Temocillin: a new candidate antibiotic for local antimicrobial delivery in orthopaedic surgery?

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Objectives: To assess the performance of the Gram-negative-specific antibiotic temocillin in polymethylmethacrylate bone cement pre-loaded with gentamicin, as a strategy for local antibiotic delivery.

Methods: Temocillin was added at varying concentrations to commercial gentamicin-loaded bone cement. The elution of the antibiotic from cement samples over a 2 week period was quantified by LC-MS. The eluted temocillin was purified by fast protein liquid chromatography and the MICs for a number of antibiotic-resistant Escherichia coli were determined. The impact strength of antibiotic-loaded samples was determined using a Charpy-type impact testing apparatus.

Results: LC-MS data showed temocillin eluted to clinically significant concentrations within 1 h in this laboratory system and the eluted temocillin retained antimicrobial activity against all organisms tested. Impact strength analysis showed no significant difference between cement samples with or without temocillin.

Conclusions: Temocillin can be added to bone cement and retains its antimicrobial activity after elution. The addition of up to 10% temocillin did not affect the impact strength of the cement. The results show that temocillin is a promising candidate for use in antibiotic-loaded bone cement.

Keywords: bone cement, elution, impact strength, LC-MS

Introduction

In the UK during 2012, bone cement was used in 54% of total primary hip replacements (including 21% of hybrid cemented/cementless procedures) and 86% of total primary knee replacements (including <1% of hybrid procedures), equating to ~150,000 arthroplastic operations. The use of antibiotic-loaded cement in primary hip replacement procedures has increased from 73% in 2004 to 89% in 2012. Similarly, the use of antibiotic-loaded cement in primary knee replacement procedures has increased from 87% in 2003 to 98% in 2012.1 Adding one or more prophylactic antibiotics to cement has been shown to reduce post-operative infection rates.2 Due to the wide range of organisms that can contribute to prosthetic joint infections and problems with antibiotic-resistant bacteria, an increasing range of antibiotics need to be available for addition to bone cement.

Temocillin is a β-lactam antibiotic resistant to hydrolysis by most β-lactamases due to the presence of a 6-α-methoxy group, which stabilizes the molecule against hydrolysis by many such enzymes.3,4 A substantial minority of prosthetic joint infections are caused by Gram-negative bacteria, most notably Enterobacteriaceae such as Escherichia coli, Klebsiella spp. and Proteus spp.5–7 Temocillin is effective against organisms expressing a range of ESBLs, including some carbapenem-resistant species.8–10 We envisage that if temocillin could be used as a locally delivered antimicrobial for orthopaedic surgery, the most likely context in which it would be employed would be in combination with gentamicin. Gentamicin is a well-established additive to bone cement that gives protection against a wide range of pathogens including staphylococci, which are the most common cause of prosthetic joint infection.11,12 In such a situation, temocillin (which does not show antagonistic interaction with gentamicin4) would give protection against Gram-negatives, including ESBL producers that were also gentamicin resistant, possibly in revision surgery in patients with a history of infection of the prosthesis with an ESBL-producing Gram-negative organism.

