The Journal of Arthroplasty 30 (2015) 1423–1429

Contents lists available at ScienceDirect

The Journal of Arthroplasty

journal homepage: www.arthroplastyjournal.org

Evaluation of Elution and Mechanical Properties of High-Dose Antibiotic-Loaded Bone Cement: Comparative "In Vitro" Study of the Influence of Vancomycin and Cefazolin

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ARTICLE INFO

Article history:
Received 12 October 2014
Received in revised form 22 February 2015
Accepted 28 February 2015

Keywords:
bone cement–PMMA
antibiotic
elution
water uptake
mechanical properties

ABSTRACT

Use of antibiotic-loaded bone cements is one of the most effective methods for the prevention and treatment of prosthetic joint infection. However, there is still controversy about the optimal combination and doses of antibiotics that provide the maximum antimicrobial effect without compromising cement properties. In this study, vancomycin and cefazolin were added to a bone cement (Palacos R + G). Antibiotic release, fluid absorption, and mechanical properties were evaluated under physiological conditions. The results show that the type of antibiotic selected has an important impact on cement properties. In this study, groups with cefazolin showed much higher elution than those containing the same concentration of vancomycin. In contrast, groups with cefazolin showed a lower strength than vancomycin groups.

Antibiotic-loaded bone cement (ALBC) is an accepted method used for prevention and treatment of prosthesis-related infection. The antibiotic is delivered directly to the surgical site. Use of bone cement as a source of antibiotics in patients with total joint arthroplasty was first reported more that 40 years ago [1]. Use of acrylic cement containing antibiotics is based on the principle that antibiotics will be gradually released from the cement over time in such a way that local antibiotic levels exceed the minimal inhibitory concentration of the pathogens of interest. The amount of antibiotics detected at local level is usually much higher than that achieved with parenteral therapy, but with lower systemic absorption. Given this, the antibiotic impregnation of cement is a logical aspect of prophylaxis and has proved effective in treating an established infection at revision surgery [2]. For this reason, it has been widely documented that a two-stage revision using a bone cement spacer (polymethylmetacrylate, PMMA) mixed with various antibiotics achieves the highest success rates in the treatment of periprosthetic joint infection (PJI) [3–5].

Orthopedic surgeons around the world routinely use ALBC to prevent infection after primary joint arthroplasties [6]. National joint arthroplasty registries have shown a reduction in the incidence of infection when ALBC was used. However, low-dose ALBC is approved by the FDA just for the use during the second stage of a revision after periprosthetic joint infection (PJI) [2].

Although incidence of PJI is low—ranging from 0.3 to 2% at 10 years [7,8]—, the there is a huge concern because of the large number of surgical arthroplasties performed every year [9], the elevated cost of the treatment, and emergent microorganism resistance to antibiotics [10]. It is estimated that direct costs of total knee arthroplasty (TKA) revision surgery will significantly increase in the next two decades, with hospital costs of these revisions expected to exceed US$ 2 billion [11]. Revision surgery procedures performed because of infection are associated with higher costs than those performed for aseptic failure [12].

The increasing resistance of periprosthetic bacteria to commonly used antibiotics, such as gentamicin and tobramycin, is an emerging problem [10]. Vancomycin is an alternative to the commonly used antibiotics [6], but has been shown to negatively affect fatigue strength of the cement when added at a 5% concentration, and it has a poor elution rate even when high vancomycin doses are used [13].

Many studies have been conducted with different types and doses of antibiotics [6] and other substances, such as xylitol [14], in order to enhance elution rate, testing their possible effect on cement properties, implant success, and effectiveness of the procedure [15], but not all

One or more of the authors of this paper have disclosed potential or pertinent conflicts of interest, which may include receipt of payment, either direct or indirect, institutional support, or association with an entity in the biomedical field which may be perceived to have potential conflict of interest with this work. For full disclosure statements refer to http://dx.doi.org/10.1016/j.arth.2015.02.040.

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http://dx.doi.org/10.1016/j.arth.2015.02.040
0883-5403/

antibiotic combinations are adequate. Klekamp et al [16] reported that vancomycin addition to tobramycin bone cement decreased tobramycin elution rate. Currently, the best way to increase vancomycin elution rate from ALBC is addition of large doses of antibiotic to bone cement, but this may have an impact on the mechanical properties of the cement [13,17]. This factor is less important when ALBC is used as a temporary treatment as spacer in PJI, where release of high amounts of antibiotic in the early hours is most important and mechanical strength is less important if performed as part of a two-staged procedure.

The aim of this study was to assess whether the type and dose of the antibiotic added have an impact on cement behavior when tested under conditions simulating a physiological environment, and to verify whether addition of two different antibiotics effective against most germs responsible for PJI affected the elution rate of each antibiotic. For this, two antibiotics (vancomycin and cefazolin) widely used for the treatment of PJI with different biochemical characteristics were added to commercial bone cement to evaluate antibiotic elution, the absorption fluid process, and mechanical properties in relation with the amount and type of antibiotic added.

