

Mechanical effects of the use of vancomycin and meropenem in acrylic bone cement

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Background The increasing resistance of certain bacteria to antibiotics commonly used in bone cements has led to a demand for alternative antibacterial agents. The antibiotics added to bone cements may, however, have detrimental effects on the mechanical properties of the cement.

Material and methods We evaluated the mechanical effects of adding vancomycin and meropenem to bone cement by compression, bending and fatigue tests.

Results Addition of vancomycin at a concentration of up to 2.5% (w/w) had no effect on the compressive strength. Bending and fatigue strength were negatively affected by vancomycin but not by meropenem.

Interpretation A cement containing 1.25% vancomycin and 1.25% meropenem might be an interesting compromise between the introduction of antibacterial properties and preservation of the mechanical properties. With this concentration of additives the compressive strength and the fatigue strength remain unchanged, while the bending strength (–14%) and the bending modulus (–9%) are only slightly reduced and remain above the limits set by the ISO5833 standard.

Local delivery of antibiotics by means of an antibiotic-loaded acrylic cement (ALAC), used to fix the implant or in revision as a temporary spacer, has become a recommended practice in the management of infected arthroplasties (Durbhakula et al. 2004, Pitto and Spika 2004). When used to fix a prosthetic device, the mechanical properties of the

bone cement are of critical importance. In particular, fatigue strength is important, since it can play a role in long-term failure of cemented implants (Topoleski et al. 1990, Lewis 2003). However, antibiotics may impair the mechanical behavior of the cement (Klekamp et al. 1999, Armstrong et al. 2002). Furthermore, the increasing resistance of periprosthetic bacteria to commonly used antibiotics, such as gentamicin and tobramycin, is an emerging problem (Neu 1992, Tunney et al. 1998). Vancomycin is an alternative to the commonly used antibiotics (Ghisellini and Ceffa 1997, Chohfi et al. 1998), but has been shown to negatively affect the fatigue strength of the cement when added at a concentration of 5% (w/w) (Klekamp et al. 1999). To the authors' knowledge, however, its effect at lower concentrations has not yet been investigated. To cover the broadest antibacterial spectrum possible and to minimize the detrimental effects of vancomycin, combination of the latter with a different antibiotic may be of value (Penner et al. 1996, Cerretani et al. 2002). Such a combination may also have a positive effect on antibiotic elution. In fact, vancomycin used alone has presented lower elution levels than other antibiotics (Klekamp et al. 1999, Cerretani et al. 2002). Meropenem is an antibacterial agent with good, proven clinical efficacy (Bradley et al. 1999). We determined the effect of vancomycin alone and vancomycin and meropenem in combination on the mechanical behavior of polymethyl methacrylate (PMMA) bone cement.

Materials and methods

Materials

We chose a commercial bone cement (Cemex XL, Tecres, Verona, Italy) as control and as the basic material for the antibiotic formulations. This cement is based on PMMA and has a powder to liquid ratio of 2.7:1. The powder contains 12% (w/w) barium sulfate to provide radiopacity. We tested vancomycin in the form of vancomycin hydrochloride with a molecular weight of 1,486 g/mol (2.5% (w/w) HCl) and meropenem (a carbapenem) in its hydrated form with a molecular weight of 438 g/mol (12% (w/w) H₂O). Both the active substances have been investigated previously as antibiotics to be added to PMMA to give the cement antibacterial properties (Ghisellini and Ceffa 1997, Chohfi et al. 1998, Cerretani et al. 2002, Bertazzoni Minelli et al. 2004).

The meropenem was a more homogeneous powder than the vancomycin, which contained particles with a much wider range of sizes (more really small particles but also particles that were much larger than the maximum size of the meropenem particles) (Table 1).

The cements containing antibiotics were prepared by mixing the vancomycin and/or meropenem powders with the PMMA powder using a powder mixer (Omomix, Tecres, Verona, Italy) before proceeding according to the recommendations of the supplier and the ISO5833 standard. Components were mixed manually in a thermostatic chamber at 23 ± 1°C and at a humidity between 40% and 60%. The following formulations were investigated (the weight per cent refers to the amount of effective antibiotic added to the PMMA powder, not to the amount of cured bone cement): (1) bone cement without antibiotics (control), (2) bone cement with 1.25% (w/w) vancomycin (1.25 VA), (3) bone cement with 1.25% (w/w) vancomycin and 1.25% (w/w) meropenem (1.25 VA + 1.25 ME), and (4) bone cement with 2.5% (w/w) vancomycin (2.50 VA).

