Effective bactericidal activity of tobramycin and vancomycin eluted from acrylic bone cement

Alejandro González Della Valle[1], Mathias Bostrom[2], Barry Brause[2], Carolyn Harney[2] and Eduardo A Salvati[2]

Departments of Orthopedics, [1]Hospital Italiano de Buenos Aires, Argentina. E-mail: agdv@bifgoot.com; [2]Cornell University Medical College, The Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021, USA
Submitted 00-05-09. Accepted 00-11-23

ABSTRACT – We studied the bioactivity of vancomycin and tobramycin eluted from methylmethacrylate bone cement. Aliquots of the drainage were obtained at 1, 6, 12 and 24 hours following total hip prosthetic implantation with vancomycin-tobramycin-loaded cement in 3 patients. The samples were analyzed with fluorescence polarization immunoassay and bioassay, using group B streptococcus for vancomycin and Escherichia coli for tobramycin. These bacteria were selected due to the effectiveness of vancomycin and poor effectiveness of tobramycin against group B streptococcus and conversely with E. coli.

The immunodetection of vancomycin averaged 14 (1 hour), 9 (6 hours), 10 (12 hours) and 11 µg/mL (24 hours). The bioassay averaged 47, 36, 79 and 41 µg/mL (p = 0.03). The immunodetection of tobramycin averaged 43, 21, 18 and 14 µg/mL; and bioassay 30, 15, 15 and 12 µg/mL (p = 0.1). Both antibiotics eluted with a highly effective bactericidal activity. Our findings indicate that the presence of tobramycin has a synergistic-like effect on the bactericidal activity of vancomycin, which has not been previously reported. We recommend a combination of vancomycin and tobramycin with cement for the treatment of orthopedic infections caused by gram-positive organisms.

Commonly used antibiotics elute from acrylic cement and the antibiotic levels vastly exceed the minimum inhibitory and bactericidal concentrations of sensitive bacteria. Thus gentamicin levels may become 7 times higher than those obtained with intravenous therapy (Salvati et al. 1986, Brien et al. 1993). With the emergence of resistant bacteria, adding a potentially synergistic combination of two antibiotics to bone cement has become a frequent practice. Vancomycin and an aminoglycoside are often combined for their potential synergistic effect in the treatment of severe infections caused by methicillin-resistant Staphylococcus aureus (Klekamp et al. 1999).

Since the early 1970s, it has been documented that beta-lactam antibiotics can combine chemically with most aminoglycosides when there is a high concentration of both drugs or when their excretion is delayed (Henderson et al. 1981). The combination of a beta-lactam molecule with that of an aminoglycoside can inactivate equimolar amounts of both antibiotics. When high doses of both antibiotics are combined with the cement, the inactivation of both drugs may jeopardize the bactericidal properties of the admixture. To our knowledge, this phenomenon has not been studied.

We determined the 'in vivo' elution and biological activity of vancomycin and tobramycin combined with bone cement.

Patients and methods

3 patients (2 women) were recruited for this study after obtaining informed consent at the Hospital for Special Surgery. 2 underwent total hip reimplantation with cement after chronic infection caused by methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis; the third patient was considered at high risk of infection because of a his-

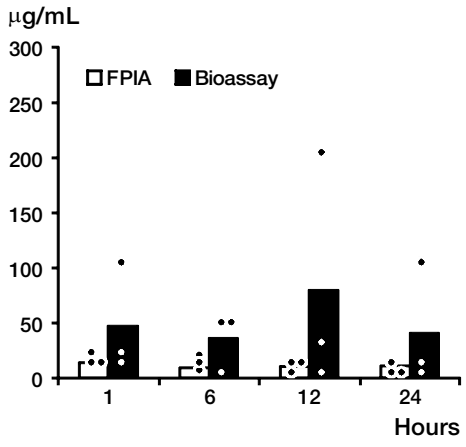

Figure 1. Comparison of FPIA and bioassay for vancomycin against group B streptococcus. Dots indicate individual values. P = 0.03 for difference between radioimmunoassay and bioassay.