Materials and Methods

Preparation of the Antibiotic-Loaded Bone Cement Samples

Palacos R + G bone cement (Heraeus Medical GmbH, Wehrheim, Germany) was loaded with different combinations of two antibiotics: vancomycin (Normon, Madrid, Spain), an antibiotic with a high molecular weight (1468 atomic mass units) and low water solubility, and cefazolin (Normon, Madrid, Spain), an antibiotic with a low molecular weight (450 atomic mass units) and high water solubility. Six groups with different amounts of antibiotic were studied; antibiotic levels used ranged from 2.5 to 10 wt. % based on powder component. Group 1 was the control group and no antibiotic was added manually. For group 2, 2.5 wt.% was used. In groups 3, 5 and 6 only vancomycin was added (2.5, 5 and 10 wt.% respectively) and group 3 contained cefazolin plus vancomycin (2.5 and 5 wt.%) (Table 1). The antibiotic was manually blended with the cement powder following the recommendations of Frommelt et al in order to homogeneously disperse the antibiotic [18].

For each of the groups, the antibiotic–cement powder blend and the liquid monomer were mixed in a commercial vacuum mixing system (HiVacVacuum Mixing System, Summit Medical, Gloucestershire, UK) with an evacuation pressure of 70 ± 0.1 kPa according to manufacturer instructions.

The properties of the different groups were studied under two test conditions: after aging, immersing in phosphate buffer saline (PBS, Sigma-Aldrich, St. Louis, MO, USA) at 37 °C to emulate a physiological (wet) medium, and without any aging process under dry conditions. Samples were prepared at room temperature (20 ± 2 °C) and relative humidity (35 ± 5%). Samples were stored for 1 week before testing or aging. Aged samples were conditioned at room temperature before testing.

Elution Study

Antibiotic elution tests were performed for each group. Test specimens in the shape of small cylinders (12 mm high and 6 mm in diameter) were formed for elution measurement and compression testing with a Teflon mold as specified in ISO 5833 standard [19]. Five cylindrical specimens of each group were immersed into individual plastic test tubes containing 5 mL of PBS. Test tubes were placed in an oven with a constant temperature of 37 °C. Samples were analyzed over 4 weeks at predefined time intervals of 3, 6, 9, 24, and 48 hours during the first days, and then at 1, 2, 3 and 4 weeks. At the predefined times, cylinders were removed from the tubes and immersed into fresh PBS.

Antibiotic concentrations were measured at each predefined elution time by UV–vis spectroscopy using a Cary 4000 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Spectrophotometric methods are convenient techniques because of their inherent simplicity, high sensitivity and low cost [20]. Cefazolin concentration was determined by direct measurement of absorbance at 272 nm [21–23]. However, vancomycin analysis required prior use of an analytical method described by Ashry et al [24] to measure absorbance at 444 nm. These methods to evaluate the elution of antibiotics have been widely reported in the literature [25,26].

Table 1
The Study Groups and the Amount of the Antibiotic Relative to the Mass of the Cement Powder.

	Cefazolin	Vancomycin
Group 1 (control)	–	–
Group 2	2.5 wt%	–
Group 3	–	2.5 wt%
Group 4	2.5 wt%	5 wt%
Group 5		5 wt%
Group 6		10 wt%

Fluid Uptake

The impact of the type and amount of antibiotic on the absorption and diffusion process of the fluid in the cement was assessed. A fluid absorption test of each of the cement groups was performed. For this, six 50 × 50 mm and 2-mm thick specimens from each group of cement were prepared and immersed into PBS at 37 ± 2 °C. Change in the mass of these samples over time was determined using an analytical balance Mettler-ToledoXS205 (Mettler-Toledo, Greifensee, Switzerland) with a precision of 0.01 mg. Weight was measured several times at specific intervals until 5 months of immersion [27].

Mechanical Properties

The effect of antibiotic addition and aging condition on the mechanical properties was evaluated with compression and bending tests. These were performed without aging and after 1 month of immersion. Compression strength was determined according to ISO 5833 standard using a universal testing machine IBTH/500 (Ibertest, Madrid, Spain) and cylindrical specimens 12-mm high and 6 mm in diameter. To analyze bending strength, four-point bending tests were performed according to ISO 5833 standard [19] using a universal testing machine ELIB 20 W (Ibertest, Madrid, Spain). Specimen dimensions were 80 × 10 × 4 mm.

Scanning Electron Microscopy

After the mechanical test, fracture surface was studied using an XL-30 scanning electron microscope (SEM) (Philips, Eindhoven, The Netherlands) together with an energy dispersive X-ray spectroscopy (SEM/EDS), which allows for visualization and analysis of surface features of materials. Energy of the electron beam was 10 kV. Samples were prepared using gold coating in a high-resolution Polaron SC7610 sputter coater (VG Microtech, Uakfield, United Kingdom) to provide a conducting medium for the electrons and adequate contrast in the SEM micrographs.

Statistical Analysis

Values were expressed as mean ± standard deviation. Results of each test were statistically analyzed using a 1-way analysis of variance (ANOVA) test, with a post-hoc Scheffe's test, using SPSS 15.0 for Windows (IBM SPSS, Chicago, USA). A *P*-value less than 0.01 was considered statistically significant.