BONE CEMENT AND GENTAMICIN IN THE TREATMENT OF BONE INFECTION. BACKGROUND AND IN VITRO STUDY

MATHEUS LEMOS AZI, MAURICIO KFURI JUNIOR, ROBERTO MARTINEZ, CLEBER ANTONIO JANSEN PACCOLA

ABSTRACT

Objective: To determine the elution characteristics of the antibiotic (gentamicin) mixed with bone cement. Methods: 480mg of gentamicin was added to 40g of bone cement. Ten specimens were immersed in buffered saline solution for 28 days. Samples of days 1, 2, 7, 14, 21 and 28 were analyzed by the fluorescence polarization immunoassay method. Results: Most of the gentamicin was eluted from the cement in the first 24 hours. A gradual downslide occurred between days 2 and 14. By the 28th day, there was no trace of the antibiotic. Conclusion: The mixture released high amounts of the antibiotic in a predictable (therapeutic) manner during at least fourteen days.

Keywords: Infection, Osteomyelitis. Polymethylmethacrylate. Gentamicin.

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INTRODUCTION

Bone infection treatment is a major therapeutic challenge and the strategy most frequently used is a combination of surgical debridement and antibiotic use. In addition there is the possible removal of the orthopedic implants and coverage with muscular flaps, allowing an improvement of vascularization at the site. Besides curing the infection, other desirable objectives are to obtain stability and avoid the dead space in the wound. Infection control occurs in 78% of the patients in the first year, and eradication of the disease is achieved in only 77% of patients.[1,2]

The penetration of antimicrobial agents in the bone depends on their pharmacological characteristics, but can be dramatically altered by local conditions. If the bacteria are inside non-vascular bone, is not possible to attain therapeutic concentrations and, if adhered to biomaterials, levels 10 to 100 times higher than usual are necessary.[3,4]

One of the main advances in the treatment was the use of local antibiotics, which make it possible to reach high concentrations in the wound, with low serum levels and low systemic toxicity.[5]

The use of antibiotic associated with polymethylmethacrylate (PMMA) as carrier agent was adopted for the first time by Buchholz et al.[6] for prophylaxis and treatment of infection in hip arthroplasties. Klemm[7] successfully introduced its use in osteomyelitis treatment, manipulating the mixture to form a necklace of beads joined by a cable that was positioned in the infection region.

The use of cement with local antibiotic associated or not with a short period of systemic antibiotic is an effective method in the treatment of bone infection. Klemm[7] reported a recurrence rate of just 9.6% in 405 cases of osteomyelitis, treated with the implantation of gentamicin beads. Calhoun et al.[8] obtained a reduction in costs and control of infection in 89.3% of the cases treated with the use of gentamicin beads, as opposed to 83.3% with the use of systemic antibiotic for four weeks. Nelson et al.[9] compared the use of gentamicin beads associated with systemic antibiotic for five days, versus systemic antibiotic for six weeks, in the treatment of infection in hip and knee prostheses, with a lower rate of reinfection with the use of local antibiotic (15% versus 30%). The prophylactic use of gentamicin beads associated with systemic antibiotic in the treatment of exposed fractures reduced the rate of infection from 17 to 4%[10] and the method has also been described successfully in the treatment of infected bone defects.[11-14]

Many factors are involved in antibiotic release by PMMA. Cement properties such as composition, porosity and quantity of the monomer, interact with environmental factors, such as tempera-

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Department of Biomechanics, Medicine and Rehabilitation of the Locomotor System - Ribeirão Preto Medical School - University of São Paulo.

Study conducted in the Department of Biomechanics, Medicine and Rehabilitation of the Locomotor Apparatus, of Faculdade de Medicina de Ribeirão Preto of Universidade de São Paulo. Mailing Address: Departamento de Biomecânica, Medicina e Reabilitação do Aparelho Locomotor. Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Av Bandeirantes 1900, CEP 14048-900, Ribeirão Preto, São Paulo, Brazil. E-mail:mlazi@hotmail.com

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ture, humidity and pressure, affecting their physical characteristics. The type of antibiotic, the quantity added and the exchange of fluids in the wound also influence them.[5,15] The three-dimensional form is of great importance, whereas the maximum elution is obtained when using small elongated beads, which provide a larger surface area.[15] The addition of antibiotic alters the mechanical properties of the cement and liquid gentamicin, in the dose of 480mg, decreases the compression resistance by 49% and traction resistance by 46%.[16]