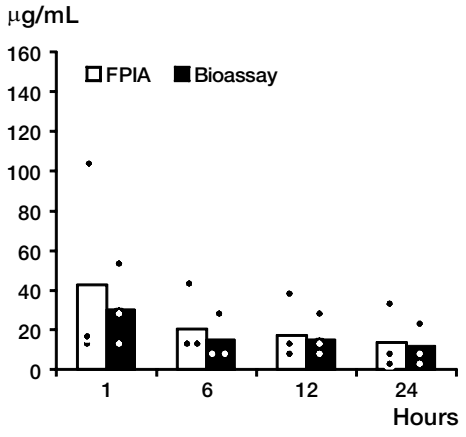

Figure 2. Comparison of FPIA and bioassay for tobramycin against Escherichia coli. Dots indicate individual values. P = 0.1 for difference between radioimmunoassay and bioassay.

tory of recurrent urinary tract infections and pneumonia, and underwent hybrid total hip replacement. Average age was 72 (64–82) years. None of the patients received vancomycin or tobramycin intravenously during surgery and for the first 24 postoperative hours.

The components were fixed with Simplex-P radiopaque bone cement (Howmedica, Rutherford, NJ, USA). 1 g of vancomycin (Eli Lilly and Company, Indianapolis, IN, USA) and 1.2 g of tobramycin (Eli Lilly and Company, Indianapolis, IN, USA) were thoroughly mixed with 40 mg of methyl-methacrylate polymer. Then, liquid monomer was added and vacuum mixed.

After implantation, 10 cc aliquots of the drainage were collected under sterile precautions at 1, 6, 12 and 24 hours. The drainage container was changed after each collection. The specimens were centrifuged and the serum frozen. The quantity of antibiotics was measured using fluorescence polarization immunoassay (FPIA) (Axsym System, Abbott Laboratories, Abbott Park, IL, USA) (Jolley 1981, Jolley et al. 1981). The results were recorded in μg/mL. The low-end sensitivity of this technique was 0.18 μg/mL. The measurements were repeated with controls.

To determine the bioactivity, aliquots from the same samples were analyzed by a standardized bioassay (National Committee for Clinical Laboratory Standards 1993). The tube dilution bioassay was employed with twofold fluid dilutions using a selected group B streptococcus for vancomycin (minimum inhibitory concentration 0.1 μg/mL) and Escherichia coli for tobramycin (minimum inhibitory concentration 0.4 μg/mL).

Statistical analysis of the differences between the radioimmunoassay and bioassay for both antibiotics was performed using the paired t-test, with an alpha of 0.05.

Results

The FPIA for vancomycin revealed an average detection of 14 (7–19) μg/mL in the first hour, 9 (5–14) μg/mL after 6 hours, 10 (6–13) μg/mL after 12 hours and 11 (8–16) μg/mL after 24 hours. The bioassay of vancomycin when exposed to group B streptococci revealed an average of 47 (13–102) μg/mL after the first hour, 36 (6–51) μg/mL after 6 hours, 79 (6–205) μg/mL after 12 hours and 41 (6–102) μg/mL after 24 hours. The difference in the detection of vancomycin with FPIA and bioassay was statistically significant (p = 0.03) (Figure 1). The enhanced bioactivity observed represents additive and potentially synergistic action against the assay organism in this study.

The FPIA for tobramycin revealed an average detection of 43 (10–103.2) μg/mL in the first hour, 21 (10–41) μg/mL after 6 hours, 18 (5–39) μg/mL after 12 hours and 14 (3–31) μg/mL after 24 hours. The bioassay of tobramycin when exposed to E.