Static mechanical testing

Specimens were produced by molding, each mold giving rise to 6 specimens. For each type of test, compression and bending, 4 molding repetitions were performed, giving 24 specimens of each mate-

Table 1. Particle sizes (µm) of the antibiotic powders

	Width mean (SD) range	Length mean (SD) range
Vancomycin	12 (11) 1–69	20 (18) 1–123
Meropenem	10 (5) 3–35	21 (12) 7–67

rial for each type of test. Specimens were stored at 23 ± 1°C for 24 ± 2 hours before testing, according to ISO 5833 recommendations.

Compression tests were performed in a materials testing machine (Instron 8502; Instron Corp., Canton, MA) according to ISO 5833 recommendations, on cylindrical specimens (diameter 6 mm, height 12 mm) at a cross-head rate of 20 mm/min.

Four-point bending tests were also performed in a materials testing machine (Mini Bionix 858; MTS Systems Corp., MN) according to ISO 5833 recommendations, on rectangular specimens (75 × 10 × 3.3 mm) at a cross-head rate of 5 mm/min. The bending test setup was equipped with an extensometer (model 632.06H-20; MTS Systems) to measure the deflection of the center of the specimen, which was done at 15 N and 50 N in order to calculate the bending modulus.

The results of the static tests were examined by analysis of variance (ANOVA) and post-hoc analysis (Scheffe's test).

Fatigue testing

Fatigue strength tests were carried out in a Mini Bionix 858 materials testing machine, according to a previously validated method (Cristofolini et al. 2000). The geometry of the specimens was of the flat dog-bone type. Their dimensions were: length 200 mm, width 10 mm (at the narrowed part) and thickness 4 mm. They were stored in water at 37°C for at least 14 days before the test, to ensure complete polymerization of the material before testing. Specimens showing macropores (defects larger than 1 mm in size) in mammographic films were discarded to eliminate effects due to improper specimen preparation. A sinusoidal uniaxial zero-tension loading was applied at a frequency of 4 Hz, this type of loading being chosen because it represents a critical condition for the accumulation of fatigue damage found in vitro and in vivo (Gates et al. 1983, McCormack et al. 1996). The

Table 2. Compressive strength and bending strength values (MPa) of the bone cement samples tested

	Compressive strength	Bending strength	Bending modulus
Control	102 (4)	59 (4)	2,528 (89)
1.25 VA	100 (3)	52 (3)	2,266 (117)
1.25 VA + 1.25 ME	98 (3)	51 (4)	2,304 (57)
2.50 VA	98 (4)	46 (3)	2,132 (55)

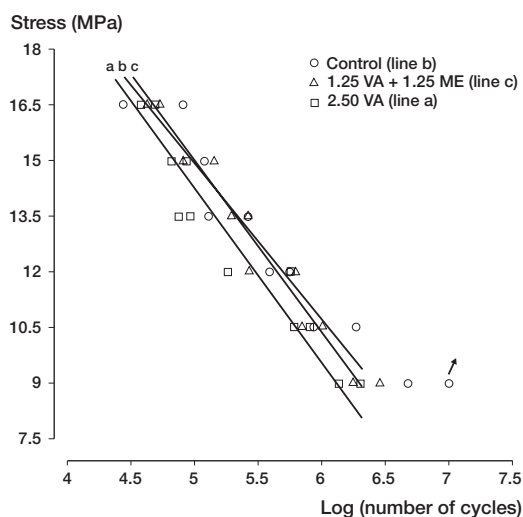
test environment was air at 23°C, representing an environmentally critical condition in terms of bone cement fatigue testing (Freitag and Cannon 1977, Nguyen et al. 1997). Tests were done at 6 stress levels (9.0, 10.5, 12.0, 13.5, 15.0 and 16.5 MPa) and 2 specimens of each material were tested at each level. The tests were stopped at failure of the specimen or at the completion of 10 million cycles. The experimental data were reported on a graph of stress plotted against the log of the number of cycles (referred to as a Wöhler diagram) and fitted with regression lines. The slope values of the three regression lines and the intersections at 2 million cycles were estimated, the latter values giving a rough estimate of the fatigue limit of the materials. The results were examined by analysis of covariance (ANCOVA) and post-hoc analysis. The three regression slopes calculated were used for the fatigue limit analysis.

Results

Static mechanical testing (Table 2)

No significant effect was found on the compressive strength of the investigated bone cements as a result of adding antibiotics. All cements presented values well above the minimum requirement of the ISO standard (70 MPa).

The addition of antibiotics affected the bending strength and the bending modulus of the material ($p < 0.001$). Post-hoc analysis showed that there was a significant difference in bending strength and in bending modulus ($p < 0.001$) between all materials, except between 1.25 VA and 1.25 VA + 1.25 ME. However, while these two materials still fulfilled the minimum requirement for the bending strength (50 MPa), the 2.50 VA cement presented a



Wöhler diagram for the bone cement samples tested.

mean value below this limit. All materials showed a bending modulus above the minimum requirement of 1,800 MPa.