The release of the antibiotic by PMMA can be qualified as bimodal. In the first hours it is quickly dispersed, presumably by dissolution on the exposed surface of the cement. After this period, there is much slower release, by means of passive diffusion through the cement mass.[5,15] In this typical biphasic pattern, a peak occurs in the first 24 hours, followed by a long period of release of low doses, which can last from days to months, with traces of the antibiotic still being found 5 years after implantation.[5,15,17]

The antibiotic should preferably be in powdered form, as the aqueous solution has limited incorporation.[5] The agent should be stable at high temperatures, as the interior of the cement mass can reach 107 ºC during polymerization.[18] It should also present a low risk of allergic reactions.[5] The quantity of antibiotic released depends on its concentration in the mixture and on the quantity of mixture implanted in the patient, whereas the doses recommended for clinical use in infection treatment are: from 2 to 3g for gentamicin, 3g for tobramycin, 4g for vancomycin, cefepime and imipenem, and 6g for cefazolin and nafcillin, for a packet of 40g of bone cement.[5,6,19,20]

Gentamicin has been the antibiotic most frequently used for local application and its mixture with PMMA is commercially available in the form of bone cement already mixed or in the form of gentamicin beads (Septopal®),[5,7] yet these presentations have a high cost.[21] On the other hand, powdered gentamicin is commercially available in Brazil at a low price, and its addition to PMMA at the time of use might be a form of reducing costs and of facilitating access to this therapeutic method.

The aim of this study was to analyze *in vitro* the elution characteristics of gentamicin added to bone cement at the time of use, with the intention of evaluating safety for clinical use of this mixture.

MATERIAL AND METHODS

Making of test specimens

A Teflon resin® mold was developed for the preparation of the test specimens, with holes measuring 15mm in depth and 6mm in diameter and a second hole at the center measuring 3mm in depth and 3mm in diameter, which reached the other side. (Figure 1)

The bone cement used was Surgical Simplex P® (Howmedica, Limerick, Ireland). The preparation was performed at room temperature under aseptic technique. The polymer was placed in a tub with 480mg of powdered gentamicin sulfate, and mixed with 40g of polymer until a homogeneous mixture was formed, at which point the monomer (liquid) was added. After this the mixture was introduced manually in the mold. (Figure 2) The surplus was removed with a spatula and the cement left to cure for thirty minutes, before the removal of the test specimens.

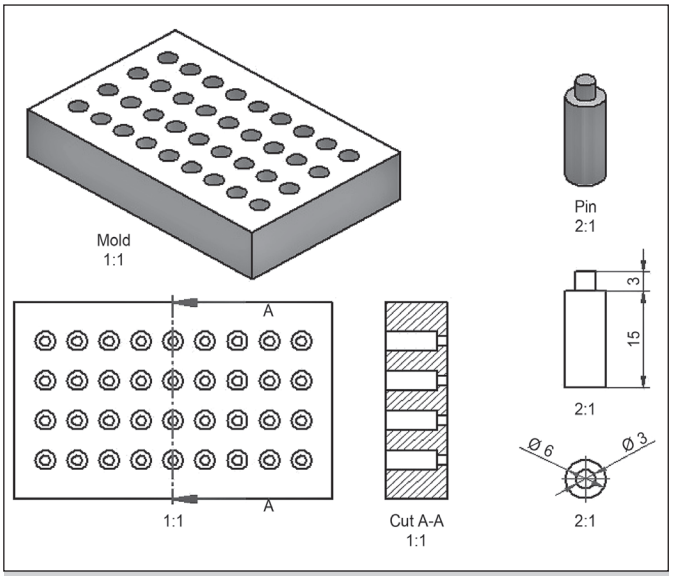

Figure 1 – *Schematic template of the mold and of the test specimen.*

Elution test

Ten test specimens were weighed and measured, and then placed individually in test tubes, with 10ml of buffered saline solution with phosphate (pH 7.4). The experiment was conducted at room temperature, protected from light and with replacement of the solution every 24 hours. The samples were stored at -20°C until the time of analysis. The concentration of gentamicin in the solutions corresponding to days 1, 2, 7, 14, 21 and 28, was measured through the Fluorescence Polarization Immunoassay (FPIA) method, with the Abbott TDx apparatus (Abbott Diagnostics, North Chicago, IL, USA),[22] where each sample was tested twice. The statistical analysis was carried out with the GraphPad Prism 3.02® program and the Kruskal-Wallis test was used with Dunn's post test and significance level of $p = 0.05$.