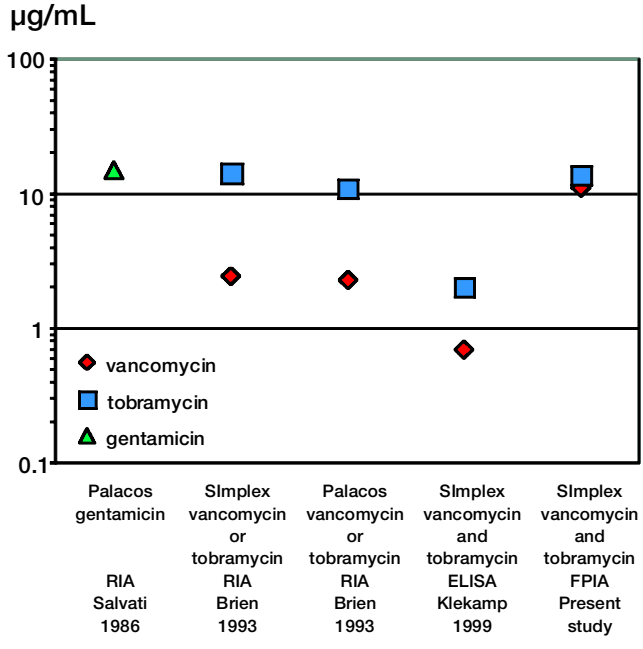

Figure 3. Local antibiotic levels of vancomycin and tobramycin combined with bone cement 24 hours after implantation.

coli revealed an average of 30 (6–51) µg/mL after the first hour, 15 (6–26) µg/mL after 6 hours, 15 (3–26) µg/mL after 12 hours and 12 (2–26) µg/mL after 24 hours. The difference in the detection of tobramycin with FPIA and bioassay was not statistically significant ($p = 0.1$) (Figure 2).

Discussion

The elution of tobramycin provides high local levels with very low transient serum levels (Salvati et al. 1986). However, vancomycin elutes erratically with suboptimal bioactivity in 30% of cases (Brien et al. 1993). The elution characteristics of vancomycin and tobramycin are also affected by the type of bone cement utilized, with the highest elution from Palacos (Greene et al. 1998).

Only a few studies are available on the elution characteristics of the combination of vancomycin and tobramycin from acrylic cement (Penner et al. 1996, Masri et al. 1998, Klekamp et al. 1999). This is a matter of concern because inactivation of penicillins and aminoglycosides when combined has been widely reported (Davies et al. 1975, Ervin et al. 1976, Henderson et al. 1981, Blair et al. 1982, Uber et al. 1991). Inactivation is thought to occur by means of nucleophilic opening of the beta lactamic ring, which then combines with an amino group of the aminoglycoside, leading to the formation of a biologically inactive amide (Ervin et al. 1976, Henderson et al. 1981, Blair et al. 1982). Traditionally, this phenomenon may be clinically relevant: when both antibiotics are mixed in the same injectable solution, when blood samples are stored at room temperature before undergoing assays for antibiotic concentration, and in patients with renal failure (Ervin et al. 1976, Henderson et al. 1981, Blair et al. 1982).

Unless an interaction between the antibiotics takes place, levels of antibiotics similar to those we observed for tobramycin would be expected in the FPIA and bioassay (Figure 2). The comparison of the FPIA and bioassay for vancomycin indicates an increased bioactivity in all patients for all samples, showing an additive or synergistic effect of tobramycin on the biological activity of vancomycin. Although this phenomenon has been found in vitro when these antibiotics are administered intravenously (Leclercq et al. 1991), to our knowledge, it has not been shown for antibiotics eluted from bone cement in vivo.

Only a few studies have analyzed the in vitro elution of vancomycin and tobramycin combined with bone cement (Penner et al. 1996, Masri et al. 1998, Klekamp et al. 1999). The in vivo activity of

this combination has not been addressed. Klekamp and coworkers studied the elution of tobramycin and vancomycin combined with Simplex or Palacos cement, using enzyme-linked immunosorbent assay. They observed that the elution of tobramycin was compromised by the presence of vancomycin. However, the elution of vancomycin was not affected by the presence of tobramycin (Klekamp et al. 1999). Penner and coworkers showed in vitro that the combination of both antibiotics in bone cement increases the elution of vancomycin to 103% and of tobramycin to 68%, compared to controls containing vancomycin or tobramycin alone (Penner et al. 1996).

