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Elution kinetics, antimicrobial activity, and mechanical properties of 11 different antibiotic loaded acrylic bone cement

Ruben Gálvez-López [a], Alejandro Peña-Monje [b], Ramón Antelo-Lorenzo [c], Juan Guardia-Olmedo [d], Juan Moliz [e], José Hernández-Quero [a], Jorge Parra-Ruiz [a,*]

[a] Servicio de Enfermedades Infecciosa, Hospital Universitario San Cecilio, Granada, Spain
[b] Service de Microbiología, Hospital Universitario San Cecilio, Granada, Spain
[c] Servicio de Cirugía Ortopédica y Traumatología, Hospital Universitario San Cecilio, Granada, Spain
[d] Laboratorio Materiales de Construcción, ETS Ingenieros de Edificación, Universidad de Granada, Granada, Spain
[e] Laboratorio de Espectometría de masas de alta resolución, Centro de Instrumentación Científica, Universidad de Granada, Granada, Spain

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ABSTRACT

Antibiotic-loaded acrylic bone cements (ALABC) spacers are routinely used in the treatment of prosthetic joint infections. The objectives of our study were to evaluate different ALABC for elution kinetics, thermal stability, and mechanical properties. A 10 or 20% mixture (w/w) beads of medium viscosity bone cement (DePuy, Inc) and vancomycin (VAN), gentamycin (GM), daptomycin (DAP), moxifloxacin (MOX), rifampicin (RIF), cefotaxime (CTX), cefepime (FEP), amoxicillin clavulanate (AmC), ampicillin (AMP), meropenem (MER), and ertapenem (ERT) were formed and placed into wells filled with phosphate-buffered saline. Antibiotic concentrations were determined using high-performance liquid chromatography. Antimicrobial activity was tested against *Micrococcus luteus* ATCC 9341 or *Escherichia coli* ATCC 25922. AmC, AMP, and FEP concentration rapidly decreased after day 2, being almost undetectable at day 4. Sustained and high elution rates were observed with VAN, GM, MOX, and RIF for the 30-day duration of the experiment. DAP, MER, ERT, and CTX elution rates constantly decreased from day 4. All antibiotics tested retained antimicrobial activity proving thermal stability. Mechanical properties of ALABC were maintained except when RIF was used.

1. Introduction

Treatment of prosthetic joint infections (PJIs) remains a challenge for clinicians. One- or two-stage exchange procedures are typically selected according to type of infection (acute or chronic), microorganism(s) involved, and patient clinical condition(s) (Esposito et al., 2009; Trampuz and Zimmerli, 2008). Antibiotic-loaded acrylic bone cements (ALABC) are widely used in 2-stage procedures. ALABC beads are often used to locally deliver high concentrations of antibiotic and maintain dead space secondary to debridement surgery (Webb and Spencer, 2007). The beads are surgically implanted in the debrided bone and covered with soft tissue. Serum, inflammatory fluid, and antibiotic eluted from the ALABC beads accumulate in the space around the beads contributing to the treatment of the PJI. The ALABC beads are left in place for 3 to 4 weeks and then surgically removed when implanting a new prosthesis (Macmull et al., 2010; Takahira et al., 2003; Youngman et al., 2003). Ideally, antibiotics mixed within the bone cement should provide eluted concentrations of active drug well above the MICs of microorganisms involved in PJI for 3–4 weeks and should not alter the mechanical properties of the cement. Vancomycin (VAN) and gentamycin (GM) are currently the antibiotics that are most commonly used for this clinical indication (Anagnostakos et al., 2006; Langlais et al., 2006). Despite the general consensus about the benefits of ALABC (Langlais et al., 2006), concerns about their usefulness have risen lately. Reasons for these concerns include questions about the thermal stability of antimicrobials (Anagnostakos et al., 2006) and poor elution kinetics of certain antimicrobials, which may result in the persistence of the infection (Dunne et al., 2008; Neut et al., 2005; Witso et al., 1999) among others. There is, however, a paucity of data regarding elution kinetics, antimicrobial activity, and mechanical properties of the majority of antimicrobials that are most commonly used in the treatment of PJIs. Therefore, the objective of this work was to evaluate *in vitro* the elution of 11 selected antibiotics from beads made with polymethylmethacrylate (PMMA) cement.

2. Materials and methods

2.1. Antimicrobials and PMMA

Moxifloxacin (MOX) analytical powder and daptomycin (DAP) for injection (CUBICIN®, Cubist Pharmaceuticals, Inc., Lexington, MA, USA) were generously provided by the manufacturers. VAN,

* Corresponding author. Tel.: +34-958-023-104; fax: +34-958-249-079.
E-mail address: jordi@ugr.es (J. Parra-Ruiz).

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ampicillin (AMP), amoxicillin clavulanate (AmC), ertapenem (ERT), meropenem (MER), cefotaxime (CTX), rifampicin (RIF), GM, and cefepime (FEP) were commercially purchased.

PMMA medium viscosity bone cement (DePuy, Inc Warsaw, IN, USA) was commercially purchased.

2.2. Methods

ALABC were prepared by mixing PMMA and antibiotic at a weight/weight ratio of 10 and 20% (1 g of antibiotic per 10 g of PMMA and 2 g of antibiotic per 10 g of PMMA, respectively). Briefly, monomethylmethacrylate was added and stirred manually according to the directions of the manufacturer, and spherical beads (0.5 cm in diameter) were made before complete hardening. Every bead was weighed, and those with differences greater than 2 SDs were discarded to ensure reproducibility.

