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Study of the Influence of Bone Cement Type and Mixing Method on the Bioactivity and the Elution Kinetics of Ciprofloxacin

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ABSTRACT

The objectives of this study were to examine ciprofloxacin release from three trademarks of bone cements (Simplex®, Lima® and Palacos®) and its bioactivity using as variables, the mixing method, the chemical form of the antibiotic and the antibiotic combination. The antibiotic amount released in base form represents 35% of antibiotic amount released when hydrochloride form is incorporated. Moreover, the combination (vancomycin and ciprofloxacin) shows a stronger release (132%) than hydrochloride ciprofloxacin alone. Three cements show equal drug release profile ($P > 0.05$). A bioactivity simulation exercise showed that until 72 hours post-surgery, ciprofloxacin concentrations in the implant would be higher than 0.1 μg/mL in 100% of the patients. After drain removal, it is expected that bioactivity would increase since drug clearance from implant would decrease.

Antibiotic-loaded acrylic bone cement, described by Buchholz and Engelbrecht [1], is a well-established tool in the prophylaxis [2,3] and treatment of orthopedic infections [4] in humans and animals [5], with meta-analyses indicating that its use reduces the infection rate [6]. Polymethylmetacrylate – PMMA – is characterized by excellent biocompatibility with low intrinsic toxicity and inflammatory activation [7], but experience has shown that not all antibiotics have the properties necessary for their incorporation in this cement. In this context, aminoglycosides and glycopeptides (vancomycin) are known to be the two groups of antibiotics that satisfy the optimal criteria to be included in these cements (availability in powder form, wide antibacterial spectrum, bactericidity at low concentrations, elution from PMMA in high concentrations for prolonged periods, thermal stability, low or no risk of allergy or delayed hypersensitivity, low influence on the mechanical properties of the cement, and low serum protein binding) [8].

50% of surgical site infections (both superficial and deep) are caused by *Staphylococcus aureus* methicillin-resistant (MRSA); thus, staphylococcal species should be the primary target of antibiotic-loaded bone cement [9]. Unfortunately, the increasing number of multidrug-resistant bacteria [10–13] limits the continued effectiveness of this tool. In addition, the prevalence of MRSA in many hospitals influences strategies for the treatment and prevention of prosthetic joint infections [14], leading to interest in incorporating alternative antibacterial agents into PMMA cement [10,14,15].

On the other hand, despite the wide use of antibiotics in orthopedic surgery for more than 30 years, the exact mechanism by which they are eluted from PMMA is still not fully understood [8]. It seems to involve a biphasic profile, consisting of an initial rapid release of drug followed by a much slower sustained release. The following factors affect the release of antibiotics from bone cement: type and quantity of antibiotic [16,17]; type and porosity of cement [18]; surface characteristics [19]; and how the cement has been prepared [20–23]. Thus, to date only a few antibiotics have been satisfactorily incorporated into cements.

In this context, it would be desirable to incorporate new drugs into bone cements in order to increase coverage to infections caused by different organisms. In the present work, ciprofloxacin (1 g antibiotic/40 g PMMA) was selected to be assayed. This synthetic fluoroquinolone is an antibacterial agent that can be administered safely and effectively to treat most clinical isolates in infections associated with joint prostheses and chronic osteomyelitis. Additionally, ciprofloxacin possesses a broad spectrum against Gram positive and negative strains [24]. However, there are few data concerning the ability of ciprofloxacin to elute from bone cement and to retain activity against resistant pathogens after elution [25,26].

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In this study we set out to characterize the elution profile of ciprofloxacin from bone cements. The following variables were evaluated: source of drug (base and hydrochloride); cement composition (three brands); mixture method (manual and vacuum); and presence of a second antibiotic in the mixture. In addition, different equations were fit to release profiles in order to explain the release mechanism. Finally, bioactivity of the mixtures was evaluated by means of a simulation exercise.

Materials and Methods

Ciprofloxacin hydrochloride, ciprofloxacin base and vancomycin hydrochloride were purchased from Aldrich (Madrid, Spain). Lima CMT1® bone cement was purchased from Lima Implantes (Barcelona, Spain), and Palacos® and Simplex® from Ibersugical (Valencia, Spain). Each cement was provided as two separate components: a powder mixture and a liquid component. The composition of the cements is shown in Table 1, according to the information provided by the manufacturers.

Palamix uno®, the vacuum mixing system employed, was supplied by Heraeus Medical GmbH (Madrid, Spain).

Buffered saline solution pH 7.4 was prepared as described in the US Pharmacopoeia: disodium hydrogen phosphate anhydrous 2.38 g, mono-potassium hydrogen phosphate anhydrous 0.19 g and sodium chloride 8 g per 1 L of purified water. All reagents were analytical grade (Panreac).

