The combination of ultrasound with antibiotics released from bone cement decreases the viability of planktonic and biofilm bacteria: an in vitro study with clinical strains

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Objectives: Antibiotic-loaded bone cements are used for the permanent fixation of joint prostheses. Antibiotic-loaded cements significantly decrease the incidence of infection. The objective of this study was to investigate whether the viability of bacteria derived from patients with a prosthesis-related infection could be further decreased when antibiotic release from bone cements was combined with application of pulsed ultrasound.

Methods: Escherichia coli ATCC 10798, Staphylococcus aureus 7323, coagulase-negative staphylococci (CoNS 7368 and CoNS 7391) and Pseudomonas aeruginosa 5148 were grown planktonically in suspension and as a biofilm on three different bone cements: Palacos R without gentamicin as control, gentamicin-loaded Palacos R-G and gentamicin/clindamycin-loaded Copal. The viability of planktonic and biofilm bacteria was measured in the absence and presence of pulsed ultrasound for 40 h.

Results: Ultrasound itself did not affect bacterial viability. However, application of pulsed ultrasound in combination with antibiotic release by antibiotic-loaded bone cements yielded a reduction of both planktonic and biofilm bacterial viability compared with antibiotic release without application of ultrasound.

Conclusions: This study shows that antibiotic release in combination with ultrasound increases the antimicrobial efficacy further than antibiotic release alone against a variety of clinical isolates. Application of ultrasound in combination with antibiotic release in clinical practice could therefore lead to better prevention or treatment of prosthesis-related infections.

Keywords: antimicrobial efficacy, gentamicin, clindamycin, prosthesis-related infections

Introduction

Bone cement is used to fix joint prostheses in total hip and knee arthroplasties. Bone cements loaded with antibiotics decrease the infection rate by local release of antibiotic, particularly in joint prosthetic revision surgery. High local antibiotic concentrations can thereby be achieved around the prosthesis and possible side-effects as arising from high systemic drug concentrations can be limited or avoided. Biomaterial-related infections cause failure of total hip and knee arthroplasties in 1–5% of the half million surgeries annually performed worldwide. This is a major medical problem as biomaterial-related infections are difficult to eradicate because of the biofilm mode of growth of the adhering bacteria. Bacteria in a biofilm are 100–1000 times more resistant to antibiotics than their planktonic counterparts, as they are embedded in an exopolymeric matrix that protects the infecting organisms against environmental attacks, such as by antibiotics and the host immune system. In most clinical cases the only way to cure a biomaterial-related infection is replacement of the infected prosthesis.

Ultrasound has been extensively used in various medical applications, including medical diagnostic imaging and...
physiotherapeutic treatment and can be considered safe for use in patients. Ultrasound has previously been shown to be effective in enhancing antibiotic efficacy, an effect earlier defined as the ‘bioacoustic effect’, whereas ultrasound itself did not influence bacterial viability.\(^4,5\) Recently, in vivo studies have shown that ultrasound in combination with gentamicin was more effective in reducing bacterial viability than the use of gentamicin alone.\(^6\)

Ideally, in order to prevent biofilm infection of implants, an effective antibiotic concentration should be achieved around an implant immediately after surgery to eradicate bacteria present as a result of inevitable bacterial contamination during surgery. For joint prostheses, this can be done through the use of antibiotic-loaded bone cements. Therefore this in vitro study investigates whether pulsed ultrasound increases the antimicrobial efficacy of antibiotic-loaded bone cements against planktonic and biofilm bacteria of bacterial strains derived from patients with prosthesis-related infections.

**Materials and methods**

**Antibiotic-loaded bone cements**

Commercially available Palacos R (Scherering-Plough, Maarsen, The Netherlands) without antibiotic-loading, Palacos R-G containing 0.84% (w/w) gentamicin base, and Copal (Biomet Merck, Darmstadt, Germany) containing 1.62% (w/w) gentamicin base and 1.62% (w/w) clindamycin base were used in this study. Mixing and preparation of the bone cement samples was done under sterile conditions according to the manufacturers’ instructions. The doughy bone cement was spread in a polytetrafluoroethylene mould, manually compressed to the manufacturers’ instructions. The doughy bone cement was degassed and placed in front of the ultrasound transducers in a water bath with degassed water. The samples were subjected to ultrasound for 40 h at 37°C. Three test tubes, one of each bone cement type, were placed in front of the ultrasound transducers in a water bath with degassed water. The samples were subjected to ultrasound for 40 h at 37°C. Three test tubes, one of each bone cement type, were not subjected to ultrasound.