Each bead was placed in 1 mL of phosphate-buffered saline (PBS) and incubated at 37 °C. A volume of 1 mL was chosen in order to approximate the volume of serum that would surround the bead when multiple beads are packed into the dead space of bone following debridement surgery (Mader et al., 1997). The solution was completely exchanged at 30 min, 1, 2, and 4 h on day 1 in order to simulate the blood efflux after ischemia. After day 1, media was completely exchanged on a daily basis until day 30.

2.3. Determination of antimicrobial concentrations and antimicrobial activity

Antimicrobial concentrations were determined using high-performance liquid chromatography (HPLC) following validated specifications (Balbao et al., 2010; Hoizey et al., 2002; Lecaroz et al., 2006; Martens-Lobenhoffer et al., 2008; McWhinney et al., 2010; Pranger et al., 2010; Urabe et al., 2009; Verdier et al., 2011). We used an HPLC WATERS ALLIANCE 2690. The assay has a sensitivity limit of 0.1 μL/mL with intra- and interday coefficients of variation of less than 5% in the concentration range of 0.1–100 μL/mL.

Antimicrobial activity after the exothermic reaction was determined by disk diffusion method with *Micrococcus luteus* ATCC 9341 for VAN, DAP, RIF, and MOX and *Escherichia coli* ATCC 25922 for AMP, AmC, FEP, CTX, GM, ERT, and MER. Briefly, samples for antimicrobial activity were tested in duplicate using blank 1-cm discs saturated with 20 μL of the appropriate solution. The discs were then placed on Antibiotic Assay Medium #1 (Difco Laboratories, Detroit, MI, USA) agar plates impregnated with 50 mg/L calcium (for DAP assay) pre-swabbed with a 0.5 McFarland suspension of the reference organism, forming a confluent lawn. Plates were incubated at 37 °C for 24 h and then were examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured

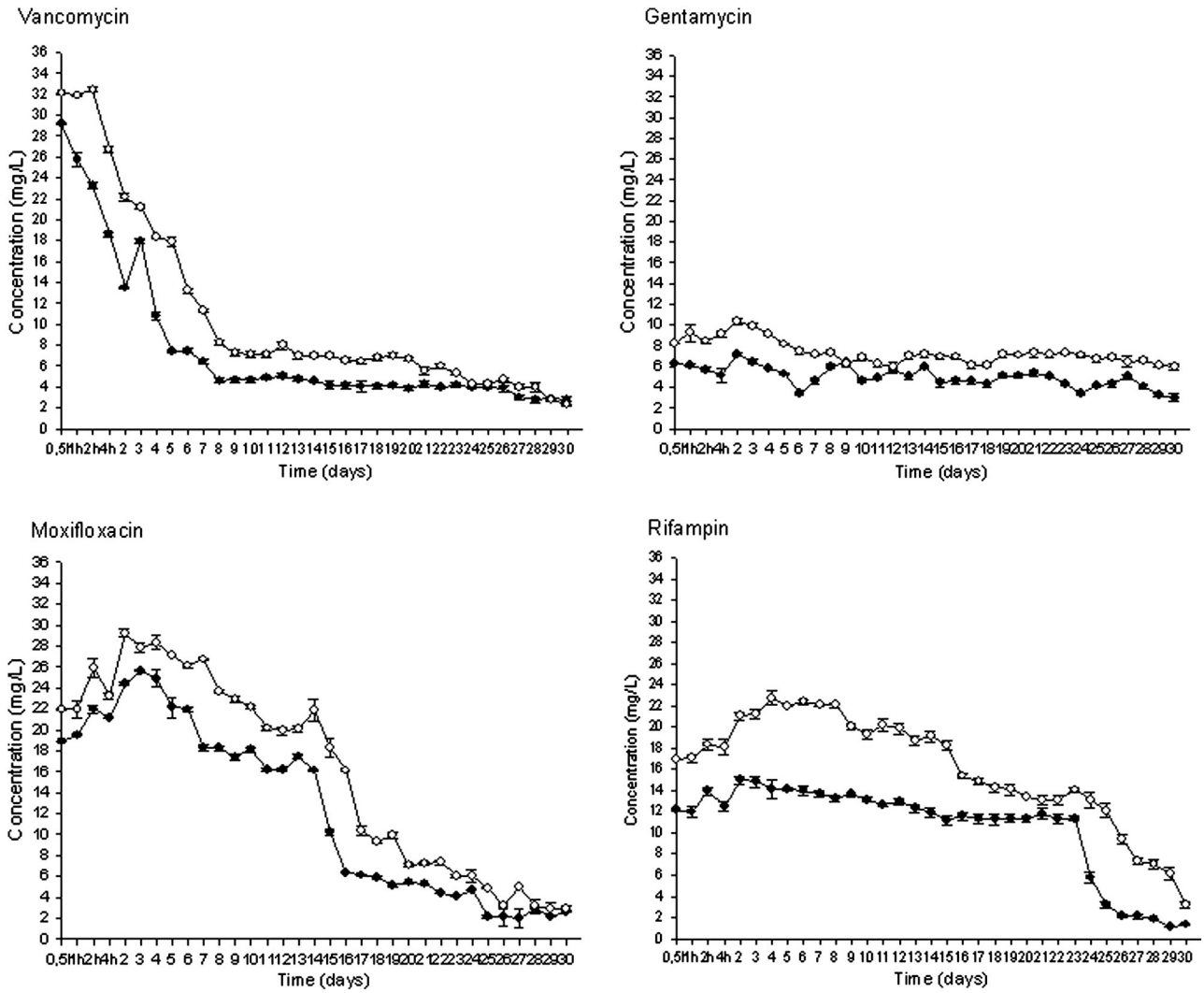

Fig. 1. Elution kinetics of VAN, GM, MOX, and RIF. Filled circles, 10% w/w concentration. Open circles, 20% w/w concentration.