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Published version

BARKER, Stewart, NICHOL, Tim, HARRISON, Patrick L., STOCKLEY, Ian, TOWNSEND, Robert and SMITH, Thomas J. (2015). Temocillin: a new candidate antibiotic for local antimicrobial delivery in orthopaedic surgery? The Journal of antimicrobial chemotherapy, 70 (3), 780-783.

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

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Running title: Assessing temocillin performance in bone cement

Keywords: Bone cement, elution, impact strength

Objectives

To assess the performance of the Gram negative-specific antibiotic temocillin in polymethylmethacrylate (PMMA) 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 two week period was quantified by HPLC-MS. The eluted temocillin was purified by fast protein liquid chromatography and minimum inhibitory concentration (MIC) for a number of antibiotic-resistant *Escherichia coli* determined. The impact strength of antibiotic-loaded samples was determined using a Charpy-type impact testing apparatus.

Results

HPLC-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: temocillin, bone cement, antibiotic elution, antimicrobial activity

Introduction

In the UK during 2012, bone cement was used in 54% of total primary hip replacements (including 21% hybrid cemented/cementless procedures) and 86% of total primary knee replacements (including <1% hybrid procedures), equating to approximately 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 postoperative 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 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 extended spectrum β-lactamases (ESBL), 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 Gram positives such as 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 gentamicin[4]) would give protection against Gram negatives, including

gentamicin resistant organisms such as ESBL-producers, 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 manufacturer's instructions and set in 5 mm × 9 mm diameter plastic moulds. Bone cement was allowed to cure for 1 h 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 phosphate buffered saline) allowed direct analysis of the eluate by HPLC using electrospray ionisation MS, 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 80 (arbitrary units), and capillary temperature 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 $[M + H]^+$. Antibiotic concentration was determined by linear regression to a standard calibration curve with a correlation coefficient (R^2) of >0.99 for each antibiotic. Method validation was carried out by analysing standard solutions of each antibiotic (n=3) at 10 mg/L 100 mg/L and 400 mg/L over 5 hours and on 3 separate days to determine interday and intraday variation respectively. Temocillin analysis showed an interday coefficient of variation (%CV) ranging from 0.98 - 5.33 and an intraday %CV ranging from 5.47 - 13.00. Gentamicin analysis showed an interday %CV ranging from 4.25 -

11.61 and an intraday %CV ranging from 12.05 - 19.98. Temocillin samples eluted during the first 24 hours of bone cement elution assays were pooled, separated from gentamicin by fast protein liquid chromatography using a HiTrap SP 5mL ion exchange column (GE Healthcare) and MICs for a number of *Escherichia coli* strains determined by broth micro-dilution method.[13] The MIC values for eluted temocillin were compared to the MIC values for 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 apparatus[14,15] and statistical analysis was carried out using 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, 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) (Fig. 1b). Similar samples containing 5% and 1.25% (w/w) temocillin produced lower concentrations of temocillin, at 1337 ± 427 mg/L and 327 ± 91 mg/L, respectively, after 336 h (Fig. 1b). In contrast gentamicin concentration was highest from the cement sample containing 1.25% temocillin (1380 ± 290 mg/L of gentamicin after 336 h) (Fig 1d). The samples containing 5% and 10% temocillin gave 400 ± 170 mg/L and 490 ± 180 mg/L of eluted gentamicin, respectively, after the same period of elution.

Activity of the eluted temocillin

MICs of the eluted temocillin were measured against 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. Results were compared against the MICs determined 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.[16] 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 ($P > 0.05$) (see supplementary Figure S1

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 the 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 MIC values 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 postoperative 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 or 10 % (w/w) also led to an unexpected decrease in gentamicin elution of approximately 3-fold by 336 h. The antibiotic elution experiments measured cumulative elution into a single buffer which was not replaced. When higher

concentrations of antibiotic were present in the buffer, this may have affected subsequent elution of antibiotic. This could explain the reduced elution of gentamicin from the bone cement containing higher concentrations of temocillin. If there was a large initial release of temocillin into the buffer as was seen at 5 and 10% temocillin concentrations, this may have inhibited the release of gentamicin into the same 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),[17] and the eluted concentration of gentamicin in this laboratory system exceed the in the MICs of susceptible organisms such as staphylococci (typically < 1 mg/L) by at least 140-fold by the time of the first sample (<1 min).[13,18] 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 detrimental effect on the impact strength of the cement. However a number of other groups have carried out tests looking at different mechanical properties of bone cement and shown a detrimental effect of high loading of antibiotic on these mechanical properties. These data should be taken into account before deciding on a concentration of temocillin within bone cement. Lautenschlager *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 above 11.25% gentamicin (w/w), compressive strength dropped below levels recommended in ASTM guidelines.[19] 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.[20,21] The data shown here indicate that temocillin is a promising candidate for inclusion in antibiotic-loaded bone cement that may be a useful tool in combating Gram negative prosthetic joint infection.