RESULTS

The test specimens presented total surface area of 367mm^2. The mean weight was 0.58 grams, and it can be deduced that each one had an average 6.8mg of gentamicin in its composition. The study was conducted at the average temperature of 25°C and the average quantity of gentamicin released in the first 24 hours was 6.15μg/ml. On the second day this value dropped to 0.31μg/ml and on days 7, 14, 21 and 28 it was 0.15μg/ml, 0.14μg/ml, 0.06 μg/ml and 0.08 μg/ml respectively, whereas the quantity of gentamicin eluted on the first day was significantly higher ($p < 0.001$) than on days 7, 14, 21 and 28. On the twenty-eighth day, 80% of the samples no longer presented detectable levels of the antibiotic. (Figure 3)

Figure 4 shows the gentamicin elution pattern, with high concentrations being obtained in the first 24 hours, followed by an abrupt drop on the second day. There was then a slow decrease between the 2nd and 14th days and, between the 14th and 21st days, another accelerated downslide. Up to the second week, the total gentamicin released by each spacer was higher than 1μg (concentration in the bone tissue expected with the systemic administration of gentamicin).[23,24]

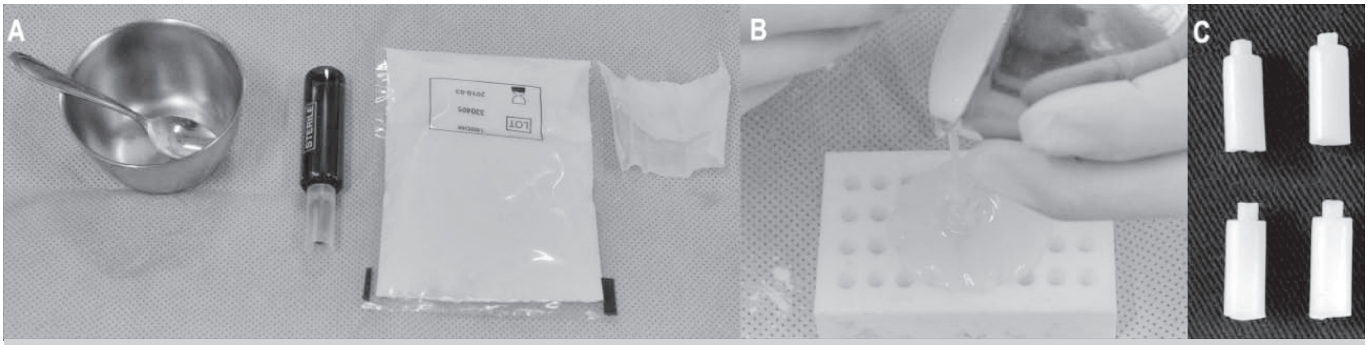

Figure 2 – *Preparation of the test specimens. From left to right: A – Tub and spoon, monomer, polymer, powdered gentamicin. B – Mixture already prepared, being poured in the mold. C – Test specimens.*

