taken. The aliquots were stored in a dark environment at −20°C.

Gentamicin concentrations were measured using a procedure proposed by Sampath and Robinson and modified by Zhang et al. Briefly, an o-phthalaldehyde reagent was made and stored for 24 h in a dark environment. The gentamicin sample, o-phthalaldehyde, and isopropanol (to avoid precipitation of the products formed) were mixed in equal proportions and stored for 30 min at room temperature. The o-phthalaldehyde reacted with the gentamicin amino groups and chromophoric products were obtained, whose absorbances were measured at 332 nm using a Spectronic® 20 Genesys™ spectrophotometer (Spectronic Instruments Inc., Rochester, NY). The gentamicin percentages released were calculated with respect to the total amount incorporated for all cements used.

Clindamycin and fusidic acid concentrations were measured using high-performance liquid chromatography. The clindamycin and fusidic acid percentages released of the total amount incorporated were calculated for the concerned cements.

Data for total antibiotic release (%) were evaluated for statistical significance using an independent sample t test. The number of study units was three for all experiments and a 95% (p < 0.05) confidence level was adapted for statistical significance.

**RESULTS**

**Biofilm formation**

Table II summarizes the effects of adding clindamycin or fusidic acid to gentamicin-loaded bone cement on bacterial growth on the cement blocks. For all runs, the number of colony forming units per square centimeter on the bone cement discs (CFU/cm²) was calculated. Combinations of 0.5 g of gentamicin with either fusidic acid or clindamycin reduced growth of the gentamicin-sensitive S. aureus 2580 to approximately 28%. The gentamicin-resistant coagulase-negative Staphylococci 2577 showed similar growth reductions.

Doubling the content of gentamicin from 0.5 to 1.0 g was less beneficial in reducing growth and colony formation. The phospholipase activity of the tested samples was also determined and expressed relative to the surface area of the cement discs (CFU/cm²). The results are shown in Table II.

**TABLE II**

<table>
<thead>
<tr>
<th>Bone Cement</th>
<th>S. aureus</th>
<th>CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 g gentamicin and 0.5 g fusidic acid</td>
<td>27.1</td>
<td>10.8</td>
</tr>
<tr>
<td>0.5 g gentamicin and 0.5 g clindamycin</td>
<td>5.9</td>
<td>3.6</td>
</tr>
<tr>
<td>0.5 g gentamicin and 1.0 g clindamycin</td>
<td>9.9</td>
<td>1.2</td>
</tr>
<tr>
<td>1.0 g gentamicin and 1.0 g fusidic acid</td>
<td>12.6</td>
<td>6.6</td>
</tr>
<tr>
<td>1.0 g gentamicin and 1.0 g clindamycin</td>
<td>4.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Copal®</td>
<td>2.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

All results are from triplicate experiments with separately cultured bacteria and different cement blocks, yielding an average standard deviation of 50%.

**Table I**

<table>
<thead>
<tr>
<th>Position</th>
<th>Type of Bone Cement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>0.5 g gentamicin, combined with 0.5 g gentamicin</td>
</tr>
<tr>
<td>3 and 4</td>
<td>1.0 g gentamicin, combined with 1.0 g gentamicin</td>
</tr>
<tr>
<td>5 and 6</td>
<td>0.5 g gentamicin, combined with 0.5 g clindamycin or 0.5 g fusidic acid</td>
</tr>
<tr>
<td>7 and 8</td>
<td>1.0 g gentamicin, combined with 1.0 g clindamycin or 1.0 g fusidic acid</td>
</tr>
<tr>
<td>9 and 10</td>
<td>Copal® (1.0 g gentamicin combined with 1.0 g clindamycin)</td>
</tr>
</tbody>
</table>

Note that flow is directed from position 1 to 10.
TABLE III
Total Amounts of Gentamicin, Clindamycin, and Fusidic Acid Released After 72 h, Expressed as a Percentage of the Total Amount Included, of Seven Different Gentamicin-Loaded Bone Cements