Acknowledegments. We would like to thank Eumedica Pharmaceuticals A.G. for providing temocillin as a gift.

Funding

Funding for this research was provided by a Student into Work grant from the Society for Applied Microbiology.

Transparency Declarations

None.

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214 Figures

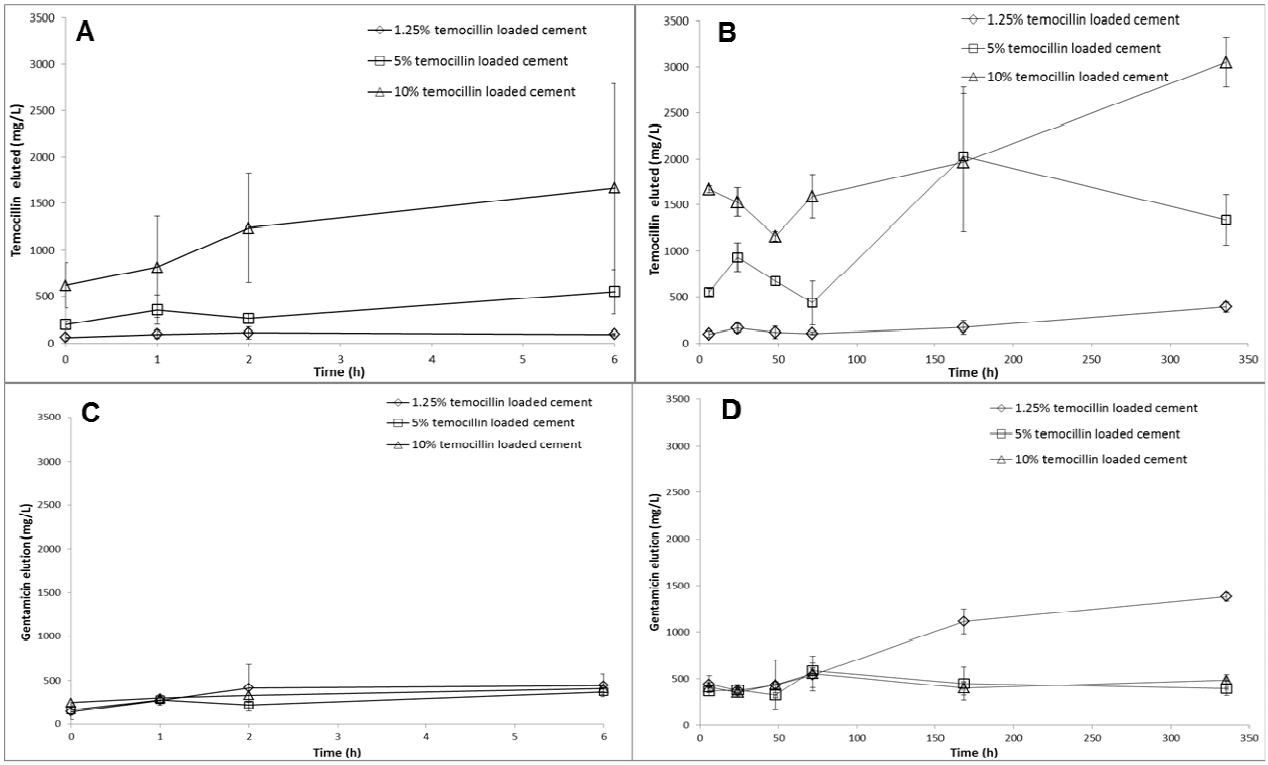

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Fig 1 A) Elution of temocillin up to 6 hours, B) elution of temocillin from 6 - 336 hours, C) elution
216 of gentamicin up to 6 hours and D) elution of gentamicin from 6 - 336 hours. Bone cement samples initially contained 1.25% (w/w) gentamicin and varying amount of temocillin as
217 indicated. Bone cement samples were immersed without buffer change and aliquots of eluate analysed over 336 hours. Results are shown as the mean of 3 separate experiments ± standard
218 deviation

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E. coli strain	Eluted temocillin[A] MIC (mg/L)	Standard temocillin MIC (mg/L)
DH5-α	3.8 - 7.6	3.1
AmpC expressing strain	1.9 - 3.8	3.1 - 6.3
SHV-1 expressing strain	3.8 - 7.6	6.3
orthopaedic isolate from infected prosthesis	15.3	6.3
temocillin susceptible ESBL producing strain -1	15.3	12.5
temocillin susceptible ESBL producing strain -2	15.3	6.3 - 12.5

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227 **Table 1.** MICs for a range of *E.coli* strains determined by broth microdilution method using eluted temocillin and standard antibiotic solutions.

[A] Temocillin samples eluted during the first 24 hours of bone cement elution assays were pooled and separated from gentamicin using ion exchange chromotography. The concentration of temocillin was quantified by HPLC and serial dilutions of the purified temocillin used to determine MICs.