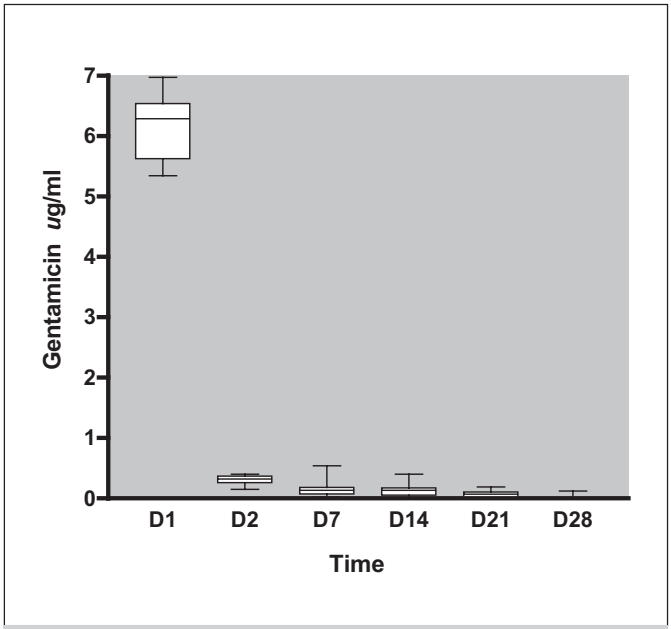

Figure 3 – *Eluted gentamicin x time (maximum value / percentile 25, 50, 75 / minimum value). D1 > D7, D14, D21 and D28 (p < 0.001). D2 > D21, D28 (p < 0.01). D7 > D28 (p < 0.05). D14 > D28 (p < 0.05). Kruskal-Wallis with Dunn's post test.*

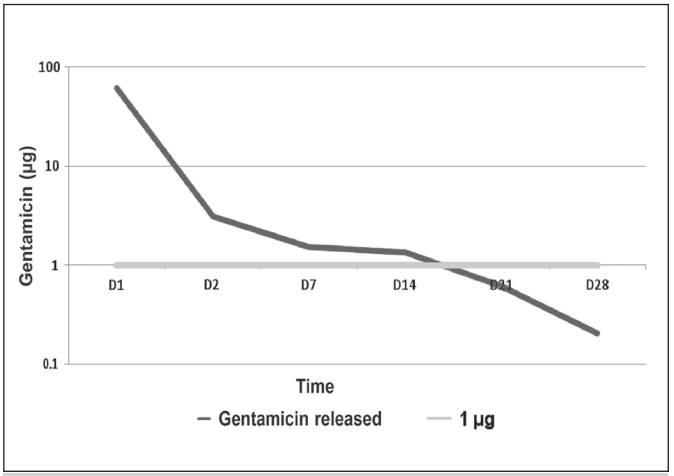

Figure 4 – *Total Gentamicin eluted (Logarithmic scale). Axis X at 1µg (concentration in the bone tissue with systemic administration of gentamicin).*

DISCUSSION

Serum peaks that range from 5 to 8µg/ml[25] are achieved with the parenteral administration of gentamicin in the recommended standard dose (1.5mg/Kg every 8 hours), yet experimental studies estimated that concentrations in the bone tissue are below 1µg/ml.[23,24]

Seldes et al.[16] performed an in vitro evaluation of the addition of 480mg of liquid gentamicin to Palacos R®, using cylindrical test specimens with an area of 282.6mm^2. Average levels of 26.4µg/ml were reached in the first 24 hours, falling to 4.15µg/ml at three weeks.

In this study with the addition of 480mg of powdered gentamicin to Simplex® and the making of test specimens with an area of 367mm^2, the participants obtained concentrations of 6.15µg/ml in the first 24 hours, dropping to 0.06 µg/ml in the third week. This difference can be explained by the variations in the experiment conditions and by the fact that the Simplex® cement has a performance inferior to Palacos® for gentamicin elution.[20]

Based on the outcome of this study, the use of 480mg of gentamicin was capable of dispersing quantities above 1µg up to the 14th day. Higher concentrations over a longer period of time should be expected in increasing the quantity of antibiotic added to the cement and the quantity of the mixture used.

CONCLUSION

The mixing of 480mg of powdered gentamicin with a 40g packet of acrylic cement presented predictable elution properties, maintaining therapeutic levels of antibiotic up to the second week, thus serving as an alternative to prepare for the expansion of local antibiotic use in the treatment and in the prophylaxis of bone infection.