<table>
<thead>
<tr>
<th>Bone Cement</th>
<th>Gentamicin (%)</th>
<th>Clindamycin (%)</th>
<th>Fusidic Acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 g gentamicin and 0.5 g gentamicin</td>
<td>6.8 ± 1.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.5 g gentamicin and 0.5 g fusidic acid</td>
<td>8.2 ± 1.4</td>
<td>—</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>0.5 g gentamicin and 0.5 g clindamycin</td>
<td>8.1 ± 2.3</td>
<td>18.6 ± 3.6</td>
<td>—</td>
</tr>
<tr>
<td>1.0 g gentamicin and 1.0 g gentamicin</td>
<td>9.3 ± 0.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.0 g gentamicin and 1.0 g fusidic acid</td>
<td>7.7 ± 2.0</td>
<td>—</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>1.0 g gentamicin and 1.0 g clindamycin</td>
<td>8.2 ± 0.9</td>
<td>32.0 ± 6.0</td>
<td>—</td>
</tr>
<tr>
<td>Copal®</td>
<td>16.8 ± 5.6</td>
<td>17.2 ± 1.5</td>
<td>—</td>
</tr>
</tbody>
</table>

The values are expressed as mean of three separate experiments ± SD.

Antibiotic release

Table III summarizes the total release (%) of gentamicin, clindamycin, and fusidic acid after 3 days for all bone cements involved in this study. Release was most rapid during the first 6 h and continued at a much lower rate thereafter for all three antibiotics. Gentamicin release from the cements containing only gentamicin amounted to 123 μg after 3 days, respectively, for the cement containing 0.5 g of gentamicin, whereas for the cement containing 1.0 g of gentamicin the number was 356 μg. Evidently, disproportionally more gentamicin is released by doubling its concentration in the bone cement. This increase in gentamicin release was statistically significant (p < 0.05).

Addition of a second antibiotic increased the total release (%) of gentamicin after 3 days (see Table III) for the 0.5-g gentamicin-loaded bone cement. In contrast, adding a second antibiotic to the 1.0-g gentamicin-loaded cement decreased the total release of gentamicin. However, this did not apply for Copal® (see Table III). Copal® showed a statistically significantly higher (p < 0.05) gentamicin release (17% of the total amount incorporated instead of 8%) than the other 1.0-g gentamicin bone cements. In addition, Copal® showed a statistically significant (p < 0.05) decrease in the clindamycin release (17% of the total amount incorporated instead of 32%) when compared with the other 1.0-g clindamycin-loaded bone cement.

Similar to gentamicin, more clindamycin and fusidic acid are released by doubling their concentration in the bone cement. This increase in antibiotic release was statistically significant (p < 0.05) both for clindamycin and fusidic acid.

DISCUSSION

Combining two antibiotics in bone cement is a common clinical practice in the United States although, until recently, only mono-antibiotic-loaded bone cement has been commercially available in Europe. The increase in gentamicin resistance among bacterial strains responsible for orthopedic infections has stimulated the industrial development of bone cements containing a combination of gentamicin and a second antibiotic. In this study, we found that bone cements loaded with combinations of gentamicin and clindamycin or fusidic acid are more effective in preventing bacterial growth than bone cements with gentamicin as a monotherapy. The choice of these additional antibiotics can be rationalized. Susceptibility to clindamycin of bacteria adhering to polymethylmethacrylate has not been shown to change because of growth in the adhered state. The combination of gentamicin and clindamycin in bone cement has recently been shown to be able to produce larger zones of inhibition against S. epidermidis than bone cements with only gentamicin. Fusidic acid, with its steroid-like structure, is a lipophilic antibiotic that could be applied as a “slime buster.” It has good efficacy against staphylococci and bone penetration and has therefore been suggested as a promising agent against bone infections.