Methods

Temocillin was added at varying concentrations to gentamicin-containing Refobacin Bone Cement R (Biomet). Bone cement was mixed in a HiVac mixing bowl according to the manufacturer’s instructions and set in
5 × 9 mm diameter plastic moulds. Bone cement was allowed to cure for 1 h and then stored at −20°C. The bone cement samples were submerged in 0.1 M ammonium acetate solution and aliquots taken at 0, 1, 2, 6, 24, 48, 72, 168 and 336 h (14 days). Eluted temocillin and gentamicin concentrations were quantified by LC-MS using a Phenomenex Luna C18(2) column coupled to a Finnigan LCQ ion-trap mass spectrometer. The isocratic mobile phase for detection of temocillin consisted of 60% (v/v) acetonitrile/0.1% (v/v) trifluoroacetic acid at a flow rate of 0.05 mL/min. For detection of gentamicin, an isocratic mobile phase of 40% (v/v) methanol/0.1% (v/v) trifluoroacetic acid at a flow rate of 0.05 mL/min was used. The use of volatile ammonium acetate solution as a buffer (rather than a standard buffer such as PBS) allowed direct analysis of the eluate by HPLC without the need for a desalting step. The mass spectrometer was operated with an ESI source in positive ion mode with a source voltage of 4.5 kV, sheath gas flow of 80 (arbitrary units) and capillary temperature of 250°C. Detection of antibiotic was carried out using selected ion monitoring at 437 m/z, corresponding to the temocillin sodium adduct [M + Na]⁺, or 478 m/z, corresponding to the protonated gentamicin C1 component [M + H]⁺. The antibiotic concentration was determined by linear regression to a standard calibration curve with a correlation coefficient (R²) of >0.99 for each antibiotic. Method validation was carried out by analysing standard solutions of each antibiotic (n = 3) at 10, 100 and 400 mg/L over 5 h and on 3 separate days to determine intraday and interday variation, respectively. Temocillin analysis showed an intraday coefficient of variation (%CV) ranging from 0.98 to 5.33 and an interday %CV ranging from 5.47 to 13.00. Gentamicin analysis showed an intraday %CV ranging from 4.25 to 11.61 and an interday %CV ranging from 12.05 to 19.98. Temocillin samples eluted during the first 24 h of elution were determined by the broth microdilution method.13 The MICs of eluted temocillin were compared with the MICs of a standard temocillin solution determined using the same method. Impact analysis of bone cement samples was carried out using a Charpy-type Hounsfield plastic impact testing apparatus14,15 and statistical analysis was carried out using the analysis of variance function in Microsoft Excel software.

### Results

#### Kinetics of antibiotic elution

When bone cement samples containing temocillin at various concentrations and gentamicin at 1.25% (w/w) were placed in buffer solution to allow elution of the antibiotic (Figure 1), the highest concentration of eluted temocillin was 3051 ± 264 mg/L after 336 h (14 days) in eluate from the cement samples containing temocillin at 10% (w/w) (Figure 1b). Similar samples containing 5% and 1.25% (w/w) temocillin produced lower concentrations of temocillin, at 1337 ± 247 and 327 ± 91 mg/L, respectively, after 336 h (Figure 1b). In contrast, the gentamicin concentration was highest from the cement sample containing 1.25% temocillin (1380 ± 290 mg/L gentamicin after 336 h) (Figure 1d). The samples containing 5% and 10% temocillin gave 400 ± 170 and 490 ± 180 mg/L eluted gentamicin, respectively, after the same period of elution.

![Figure 1](image-url) (a) Elution of temocillin up to 6 h. (b) Elution of temocillin from 6 to 336 h. (c) Elution of gentamicin up to 6 h. (d) Elution of gentamicin from 6 to 336 h. Bone cement samples initially contained 1.25% (w/w) gentamicin and varying amounts of temocillin as indicated. Bone cement samples were immersed without buffer change and aliquots of eluate analysed over 336 h. Results are shown as the mean of three separate experiments (±SD).
Table 1. MICs for a range of E. coli strains determined by the broth microdilution method using eluted temocillin and standard antibiotic solutions

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Eluted temocillin&lt;sup&gt;a&lt;/sup&gt; MIC (mg/L)</th>
<th>Standard temocillin MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH5α</td>
<td>3.8–7.6</td>
<td>3.1</td>
</tr>
<tr>
<td>AmpC-expressing strain</td>
<td>1.9–3.8</td>
<td>3.1–6.3</td>
</tr>
<tr>
<td>SHV-1-expressing strain</td>
<td>3.8–7.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Orthopaedic isolate from infected prosthesis</td>
<td>15.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Temocillin-susceptible ESBL-producing strain</td>
<td>15.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Temocillin-susceptible ESBL-producing strain</td>
<td>15.3</td>
<td>6.3–12.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Temocillin samples eluted during the first 24 h of bone cement elution assays were pooled and separated from gentamicin using ion-exchange chromatography. The concentration of temocillin was quantified by HPLC and serial dilutions of the purified temocillin used to determine MICs.