After the experiment, growth of planktonic bacteria in TSB and biofilm growth on the bone cement discs in the absence or presence of ultrasound was determined by plate counting on TSB agar. The bone cement discs were carefully removed from the test tubes and placed in tubes filled with 2 mL of 0.9% NaCl vortexed for 10 s and subsequently sonicated in a water bath for 60 s to remove the biofilm from the bone cement. Plates were incubated for 24 h at 37°C after which the numbers of cfu were determined and expressed relative to the surface area of the cement discs (cfu/cm\(^2\)) for biofilm bacteria and expressed relative to the amount of TSB for planktonic bacteria (cfu/mL). Experiments were performed in 3-fold per strain. Results obtained for Palacos R, the bone cement without antibiotic, were taken as a (negative) control.

In order to quantitatively express the results found in this study, the percentages of bacterial growth reduction on antibiotic-loaded bone cements with and without ultrasound were calculated with respect to the control cement.

**Table 1.** Number of planktonic (log\(_{10}\) cfu/mL) and biofilm (log\(_{10}\) cfu/cm\(^2\)) bacteria on Palacos R-G or Copal bone cement with ultrasound (US+) and without ultrasound (US–)

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<tr>
<td><strong>Planktonic (log(_{10}) cfu/mL)</strong></td>
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<tr>
<td><em>E. coli</em> ATCC 10798</td>
<td>9.0 ± 0.3</td>
<td>8.9 ± 0.5</td>
<td>2.2 ± 1.9</td>
<td>1.0 ± 1.7</td>
<td>2.6 ± 2.7</td>
<td>1.6 ± 2.8</td>
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<td><em>S. aureus</em> 7323</td>
<td>8.5 ± 0.1</td>
<td>9.0 ± 0.1*</td>
<td>6.3 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>6.4 ± 1.1</td>
<td>6.0 ± 1.1</td>
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<td>CoNS 7368</td>
<td>5.5 ± 1.0</td>
<td>4.5 ± 0.7</td>
<td>3.3 ± 0.1</td>
<td>2.5 ± 0.4</td>
<td>3.4 ± 0.2</td>
<td>2.1 ± 0.6</td>
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<tr>
<td>CoNS 7391</td>
<td>8.5 ± 0.1</td>
<td>8.9 ± 0.4</td>
<td>6.9 ± 0.5</td>
<td>6.5 ± 0.6*</td>
<td>7.5 ± 0.7</td>
<td>6.2 ± 0.8*</td>
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<td><em>P. aeruginosa</em> 5148</td>
<td>9.0 ± 0.5</td>
<td>9.2 ± 0.1</td>
<td>5.3 ± 1.2</td>
<td>5.0 ± 1.3</td>
<td>5.8 ± 0.6</td>
<td>5.4 ± 1.3</td>
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<td><strong>Biofilm (log(_{10}) cfu/cm(^2))</strong></td>
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<tr>
<td><em>E. coli</em> ATCC 10798</td>
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<td>7.2 ± 0.4</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1.1 ± 1.9</td>
<td>0 ± 0</td>
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<tr>
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<td>6.1 ± 0.4</td>
<td>4.7 ± 0.1</td>
<td>4.5 ± 0.6</td>
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<tr>
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<tr>
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<td>3.1 ± 0.9</td>
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The bacterial growth results are averages including standard deviations from three experiments with separately cultured bacteria and different cement blocks.

*Statistical significant difference (\(P < 0.1\)).
A 90% ($P < 0.1$, two-tailed) confidence interval was applied for statistical significance.

**Results**

During bacterial growth in the presence of an antibiotic-releasing bone cement in combination with application of ultrasound, bacterial viability can be affected by ultrasound, antibiotic release and its combination. Table 1 shows that ultrasound alone did not negatively significantly affect bacterial viability, neither planktonically nor in a biofilm.

