Release of gentamicin and vancomycin from temporary human hip spacers in two-stage revision of infected arthroplasty

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Aim: Evaluation of the delivery of gentamicin and vancomycin from polymethylmethacrylate (PMMA) spacers before and after implantation for the treatment of total hip replacement infections.

Methods: Twenty industrially produced spacers containing gentamicin (1.9%) were utilized. Vancomycin (2.5%) mixed with PMMA cement was used to fill holes drilled in the cement of 14 of the 20 spacers immediately before implantation. The spacers were removed from 20 patients 3–6 months after implantation and then immersed in phosphate buffer at 37°C for 10 days. Antibiotic concentrations were determined by fluoroscent polarization immunoassay.

Results: Gentamicin and vancomycin were still present in all the spacers removed from the patients. The release of gentamicin alone and in combination with vancomycin was in the range 0.05%–0.4% of the initial amount present, whereas the release of vancomycin was in the range 0.8%–3.3%. The release kinetics showed a similar pattern for both drugs. After a high initial release of drug, a reduced, but constant, elution was observed over the next few days.

Conclusions: The delivery of gentamicin and vancomycin from PMMA cement was high initially, with sustained release over several months. Incorporation of vancomycin into the surface of the spacers permitted spacers to be prepared with multiple antibiotics present and without adversely affecting the release kinetics of the agents. The gentamicin–vancomycin combination shows potential for the treatment of infection following total hip replacement in specific patients.

Keywords: antibiotic combination, antibiotic-loaded cement, drug delivery system, surgical wound infection, topical administration

Introduction

Total hip replacement (THR) is a successful procedure for restoring hip function, but bacterial infection constitutes a major complication sometimes associated with this technique. Delivery of antibiotics to the surgical area as prophylaxis is normally used to reduce the frequency of infections in THR, and the use of antibiotic-containing cement for THR is now recognized as an effective component of such prophylaxis. A recent study that evaluated 10 950 primary THRs demonstrated that there is a lower incidence of THR revision when systemic antibiotic therapy is used in combination with antibiotic-containing cement. For the treatment of THR infection, a two-stage protocol is commonly used, characterized by the implantation of temporary antibiotic-loaded polymethylmethacrylate (PMMA) hip spacers, after removal of the infected prosthesis and adequate surgical debridement.

However, the increase in antibiotic resistance rates in bacteria isolated from infected hip joints, particularly staphylococci, is an emerging problem and has prompted us to investigate the usefulness of antibiotic combinations such as gentamicin plus vancomycin in PMMA spacers.

PMMA bone cements containing different antibiotics, such as penicillins, fluoroquinolones and aminoglycosides are now under investigation as carrier systems for the local delivery of antibiotics. However, data from the literature are difficult to compare because of the difference in the elution methods, experimental conditions, cements and in vivo models used. Few experimental data have been obtained to describe the in vivo release of antibiotics beyond the initial period of prosthesis implantation, and to the best of our knowledge no data are available on the release of antibiotics in the months following prosthesis implantation.

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In a preliminary in vitro study, we observed that vancomycin was released poorly from PMMA test specimens; and that this release was further decreased by the presence of gentamicin. Therefore, in this study we placed vancomycin-containing PMMA cement into holes drilled in the surface of industrially produced spacers impregnated with gentamicin to evaluate: (i) whether spacers removed after prolonged implantation are still capable of releasing antibiotics from PMMA cement; and (ii) the release rates of gentamicin and vancomycin alone and in combination from PMMA cements.

Patients and methods

Temporary hip prostheses (spacers)

The spacers loaded with gentamicin (n = 20) were commercially available products (Spacer-G; Tecres SpA, Verona, Italy) resembling hip prostheses in shape and prepared as follows: 1.9 g of gentamicin powder (E. Ph. grade) was mixed with 100 g of PMMA polymer powder (Cemex, Tecres, Verona) in a plastic container. The liquid monomer (35 mL) was then added and an exothermic polymerization reaction occurred yielding solidification over a period of 10 min. Before solidification, the cement mixture was placed in a mould under aseptic conditions. After 1 h the mould was disassembled and the spacer surface sterilized with ethylene oxide. The weight of the spacers (including the central stainless steel cylindrical bar) was 177.5 ± 2.1 g with a surface area of 165–180 cm².