Long-term elution of vancomycin and tobramycin from bone cement was studied in vivo by Masri and coworkers (1998). 49 patients who had had an infected total hip or knee replacement removed and temporary implantation of an antibiotic-loaded prosthesis (PROSTALAC) had the antibiotic levels of vancomycin and tobramycin measured by FPIA at the second-stage reimplantation, after an average of 118 (42–340) days (Masri et al. 1998). They found that tobramycin elution was better than that of vancomycin, but the elution of vancomycin was increased by the presence of tobramycin. No bioassays were performed.

Our study shows that vancomycin and tobramycin eluted from acrylic bone cement have adequate bactericidal levels and biological activity. We also found an additive or synergistic effect of the biological activity of vancomycin when combined with tobramycin in acrylic cement.

Blair D C, Duggan D O, Schroeder E T. Inactivation of amikacin and gentamicin by carbenicillin in patients with end-stage renal failure. Antimicrob Agents Chemother 1982; 22 (3): 376-9.

Brien W W, Salvati E A, Klein R, Brause B, Stern S. Antibiotic-impregnated bone cement in total hip arthroplasty: an in vivo comparison of the elution properties of tobramycin and vancomycin. Clin Orthop. 1993; 296: 242-8.

Davies M, Morgan J R, Anand C. Interactions of carbenicillin and ticarcillin with gentamicin. Antimicrob Agents Chemother 1975; 7 (4): 431-4.

Ervin F R, Bullock W E, Nuttal C E. Inactivation of gentamicin by penicillins in patients with renal failure. Antimicrob Agents Chemother 1976; 9 (6): 1004-11.

Greene N, Holtom P D, Warren C A, Ressler R L, Shepherd L, McPherson E J, Patzakis M J. In vitro elution of tobramycin and vancomycin polymethylmethacrylate beads and spacers from Simplex and Palacos. Am J Orthop 1998; 27 (3): 201-5.

Henderson J L, Polk R E, Kline B J. In vitro inactivation of gentamicin, tobramycin, and netilmicin by carbenicillin, azlocillin, or mezlocillin. Am J Hosp Pharm 1981; 38: 1167-70.

Jolley M E. Fluorescence polarization immunoassay for determination of therapeutic drug levels in human plasma. J Anal Tox 1981; 5: 236-40.

Jolley M E, Stroupe S D, Schwenzer K S, et al. Fluorescence polarization immunoassay. III. An automated system for therapeutic drug determination. Clin Chem 1981; 27: 1575-9.

Klekamp J, Dawson J M, Haas D W, DeBoer D, Christie M. The use of vancomycin and tobramycin in acrylic bone cement: biomechanical effects and elution kinetics for use in joint arthroplasty. J Arthroplasty 1999; 14 (3): 339-46.

Leclercq R, Bingen E, Su Q H, Lambert-Zechovski N, Couvalin P, Duval J. Effects of combinations of beta-lactams, daptomycin, gentamicin, and glycopeptides against glycopeptide-resistant enterococci. Antimicrob Agents Chemother 1991; 35 (1): 92-8.

Masri B A, Duncan C P, Beauchamp C P. Long-term elution of antibiotics from bone cement. An in vivo study using the prosthesis of antibiotic-loaded acrylic cement (PROSTALAC) system. J Arthroplasty 1998; 13 (3): 331-8.

National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically - Third Edition; Approved Standard. NCCLS document M7-A3. NCCLS, Villanova, Pennsylvania 1993.

Penner M J, Masri B A, Duncan C P. Elution characteristics of vancomycin and tobramycin combined in acrylic bone cement. J Arthroplasty 1996; 11 (8): 939-44.

Salvati E A, Callaghan J J, Brause B D et al. Reimplantation in infection: elution of gentamicin from cement and beads. Clin Orthop 1986; 207: 83-93.

Uber W E, Brundage R C, White R L, Brundage D M, Bromley H R. In vivo inactivation of tobramycin by piperacillin. DICP 1991; 25: 357-9.