Original Research
Medical Journal of the Islamic Republic of Iran. Vol. 22, No. 3, November, 2008. pp. 125- 131

Acrylic antibiotic-loaded bone cement: a basic study

Hossein Farahini, MD.[1], Mehdi Ghorbani, MD.[2], Ehsan Akbarian, MD.[3]

Department of Orthopedic Surgery, Rasool Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

Abstract

Objective: The aim of this study was to evaluate the efficacy of antibiotic-loaded bone cement in controlling local infection and in regard to its physical characteristics, elastic modulus, and tensile strength in-vitro.

Methods: Acrylic bone cement, based on polymethylmethacrylate (PMMA) was mixed with the powder form of three antibiotics, i.e., gentamicin, tobramycin, and cefuroxime with different doses below 2gr per 40gr of cement powder; thereafter, liquid monomer was added to process the cement. Sensitivity to common clinical isolates was assessed by counting the inhibition zone of each ALBC disc in cultured strains. Elution with normal saline was performed to evaluate the effects on ALBC disks and their antimicrobial efficiency. Cement structure, tensile strength, and elastic modulus were assessed by biomechanical tests to understand the characteristics of ALBCs after loading antibiotics with different doses and two methods of vacuum and manual mixing.

Results: Gentamicin, tobramycin, and cefuroxime reduced bacterial growth significantly with doses more than 1gr of antibiotics in 40gr of the cement. Cefuroxime was less efficient than the other two antibiotics in controlling pseudomonas. Elution with normal saline has not affected antibacterial results, significantly. All the 3 antibiotics had the same pattern of physical characteristics while loaded in bone cement. Gross structure of ALBCs with different doses of the three antibiotics was the same as non-ALBC and the elasticity or strength did not decline after loading antibiotics. The elastic modulus of ALBC was increased by boosting the doses of antibiotics; however, doses of 1gr to 1.5gr were the optimal doses in this regard. The tensile strength of ALBC was increased by doses of 1gr to 1.5gr of antibiotics; however, below and above these doses, the strength was decreased, but it did not exceed the basic strength of non-ALBC. Vacuum mixing method increased strength and elasticity more than manual one, remarkably.

Conclusion: Optimal protective effects of ALBCs against infection could be seen with mixing doses of about 1gr to 1.5gr of antibiotics in 40gr of acrylic bone cements by vacuum method, while optimal elastic modulus and tensile strength could be achieved at the same doses.

Keywords: ALBC, acrylic bone cement, antibiotic, microbiology, biomechanics.

Introduction

Acrylic bone cement is used on a daily basis by orthopedic surgeons as an invaluable aid in securing implants for hip or knee replacements. However, 10-year prosthesis loosening rates of cemented prostheses have ranged from 5% to 10% [1]. Although loosening is often ascribed to suboptimal performance of the cement, the conditions of cement use during implantation are probably the main problem. Biomaterial-re-

1. Associate Professor of Iran University of Medical Sciences, Orthopedic Surgeon and Fellowship of Knee and Arthroscopy, Rasool Akram General Hospital.
2. Resident of orthopedic surgery, Rasool Akram General Hospital.
3. **Corresponding author,** General Practitioner, Shahid Beheshti University of Medical Sciences. Tel: +98912 172-5275.
Email: eakbarian@gmail.com.

lated infections constitute a major threat to the current use of biomaterials. In orthopedics, use of polymethylmethacrylate (PMMA) bone cement loaded with one antibiotic to prevent biomaterial-related infections is widespread. Several studies have shown antibiotic-loaded bone cement to be beneficial in prophylaxis for primary or revision arthroplasty [2,3].

Bacteria adhere to the surface of most materials used in joint replacement surgery. Furthermore, bone tissue changes related to surgery can promote infection. The bone in contact with the implant can undergo necrosis related to vibrations from the oscillating saws used to cut the bone, heat release by the cement, or toxicity of the cement monomer. Thus, prophylactic systemic antibiotic therapy is probably not sufficient to ensure full protection from infection during and after joint replacement. Local release of an antibiotic from the cement used to secure the implant may be the most effective means of reaching the organisms located at the cement-bone or cement-metal interface. ALBC (Antibiotic-Loaded Bone Cement) is the method that provides the highest antibiotic levels in the joint, neighboring bone tissue, and cement-prosthesis interface during the first 2 weeks after surgery [1]. Staphylococcus, streptococcus, Escherichia coli, and pseudomonas are the most important bacteria which are responsible for infections of an arthroplasty [4-21].

ALBC was first used by Buchholz and Engelbrecht [4] who added gentamicin to standard cement for the prophylactic treatment of infection in total hip arthroplasty and they decreased the infection rate after total hip arthroplasty from 6% to 1.6% [5-7]. Subsequently, gentamicin-loaded bone cement was found helpful in the treatment of documented infection of total hip prostheses. The available data is applicable to all joint prostheses. Although the local concentrations of gentamicin are very high, diffusion of the antibiotic into the bloodstream is minimal in animal models [8] and in humans [9,10]. Also, a vast literature review found no reports of allergies to gentamicin in bone cement [11]. Adding antibiotics in the amounts used in clinical practice (antibiotic/bone cement: 2gr/40gr) caused no detectable alterations in the mechanical properties of the cement [12-16]. In the single prospective randomized trial comparing cement with and without antibiotics [17], the rate of aseptic loosening was similar in the two groups (29% with standard cement and 24% with antibiotic-loaded cement; n = 1698 total hip prostheses). The aim of this study was to investigate the bactericidal effects of antibiotic loaded bone cements using different doses of gentamicin, tobramycin, and cefuroxime and the mechanical characteristics of these ALBCs.