Spacers loaded with the antibiotic combination

Vancomycin-loaded cement was prepared by mixing 40 g of powdered cement polymer (Cemex, Tecres) and 1 g of vancomycin (Vancocin, Eli Lilly, Milan, Italy) in a plastic container, after which 35 mL of liquid monomer was added. After careful mixing with a metal spatula, the cement mixture was added to 17–18 holes (10–12 mm diameter, 2–3 mm deep) drilled immediately before implantation on the surface of the spacers (Spacer-G) as shown in Figure 1. The amount of cement utilized in each prosthesis was 6–7 g, corresponding to 150–170 mg of vancomycin.

Of the 20 spacers, 14 were used for the combination with vancomycin.

Patients

Twenty patients (13 male, seven female, aged 69.3 ± 7.1 years, mean ± s.d.) who presented with clinical evidence of THR infection were treated with a two-stage revision procedure. Removal of the prosthesis and debridement of the infected periprosthetic tissues was followed by implantation of an antibiotic-loaded pre-formed PMMA spacer in the femoral canal during the same surgical procedure. Samples of periprosthetic tissues and/or pus obtained from the patients were analysed by routine methods in the microbiological department of our hospital. The organisms identified were Staphylococcus aureus (4), Staphylococcus epidermidis (2), Streptococcus β-haemolyticus (1), Escherichia coli (1) and Pseudomonas aeruginosa (1).

Six patients were implanted with PMMA spacers impregnated with gentamicin alone (1.9% final concentration) and 14 with the combined gentamicin–vancomycin spacers.

The spacers were removed after 12–36 weeks in the infection site when the inflammation and infection parameters, erythrocyte sedimentation rate and C-reactive protein had returned to normal values, or, in the absence of this, if the case was considered a failure and therefore not suitable for a prosthetic reimplantation (two patients). The spacers were removed, without instrumentation, by the excision of the soft tissues interfering at the proximal femur, and carefully handled taking care to avoid mechanical damage of the cement surface. The spacers removed from patients were rinsed with sterile water and kept in sterile conditions at 4°C until analysis. Samples of periprosthetic tissues were also obtained during surgery for bacteriological assessment.

Following implant of the spacers, all patients were treated with a 4 week course of intravenous teicoplanin (400 mg daily) followed by a 4 week oral treatment with ciprofloxacin (1.5 g daily).

The patients taking part in the study gave their informed consent, and the protocol was approved by the local hospital ethics committee.

Antibiotic determination

Elution of antibiotics. The spacers were immersed in Pyrex tubes with 500 mL of phosphate buffer [0.2 M, pH 8.0, phosphate buffer (PB)] at 37°C for 10 days. The PB was removed and replaced with the same volume of fresh PB after 1, 3 and 10 days of immersion. The removed buffer was subdivided into small aliquots and frozen at −24°C.

The PB samples from each prosthesis were analysed in the same experiment. One non-implemented spacer with gentamicin and one with gentamicin–vancomycin served as controls.

Fluorescence polarization immunoassay

Concentrations of gentamicin and vancomycin in the PB samples were determined by fluorescence polarization immunoassay (FPIA; TDx, Abbott). Vancomycin and gentamicin can be measured separately by the FPIA method even when used in combination. The kits, calibrators, controls and buffers were purchased from an Italian supplier. The method was calibrated and applied according to the manufacturer’s recommendations (TDx, Abbott). The lowest measurable level of drug concentration was 0.27 mg/L for gentamicin and 2.0 mg/L for vancomycin, and all tests were carried out in duplicate.

Since we could not measure the local concentration of antibiotics released from the spacers in vivo, we were unable to determine the drug concentrations at the site of infection. We therefore decided to relate the total amount of antibiotics released during 10 days to the spacer surface area. These values were expressed in µg per cm², a non-conventional unit, that could, in our opinion, be considered as representative of the conditions at the implantation site and also of the residual release capacity of the removed spacers.