Figure 1 shows the effect of antibiotic release and the enhanced efficacy of the antibiotics due to application of ultrasound for both planktonic and biofilm bacteria grown on the antibiotic-loaded cements, respectively. First, bacterial viability in general is greatly reduced by antibiotic release. Secondly, antibiotic release combined with ultrasound additionally reduced bacterial viability for both planktonic as well as for biofilm bacteria. The percentage reduction was in most cases higher for biofilm bacteria than for bacteria in a planktonic state, which is probably due to the slow diffusion of the released antibiotic through the biofilm in the suspension, causing locally a higher concentration of antibiotic.

**Figure 1.** (a) Percentages of planktonic growth reduction with respect to the control cement of several clinical strains due to antibiotic release from antibiotic-loaded bone cements with and without ultrasound application. Note, the percentage reduction represents a change in the log$_{10}$ values of the viability. The bars represent means of three separate experiments, with SD given by the error bars. *Statistical significant difference ($P < 0.1$). (b) Percentages of bacterial growth reduction with respect to the control cement of several clinical strains in the biofilm mode of growth on antibiotic-loaded bone cements with and without ultrasound application. Note, the percentage reduction represents a change in the log$_{10}$ values of the viability. The bars represent means of three separate experiments, with SD given by the error bars. *Statistical significant difference ($P < 0.1$).
in the biofilm than in suspension. Interestingly, growth of gentamicin-resistant CoNS 7391 was strongly reduced in the presence of ultrasound, especially in a biofilm mode of growth. Application of ultrasound yielded a statistically significant enhancement of the antibiotic efficacy in three of the five bacterial strains (see Figure 1).

Discussion

This study is the first to show that the application of ultrasound in combination with antibiotic release from bone cements yields enhanced antibiotic efficacy against clinical bacterial isolates in a planktonic and biofilm mode of growth. This is an important finding as biofilms formed in biomaterial-related infections are usually most difficult to treat due to a greater bacterial antibiotic resistance.1 Previously it was hypothesized that ultrasound increases the transport of gentamicin across and within the biofilm, while it presumably also increases the transport of oxygen and other small molecules which may increase the metabolic state and render cells more susceptible to the antibiotic.2 Thus higher gentamicin concentrations could be reached within the biofilm upon application of ultrasound.

Higher gentamicin release (Copal versus Palacos R-G), or clindamycin release (Copal) seems to increase the effect of ultrasound, as in almost all cases the mean percentage of bacterial growth reduction is higher for Copal than for Palacos R-G. The extremely good release of gentamicin from Copal bone cement is additionally favoured by the release of the second antibiotic.3

Figure 1 (a and b) shows that the bacterial growth reductions for the gentamicin-resistant (MIC > 256 mg/L) CoNS 7391 were lower than for other (gentamicin-susceptible, MIC 0.38–6 mg/L) strains. Yet, gentamicin release combined with ultrasound was effective, as shown by the high bacterial growth reductions for this strain, especially in the biofilm mode of growth (Figure 1b). Probably the ultrasound causes a very high local gentamicin concentration (>256 mg/L) and a better penetration in the biofilm and bacterial cell. This can be considered an important finding as gentamicin-resistant strains are emerging more and more in prosthesis-associated infections.

Remarkably, ultrasound did not additionally reduce bacterial viability of P. aeruginosa 5148. This might be explained by the high amount of extracellular polymeric substances excreted by P. aeruginosa, which was even suggested to increase upon exposure to an antibiotic.4 Higher acoustic intensities may be required for P. aeruginosa to reach a bioacoustic effect comparable to other bacterial strains.5

In conclusion, this study shows that ultrasound increases the efficacy of antibiotics against clinical isolates from infected joint replacements both in a planktonic and biofilm mode of growth, except for a P. aeruginosa isolate. The enhanced efficacy is clinically most important with regard to the treatment of infected joint prostheses, especially as also enhanced antibiotic efficacy was found against a gentamicin-resistant strain. Ultrasound may also be applied combined with antibiotics in the early post-operative period to prevent infection, because planktonic bacteria present in the wound and wound area due to inevitable contamination during surgery can then be more effectively prevented to form a biofilm.

Transparency declarations

None to declare.

References