Antibiotic-loaded bone cement cylinders were prepared as follows: 1 g of the drug was added to 40 g of solid component of the cement, and, after mixing the powder, the liquid component was added following the manufacturer's instructions. Cylinders of antibiotic bone cement were made for each batch in a standardized fashion according to the ISO normative 5833 (Annex E). Samples were prepared using Teflon molds in which they were kept for 1 hour until completely hardened into a cylinder/disk shape. Each specimen was carefully weighed and measured and the theoretical amount of loaded ciprofloxacin calculated. This value was used for calculating the exact percentage released from each sample.

When two antibiotics were incorporated into the cement the total amount of antibiotic in the mixture was 1 g (50% each one).

Samples were immersed in a water bath in 10 ml phosphate buffer saline pH 7.4 at 37 °C and stirred for 8 weeks. Samples were taken 1, 3, 5, 7, 24, 32, 48 56, 72, and 168 hours after immersion, and subsequently once a week for a period of 8 weeks (final sample was taken 56 days after immersion). Three samples per batch were tested. Antibiotic homogeneity distribution within batches was indirectly evaluated by means of the statistical analysis of the percentage of the total antibiotic released from the samples assayed. The phosphate buffer was replaced every time a sample was taken in order to maintain the sink condition (defined as the volume of medium at least three times that required in order to form a saturated solution of drug substance). All samples taken were frozen at −20 °C until analyzed. Table 2 summarizes the test conditions (a total of 61 samples were processed).

Ciprofloxacin concentration was assayed by HPLC, using a Perkin Elmer® Series 200 equipped with a Waters 484® UV detector (λ = 254 nm). The mobile phase consisted of acetic acid solution 0.1 M: acetonitrile (80:20) and was filtered through a 0.45 μm membrane filter before use. The mobile phase was eluted at a flow rate of 1 ml/min. The column was a Kromasil® C-18 with a pore size of 5.0 μm, measuring 150 mm (length) × 4.6 mm (diameter) [27].

Table 2
Samples Assayed in Each Condition Tested.

Mixture	Antibiotic	Bone Cement	Sample
Manual	Ciprofloxacin hydrochloride	Simplex®	A
		Lima®	B
		Palacos®	C
	Ciprofloxacin base	Lima®	D
	Ciprofloxacin hydrochloride and vancomycin	Simplex®	E
		Lima®	F
		Palacos®	G
Vacuum	Ciprofloxacin hydrochloride	Simplex®	H
		Lima®	I
		Palacos®	J

The elution rate at each time interval (mg/h) was obtained by dividing the total quantity of antibiotic released in each interval by the elution time (in hours). The elution rates and the total amount of antibiotic released (expressed as a percentage) at each time point were compared using one-way analysis of variance (ANOVA).

Zero order (Eq. (1)), first order (Eq. (2)), Higuchi (Eq. (3)) and Korsmeyer–Peppas (Eq. (4)) equations were fit to data to characterize elution parameters and the mechanism of release of ciprofloxacin from bone cement:

$$Q_t = k_0 t \tag{1}$$

$$Q_t = Q_0 \cdot e^{-k1t} \tag{2}$$

$$\frac{Q_t}{Q\infty} = K_h \cdot t^{0.5} \tag{3}$$

$$\frac{Q_t}{Q_\infty} = K_k t^n \tag{4}$$

where t is time, Q_t is the amount of drug released at time t, Q_0 is the initial amount of drug in the specimen, Q_∞ is the amount of drug released at time ∞, n is the release exponent and K_0, K_1, K_h and K_k are the ciprofloxacin elution rate constants of each of the kinetics.

Turface morphology and internal structure of the samples were characterized using a scanning electron microscope (SEM, S-4100 Hitachi, Madrid, Spain). The samples were mounted on an aluminum stub using double-sided tape. They were made electrically conductive by coating with gold-palladium under vacuum. The SEM picture was taken at an excitation voltage of 20 kV. For the internal structure evaluation samples were fractured and the broken surfaces sputter-coated with gold and a layer of palladium for examination at 20.0 kV.

The elution rate from different cements was used to simulate biophase concentration for 100 patients using NONMEM version VII.

Table 1
Composition of the Different Acrylic Bone Cements, As Provided by the Manufacturer.

	Lima CMT 1	Simplex	Palacos
Solid component (40 g)	Methyl methacrylate 87.6% Benzoyl peroxide 2.4% Barium sulfate 10%	Methyl methacrylate-styrene copolymer 30 g Polymethyle methacrylate 6 g Barium sulfate E.P. 4 g	Poly(methylacrylate. methyl methacrylate) 33.8 g Zirconium dioxide 6.0 g Benzoyl peroxide 0.2 g Colorant E141 0.008 g
Liquid component (20 mL)	Methyl methacrylate 84.4% Buthylmethacrylate 13.2% N, N-dimethyl pare toluidine 2.4% Hydroquinone 20 ppm	Methyl methacrylate 19.5 mL N, N-dimethyl pare toluidine 0.5 mL Hydroquinone, USP 1.5 mg	Methyl methacrylate 18.4 g N, N-dimethyl-p-toluidine 0.4 g Hydroquinone Colorant E141 0.005 g
Viscosity	Standard	Medium	High