Figure 1. Spacers with gentamicin and vancomycin after surgical preparation using the ‘surface drill hole’ technique. On the right, a removed spacer; on the left, a control spacer.
Gentamicin and vancomycin delivery from human hip spacers

Table 1. Release of gentamicin from control spacers and spacers removed from patients in PB at 37°C after 10 days of elution

| Spacers no. | Duration of implantation (months) | total release (µg) | % of initial amount | total release (µg/cm²) 
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1</td>
<td>4.0</td>
<td>1350</td>
<td>0.07</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>1030</td>
<td>0.05</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>1800</td>
<td>0.09</td>
<td>10.0</td>
</tr>
<tr>
<td>4</td>
<td>6.5</td>
<td>1500</td>
<td>0.08</td>
<td>8.3</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>850</td>
<td>0.05</td>
<td>4.7</td>
</tr>
<tr>
<td>19</td>
<td>5.0</td>
<td>1320</td>
<td>0.07</td>
<td>7.3</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>4.8 ± 1.3</td>
<td>1308.3 ± 337.0</td>
<td>0.07 ± 0.02</td>
<td>7.3 ± 1.9</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>15 550</td>
<td>0.82</td>
<td>86.1</td>
</tr>
</tbody>
</table>

*The surface area of the spacers was taken to be 180 cm².

Results

Spacers loaded with gentamicin

The release of gentamicin from the non-implanted spacer (control) amounted to 0.82% of the initial concentration, showing good, early release of the antibiotic. After 12–24 weeks in the hip, the removed spacers still released appreciable amounts (850–1800 µg) of gentamicin, representing 0.05%–0.09% of the initial total amount, and in the range 4.7–10.0 µg/cm² (Table 1).

The release of gentamicin from the removed spacers was initially high in the first 24 h of immersion, followed by low, but constant, release over the following days (Figure 2) with a pattern of release similar to that obtained with the control spacer.

Spacers with the gentamicin–vancomycin combination

Spacers with gentamicin and surface-filled holes containing vancomycin maintained their ability to release both antibiotics. The residual release of gentamicin from the removed spacers modified by the surface drill hole technique was higher (3889.6 ± 1806.5 µg, mean ± S.D.) than that seen for spacers with gentamicin alone (1308.3 ± 337.0 µg, mean ± S.D.), as shown in Tables 2 and 1, respectively. This difference is possibly due to the increase in surface area, due to drilling the holes, and factors such as the roughness of the new surface and the different porosity of the cement.

Vancomycin from the surface-drilled holes was released in large amounts (24 114 µg, corresponding to 16.1% of the initial amount) from the control spacer and was still present (release 0.8%–3.3% of the initial amount) in the spacers removed several months after implantation (Table 2).

The initial high release of vancomycin from both control and removed spacers during the first 24 h of immersion was followed by a lower elution over the following days and was similar to that seen with gentamicin (Figure 2).

The ‘surface drill hole’ technique for vancomycin on spacers loaded with gentamicin resulted in equivalent release of the two antibiotics, giving 1:1 concentration ratios (Figure 3). In the first 24 h the elution of gentamicin and vancomycin was 1285.4 ± 563.3 µg (mean ± S.D.) and 1158.2 ± 456.7 µg, respectively, whereas in the following 9 days the amount corresponded to 2589.3 ± 1331.4 µg and 2318.8 ± 787.7 µg, respectively.

The total release of gentamicin (23.0 ± 11.1 µg/cm²) and vancomycin (20.9 ± 6.4 µg/cm²) after several weeks *in situ* would appear, in our opinion, to be enough to inhibit the common susceptible microorganisms involved in orthopaedic infections.

Although there was a wide range in the release of antibiotic between patients, the differences were only partly related to the duration of the implantation period, as we found similar antibiotic elution in spacers implanted for 3–4 months and in those implanted for 6.5 months.