Beeching et al., who also studied a combination of gentamicin with fusidic acid, demonstrated an enhanced inhibitory effect for the first 3 days that disappeared after 5 days. The disappearance of an enhanced effect was attributed to a possible physical incompatibility of gentamicin and fusidic acid in solution. An advantage of combining gentamicin with fusidic acid in orthopedic infections might be the lipophilic character of fusidic acid that enables it to be more homogeneously spread throughout the polymer matrix. High homogeneity is required for a sustained and prolonged antibiotic release and effective inhibition of bacterial colonization. Combined with the ability to act as a “slime buster,” fusidic acid seems an ideal antibiotic to incorporate in bone cement. The present study showed that despite a low total release of fusidic acid after 3 days, bone cements loaded with a combination of gentamicin and clindamycin or fusidic acid yield additional antimicrobial efficacy. For fusidic acid, this may signify that the lipophilic character of
fusidic acid enables it to be more effective against bacteria colonizing bone cement.

The type of the antibiotic influences its release from bone cement. For example, within the first few hours, gentamicin elutes in much higher concentrations than does clindamycin.\textsuperscript{4} The elution rate of both antibiotics decays with time but the elution rate of gentamicin decays much faster than that of clindamycin.\textsuperscript{4} Although other authors have reported clindamycin release characteristics from bone cement that are superior to those of gentamicin,\textsuperscript{20} the opposite has also been found.\textsuperscript{21} Literature is equally divided as to the release of fusidic acid from bone cement.\textsuperscript{15,20,21} Also, the concentration and possible combinations that are used influence the release of antibiotics.\textsuperscript{10,22} This might be attributable to the existence of more and larger pores and cracks in the bone cement\textsuperscript{10} as a result of greater disturbances of the polymethylmethacrylate matrix. Our release experiments showed that the total gentamicin release after 3 days was significantly higher when more gentamicin was incorporated in the experimental cements. The existence of more pores and cracks in the bone cement might be beneficial to the antibiotic release, but the strength of the bone cement is affected by adding antibiotics to it.\textsuperscript{23,24} High quantities of antibiotics or combined antibiotics may lead to incomplete polymerization of the cement and significantly prejudices its mechanical properties.

The elution kinetics of different combinations of antibiotics depends not only on the type of antibiotic used and the mixing ratio, but also on the type of bone cement used. Our results indicated that Copal\textsuperscript{®} released much more gentamicin than the experimental bone cement with 1.0 g of gentamicin and clindamycin, despite a similar antibiotic content. This could be explained by the difference in the porosity of bone cement. Copal\textsuperscript{®} is made of Palacos cement, whereas the experimental cements were made of CMW cements. Palacos bone cement has a higher porosity than CMW cements, yielding higher gentamicin release rates.\textsuperscript{25} Strikingly, Copal\textsuperscript{®} released far less clindamycin than the experimental bone cement with a similar clindamycin content. The difference in hydrophobicity of Palacos and CMW cements may explain this.\textsuperscript{25}

Doubling the gentamicin content in experimental bone cement yielded a reduction in bacterial growth of the gentamicin-resistant bacterial strain because of the very high local concentrations achieved. The gentamicin-resistant strain used has a gentamicin MIC value >256 \mu g/mL, whereas local antibiotic concentrations achieved with antibiotic-loaded bone cement can increase above 450 \mu g/mL.\textsuperscript{26} However, the higher gentamicin release from Copal\textsuperscript{®} after 3 days, as compared with the experimental bone cement with the same antibiotic content, is not accompanied by an additional antimicrobial effect found in the Robbins device experiment and the gentamicin release showed no relation in either the 0.5-g gentamicin group or the 1.0-g gentamicin group. The reason for the additional antimicrobial effect for the bone cements containing combinations of antibiotics must therefore be found in the presence of the second antibiotic (so called multidrug targeting), and not in the increase of gentamicin release.

In conclusion, the addition of a second antibiotic to gentamicin-loaded bone cements shows an additional antimicrobial effect against pathogens occurring in orthopedic joint surgery, both against gentamicin-resistant and gentamicin-sensitive strains. Furthermore, gentamicin-loaded bone cement with clindamycin or fusidic acid added releases after 3 days similar percentages of gentamicin.

References