Activity of the eluted temocillin

The MICs of the eluted temocillin were determined for several strains of E. coli (Table 1) after chromatographic separation from the eluted gentamicin, in order to confirm that the temocillin retained its antimicrobial activity after incorporation into the bone cement and subsequent elution. These results were compared with MICs determined using a standard solution of temocillin that had not been in contact with bone cement. The MICs determined using the eluted and standard temocillin solutions were comparable for all strains tested and in line with published data for temocillin-susceptible strains. Hence, temocillin retained its antimicrobial activity after elution from the bone cement. The range of MICs observed for the different strains can be attributed to varying (low) levels of resistance that would be expected between different isolates of these types.

Impact analysis

The results of the impact analysis showed no significant difference in the impact strength of bone cement containing 1.25% (w/w) gentamicin with or without temocillin at ≤10% (w/w) concentration (P>0.05) (see Figure S1, available as Supplementary data at JAC Online).

Discussion

The data presented here show that temocillin is a promising candidate for antibiotic-loaded bone cement delivery strategies. The temocillin is not degraded by the elevated temperatures during the cement curing process and retains its antimicrobial activity, which is still detectable in the eluate up to 2 weeks later. Antimicrobial activity of the eluted temocillin was confirmed with a range of E. coli strains, including a laboratory strain (DH5α) and recent clinical isolates expressing ESBLs of the type that might require the use of temocillin in bone cement during a joint revision operation in a patient with a history of periprosthetic infection with such an organism. The concentrations of eluted temocillin and gentamicin exceeded MICs for susceptible strains within the first hour of elution in this laboratory system. This result may be important in a clinical setting since it indicates that the antibiotic-loaded bone cement could provide effective antimicrobial prophylaxis during the perioperative period (hip and knee replacement operations typically take 1–2 h) and it may be beneficial that active antibiotic continues to elute during the post-operative period. Increasing the percentage of temocillin within the bone cement produced larger concentrations of eluted temocillin. An increase in the amount of temocillin from 1.25% (w/w) to 5% (w/w) or 10% (w/w) also led to an unexpected decrease in gentamicin elution of ~3-fold by 336 h. The antibiotic elution experiments measured cumulative elution into a single volume of buffer, which was not replaced during the experiment. One possible explanation for the reduced elution of gentamicin from cement containing higher concentrations of temocillin is that the elution buffer was not changed during each individual experiment and so elution of gentamicin may have been retarded by the higher concentrations of eluted temocillin in the buffer. Although substantial, we believe that this difference in gentamicin elution is unlikely to be clinically significant because no reduction in gentamicin elution was observed during the critical perioperative period (typically 1–2 h) and the eluted concentration of gentamicin in this laboratory system exceeded the MICs for susceptible organisms such as staphylococci (typically ≤1 mg/L) by ≥140-fold by the time of the first sample (<1 min). In addition to its favourable elution and antimicrobial properties, up to 10% (w/w) of temocillin can be added to commercial gentamicin-containing bone cement without a detrimental effect on the impact strength of the cement. A number of other groups have carried out tests looking at different mechanical properties of bone cement and showed a detrimental effect of high loading of antibiotic on these mechanical properties. Lautenschläger et al. showed that increasing amounts of gentamicin caused a gradual, proportional decrease in the compressive and diametral tensile strength of the bone cement. It was noted that at ≥11.25% (w/w) gentamicin, the compressive strength dropped below levels recommended in American Society for Testing and Materials (ASTM) guidelines. Studies looking at increasing amounts of daptomycin and gentamicin showed that fatigue limits decreased with increasing antibiotic concentrations and gave optimum loading concentrations of 3.4% and 4.78%–6.5%, respectively. If temocillin is used as an additive to bone cement, these data should be taken into account before deciding on the concentration of temocillin to add. The data shown here indicate that temocillin is a promising candidate for inclusion in antibiotic-loaded bone cement, which may be a useful tool in combating Gram-negative prostatic joint infection.

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Transparency declarations
None to declare.

Supplementary data
Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References