Clinical results

Seventeen patients achieved a substantial decrease in serological infection markers. In three cases no such decrease occurred, and this was considered a contraindication to prosthesis re-implantation; the surgical procedure therefore consisted of removal of the spacer. The microorganisms responsible for the infections in these three cases were *Mycobacterium tuberculosis* in one case and a multiresistant *S. aureus* strain in another, whereas in the third case the pathogen was not detected.

Samples for bacteriological examination taken in the operating room from all re-implanted patients yielded negative findings.

The patients were assessed after the prosthesis re-implantation procedure over a mean follow-up period of 49 months (minimum 24, maximum 77), which revealed no clinical, radiological or laboratory signs of recurrence of infection.

Discussion

Our results demonstrate that gentamicin and vancomycin are present in spacers removed after prolonged periods of implantation. There would appear to be a prompt, appreciable release of antibiotics from spacers during the implantation period.

Antibiotic-impregnated PMMA cements are regarded as a safe method of delivering antibiotics to the infection site, with a high initial release of drug followed by elution that progressively decreases over a period of time ranging from a few weeks to several months.5,13 In this study, the drug elution kinetics from the spacers were similar before (control) and after prolonged implantation.

The release of both gentamicin and vancomycin was biphasic, with good, early release from the cement in the first 24 h of elution followed by a gradual release over the following days. These results are similar to the findings of studies on the elution kinetics of aminoglycosides and vancomycin from acrylic bone cements.5,7 The high initial release of antibiotics from removed spacers is thought to be a surface mechanism resulting from mechanical erosion of the head of the device, whereas the prolonged release relates more to the antibiotic-loaded cement and elution system (environment).14 We opted for surface application of the vancomycin-containing cement on the basis of previous studies we had conducted on cylinder test specimens.8 Vancomycin mixed with PMMA cement (Cemex) eluted less effectively than gentamicin. These results are consistent with the data reported by other authors5,15 utilizing different brands of acrylic cement (Palacos and Simplex), and highlight the difficulties in preparing cements that have good vancomycin-elution kinetics.5,13 Our results with the combination differ from those obtained by Klekamp and co-workers,16 in that we found a further decrease in the amount of vancomycin released from the cement in the presence of gentamicin.
The poor elution of vancomycin may depend on many factors, such as the physicochemical characteristics of vancomycin, molecular weight, interference with cement polymers, and the stability of the drug in the presence of heat and biological fluids, as well as the different consistency of the cement itself (degree of porosity, roughness, size of preparation and surface area, etc.). The results reported in the literature, however, are difficult to compare because of the different elution methods, types of cement, size of specimens used and the antibiotic determination methods.

Spacers can be supplied pre-formed, but can also be prepared in the operating room by the surgeons themselves. In this case, one has to consider the potential disadvantages of a lack of uniform mixing of the drug in the cement and of irregular porosity and size, resulting in unpredictable antibiotic availability. The superficial application of vancomycin using the ‘surface drill hole’ technique eliminates the problem of interference between release of gentamicin and vancomycin from PMMA cement. Moreover, the concentrations of gentamicin (1.9%) and superficial vancomycin (2.5%) enabled us to obtain an optimal ratio (1:1) in this elution system.

The release of antibiotics from the non-implanted spacers was much higher than that from the removed spacers. It is reasonable to assume that the measured antibiotic amount in the spacer before implantation would reflect the amount of drug available for release at
Gentamicin and vancomycin delivery from human hip spacers

Table 2. Release of gentamicin and vancomycin from control spacers and spacers removed from patients after 10 days of elution at 37°C