Fatigue testing

The data on stress and number of cycles used to calculate the slope part of the Wöhler curve for each bone cement mixture are shown in the Figure. The calculated coefficients of determination, R^2 , were greater than 0.9. There was no significant difference between the slopes of the three regression lines ($p = 0.8$). An effect due to the addition of antibiotics was, however, found in the fatigue limit of the cements ($p = 0.002$). Post-hoc analysis showed that there was a significant difference in fatigue limit only between the control and 2.50 VA ($p = 0.002$). 1.25 VA was not subjected to fatigue testing since 1.25 VA + 1.25 ME did not have a significant effect on the fatigue strength of the material.

Discussion

Our findings confirm that the addition of antibiotics may affect the mechanical behavior of the bone cement. We found no significant reduction in the compressive strength of the bone cement mixtures investigated, which is in accordance with previous studies (Chohfi et al. 1998, Klekamp et al. 1999). However, while we found no reduction or accept-

ably little reduction in the mechanical behavior of 1.25 VA cement and 1.25 VA + 1.25 ME cement in comparison with the control, 2.5 VA cement showed reduced bending and fatigue strength compared to the control cement. Our findings regarding the reduced bending strength are consistent with those of a previous study (Armstrong et al. 2002).

Previous studies of the fatigue properties of PMMA containing antibiotics are contradictory: sometimes no significant reduction in fatigue strength has been found after adding gentamicin or erythromycin and colistin (Davies et al. 1989, Baleani et al. 2003) or tobramycin (Davies and Harris 1991), while in other cases a significant reduction in fatigue strength has been found after adding gentamicin (Schurman et al. 1978, Davies et al. 1989) or vancomycin (Klekamp et al. 1999). Several possible explanations for these differences can be found. Specimens with pores larger than 1 mm may not have been discarded in the former cases, which would lead to greater data scattering (Cristofolini et al. 2000) and hence significant differences are harder to find. The amount of antibiotic added to the bone cement may also vary. Furthermore, different antibiotics may have different effects on the bone cement. In fact, vancomycin is an amphoteric substance and has a higher molecular weight than the antibiotics investigated previously, including meropenem. This could possibly bring about an interaction of part of the monomer liquid with the vancomycin, which might lead to a lower molecular weight of the final polymeric chains and thus to a compromising of the mechanical properties of the cement. In addition, there is the problem that the antibiotic powder exists as an inclusion in the cement and might act as a stress riser (Topoleski et al. 1990). In the case of vancomycin, this problem may be intensified by the fact that a proportion of the vancomycin particles were found to be much larger than those of meropenem and those of another commonly used antibiotic (gentamicin) (Lewis et al. 2005).

That the vancomycin molecule is larger than those of other antibiotics may also be a reason for the lower elution rates of this antibiotic from PMMA compared to others (Klekamp et al. 1999, Cerretani et al. 2002). However, when added in amounts of 2.5% (w/w), vancomycin can elute several months after implantation and the levels of this antibiotic

found in periprosthetic tissues have been found to lie above the minimal inhibitory concentration (MIC) for most of the common bacteria involved in orthopedic prosthesis infections (Bertazzoni Minelli et al. 2004). In vitro elution tests have also shown that with 2.5% vancomycin, the antibiotic levels are above the MIC necessary to eliminate all relevant bacteria still after 1 week (Penner et al. 1996, Tunney et al. 1998). However, our data show a detrimental effect on the mechanical behavior of PMMA when 2.5% vancomycin is added. While this detrimental effect of the antibiotic on the mechanical properties is negligible when the bone cement is used as a temporary spacer, it becomes a problem when the cement is used as a filler during prosthesis reimplantation. It has, however, been found that vancomycin elutes more effectively when combined with other antibiotics (Penner et al. 1996), including imipenem/cilastatin which is a carbapenem like meropenem (Ghisellini and Ceffa 1997, Cerretani et al. 2002). This is probably due to what Penner et al. have called “passive opportunism”, i.e. when the second antibiotic dissolves it brings about not only antibacterial activity, but also an increased porosity that facilitates the elution of the first one (Penner et al. 1996). Thus, the formulation 1.25 VA + 1.25 ME (containing a total of 2.5% (w/w) of antibiotics) seems to represent a potentially interesting formulation to gain antibacterial properties to be investigated without significantly reducing the mechanical strength of the PMMA—except for the bending modulus (–9%) and the bending strength (–14%), which still, however, fulfill the minimum requirements stipulated for these properties.

Contributions of authors

CP: fatigue testing, data analysis and writing of the paper. MB: static mechanical testing, fatigue testing, data analysis and writing of the paper. LG: static mechanical testing. DT: research promoter and writing of the paper. MV: scientific head of the laboratory.

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