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REFERENCES

1. Stengel D, Bauwens K, Sehouli J, Ekkemkamp A, Porzolt F. Systematic review and meta-analysis of antibiotic therapy for bone and joint infections. Lancet. 2001;1:175-88.
2. Lazzarini L, Mader JT, Calhoun J H. Osteomyelitis in long bones. J Bone Joint Surg Am. 2004;86:2305-18.
3. Gristina AG, Costerton J W. Bacterial adherence to biomaterials and tissue: the significance of its role in clinical sepsis. J Bone Joint Surg Am. 1985;67:264-73.
4. Nelson CL. The current status of material used for depot delivery of drugs. Clin Orthop Relat Res. 2004;(427):72-8.
5. Zalavras C, Patzakis MJ, Holtom P. Local antibiotic therapy in the treatment of open fractures and osteomyelitis. Clin Orthop Relat Res. 2004;(427):86-93.
6. Buchholz HW, Elson RA, Engelbrecht E, Lodenkämper H, Röttger J, Siegel A. Management of deep infection of total hip replacement. J Bone Joint Surg Br. 1981;63:342-53.
7. Klemm KW. Antibiotic bead chains. Clin Orthop Relat Res. 1993;(295):63-76.
8. Calhoun JH, Henry SL, Anger DM, Cobos JA, Mader JT. The treatment of infected nonunions with gentamicin- polymethylmethacrylate antibiotic beads. Clin Orthop Relat Res. 1993;(295):23-7.
9. Nelson CL, Evans RP, Blaha JD, Calhoun J, Henry S, Patzakis MJ. A comparison of gentamicin-impregnated polymethylmethacrylate bead implantation to conventional parenteral antibiotic therapy in infected total hip and knee arthroplasty. Clin Orthop Relat Res. 1993;(295):96-101.
10. Ostermann PAW, Henry SL, Seligson D. The role of local antibiotic therapy in the management of compound fractures. Clin Orthop Relat Res. 1993;(295):102-11.
11. Christian EP, Bosse MJ, Robb G. Reconstruction of large diaphyseal defects, without free fibular transfer, in grade-IIIB tibial fractures. J Bone joint Surg Am. 1989;72:427-38.
12. Patzakis MJ, Mazur K, Wilkins J, Sherman R, Holtom P. Septopal beads and autogenous bone grafting for bone defects in patients with chronic osteomyelitis. Clin Orthop Relat Res. 1993;(295):112-8.
13. Masquelet AC, Fitoussi F, Begue T, Muller GP. Reconstruction des os longs par membrane induite et autogreffe spongieuse. Ann Chir Plast Esthet. 2000;45:346-53.
14. Masquelet AC. Muscle reconstruction in reconstructive surgery: soft tissue repair and long bone reconstruction. Langenbecks Arch Surg. 2003;388:344-6.
15. Seeley SK, Seeley JV, Telehowski P, Martin S, Tavakoli M, Colton SL, et al. Volume and surface area study of tobramycin-polymethylmethacrylate beads. Clin Orthop Relat Res. 2003;(420):298-303.
16. Seldes RM, Winiarsky R, Jordan LC, Baldini T, Brause B, Zodda F, et al. Liquid gentamicin in bone cement: a laboratory study of a potentially more cost-effective cement spacer. J Bone Joint Surg Am. 2005;87:268-72.
17. Hendriks JGE, Van Hornb JR, Van Der Mei HC, Busscher HJ. Backgrounds of antibiotic-loaded bone cement and prosthesis-related infection. Biomaterials. 2004;25:545–56.
18. Leeson MC, Steven BL. Thermal aspects of the use of polymethylmethacrylate in large metaphyseal defects in bone: a clinical review and laboratory study. Clin Orthop Relat Res. 1993;(295):239-45.
19. Buchholz HW, Elson RA. Antibiotic-loaded acrylic cement: current concepts. Clin Orthop Relat Res. 1984;(190):96-108.
20. Nelson CL, Griffin FM, Harrison BH, Cooper RE. In vitro elution characteristics of commercially and noncommercially prepared antibiotic PMMA beads. Clin Orthop Relat Res. 1992;(284):303-9.
21. Fischmann AMA. Guia farmacêutico brasíndice. São Paulo: Andrei Publicações médicas, farmacêuticas e técnicas Ltda; 2008.
22. Jolley ME. Fluorescence polarization immunoassay for the determination of therapeutic drug levels in human plasma. J Anal Toxicol. 1981;5:236-40.
23. Wiggins CE, Nelson CL, Clarke R, Thompson CH. Concentration of antibiotics in normal bone after intravenous injection. J Bone Joint Surg Am. 1967;60:60-93.
24. Perry CR, Rice S, Ritterbusch JK, Burdge RE. Local administration of antibiotics with an implantable osmotic pump. Clin Orthop Relat Res. 1985;(192):284-90.
25. Schafer JA, Hovde LB, Rotschafer JC. Consistent rates of kill of Staphylococcus aureus by gentamicin over a 6-fold clinical concentration range in an in vitro pharmacodynamic model. J Antimicrob Chemother. 2006;58:108-11.