<table>
<thead>
<tr>
<th>Spacer no.</th>
<th>Duration of implantation (months)</th>
<th>Gentamicin</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total release (µg)</td>
<td>% of initial amount (1.9 g)</td>
<td>µg/cm²</td>
</tr>
<tr>
<td>6</td>
<td>5 1095</td>
<td>0.06</td>
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<td>7</td>
<td>9 2725</td>
<td>0.14</td>
<td>15.1</td>
</tr>
<tr>
<td>8</td>
<td>4 2560</td>
<td>0.13</td>
<td>15.5</td>
</tr>
<tr>
<td>9</td>
<td>7 3684</td>
<td>0.19</td>
<td>22.3</td>
</tr>
<tr>
<td>10</td>
<td>3 3498</td>
<td>0.18</td>
<td>21.2</td>
</tr>
<tr>
<td>11</td>
<td>5 3636</td>
<td>0.19</td>
<td>22.0</td>
</tr>
<tr>
<td>12</td>
<td>3 8004</td>
<td>0.47</td>
<td>48.5</td>
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<td>13</td>
<td>5.5 4914</td>
<td>0.26</td>
<td>29.8</td>
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<td>14</td>
<td>5 3762</td>
<td>0.20</td>
<td>22.8</td>
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<tr>
<td>15</td>
<td>3 1854</td>
<td>0.10</td>
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</tr>
<tr>
<td>16</td>
<td>3 6192</td>
<td>0.33</td>
<td>37.5</td>
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<td>17</td>
<td>6.5 4039</td>
<td>0.21</td>
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<td>9 4602</td>
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<td>20</td>
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<tr>
<td>Mean ± S.D.</td>
<td>3889.6±1806.5</td>
<td>0.21±0.11</td>
<td>23.0±11.1</td>
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<tr>
<td>Control</td>
<td>14220</td>
<td>0.75</td>
<td>86.2</td>
</tr>
</tbody>
</table>

ND, not determined.

Figure 3. Release of gentamicin and vancomycin from removed spacers determined with FPIA after 24 and 240 h of elution. Values (µg) are expressed as mean ± S.D.

the moment of the implantation. This amount is very high and can be bactericidal, as shown by other authors with this device.22

These results enable us to assess the effects of time and implantation conditions (such as the presence of fluids, cells, pus, blood circulation, absorption, microorganisms, etc.) upon the release of antibiotics after a prolonged implantation period in human subjects. Moreover, in addition to the cement and drug characteristics, other factors, such as the dynamic fluid flow system, tissue absorption capability, limb mobility, and so on, can influence drug delivery from cement and contribute to the wide range of residual antibiotic recorded in different patients.

Although it is always difficult to relate the results from in vitro studies to the situation in vivo, in two cases it was possible to determine the antibiotic levels in periprosthetic tissue samples (repair tissue) obtained during the explantation. Drug concentrations corresponded to 12.0 ± 1.5 µg/g and 10.3 ± 1.5 µg/g for gentamicin and vancomycin, respectively—values higher than those determined in the elution studies. These antibiotic concentrations obtained at the infection site exceed the MICs of most of the common pathogens involved in orthopaedic prosthesis infections.24–26

The results of other authors20–22 reporting high concentrations of antibiotics in drainage fluids in the first few days after implantation in patients with primary THR and in experimental studies24 with bone cement in vivo support our conclusions.

The treatment was well tolerated and no serious adverse reactions, including allergic reactions, were observed. In general, a low frequency of local and systemic adverse reactions has been reported with antibiotic-containing spacers,7 and the time of recovery and clinical outcome are satisfactory.27

Our results appear to support the potential clinical efficacy of the vancomycin–gentamicin combination in treating orthopaedic THR infections in two-stage revision.
However, the therapeutic rationale for using a combination of antibiotics depends on the susceptibility of the infecting pathogens, and the use of vancomycin should be reserved for infections likely to be caused by more resistant Gram-positive bacteria, such as *S. epidermidis*, methicillin-resistant *staphylococci*, coagulase-negative *staphylococci* or *enterococci*.28,29

In conclusion, these results provide data on the release of gentamicin from antibiotic-containing acrylic cement after prolonged implantation in a human infection site, and methods for the modification of commercially available spacers to provide the delivery of multiple antibiotics. Such spacers gave good release kinetics for vancomycin and gentamicin and would appear to warrant further investigation in the management of patients with infected THR using the two-stage revision procedure.

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References