Methods

Bone Cement Preparation: Acrylic bone cement, based on polymethylmethacrylate (PMMA), made by Tianjin Institute Synthetic Material Industry (Tianjin, China) was used in our study. For preparing ALBC, we mixed the powder form of gentamicin, tobramycin, and cefuroxime sodium (Zinacef) antibiotics with the cement. In case of vacuum mixing, vacuum mixing chamber (Stryker Orthopedics, Mahwah, NJ, USA) was used. Cements were prepared by mixing the powdered polymethylmethacrylate and the antibiotic powder (after manual or vacuum mixing) with the liquid monomer in a bowl with a spatula. Manual mixing was done according to the manufacturer's instructions and resulted in liquid cement. The liquid cement was spread over a cement plate mold (6mm diameter and 3mm thickness). The filled mold was manually pressed between two glass plates, covered with copier overhead film to facilitate removal after hardening, up to the time specified for final hardening. After 24 hours, the cement discs were pulled out of the mold and stored under dark, sterile conditions at room temperature. All procedures were carried out under sterile techniques.

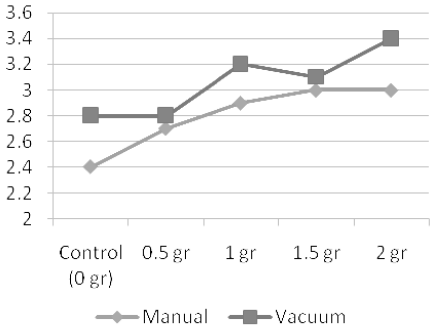

Figure 1. Elastic modulus of ALBC regarding doses of antibiotics.

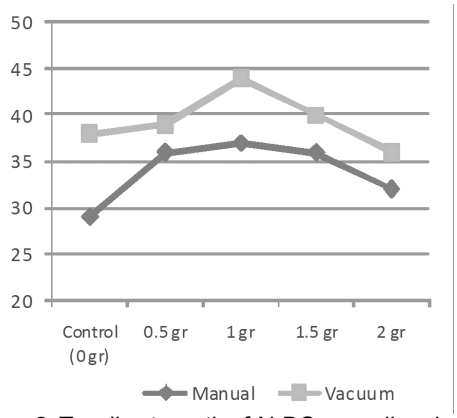

Figure 2. Tensile strength of ALBC regarding doses of antibiotics.

Sensitivity of clinical isolates to different bone cements: We studied the cement discs on three kinds of bacterial strains in culture plates: Staphylococcus aureus, Escherichia coli, and Pseudomonas pyocyanea. The bacteria were cultured into agar plates at 37°C in ambient air for 24 hours and suspended in 0.9% saline to a concentration of 10^5 bacteria/mL. The thickness of the agar was approximately 5mm. Ten minutes after inoculation, the bone cement discs were placed firmly in the center of each plate and the plate was subsequently incubated aerobically at 37 °C. Zones of inhibition around the discs were measured after overnight incubation. Every 24 hours until a week, the discs would be transfered into a fresh culture plate containing of the same strain with the same preparation for evaluating the maintenance of the antibiotic function of the ALBC. The zone of inhibition was considered as the clear area around a disc in which bacteria were not able to grow. The diameter of each zone was measured in two directions and the mean zone of inhibition was calculated. The size of the zone of inhibition is used to determine whether the bacteria are sensitive or resistant to the antibiotic(s) in the used bone cement discs. Sensitive strains possess a clear inhibition zone and larger diameters indicate a higher sensitivity. Sensitivity was recorded if a zone of inhibition of at least 12 mm was present. The absence of a zone of inhibition was taken as an indication of antibiotic resistance. All the processes of culturing strains and testing the efficacy of antibiotics were carried out under supervision of a microbiologist.

Elution of Antibiotic In-Vitro: Elution of antibiotics from acrylic cements with normal saline has been measured. In this test, we only studied the antibiotic tobramycin with different doses of 0, 0.5, 1.0, 1.5, and 2 grams in 40 grams of the cement. ALBC discs which were prepared as the protocol were placed into 10 ml of normal saline, at 37 °C for ten days. Then the discs were transferred into the mentioned culture plates with the same method. Sizes of the zones of inhibition were calculated after 24 hours.

Mechanical Characteristics of ALBCs: Gross structure, elastic modulus, and tensile strength of the bone cement either loaded with different doses of antibiotics (gentamicin, tobramycin, and cefuroxime) or not loaded were examined. We added fine powder form of antibiotics in a variety of doses below 2gr/40gr to the cement by both methods of manual mixing and vacuum mixing. Further, before assessment of the tensile strength, the prepared ALBC and the non-loaded antibiotic bone cement were placed into aluminium molds (60×10×2 millimeters) and left for 24 hours to solidify.

Day	Staphylococcus Aureus			Escherichia Coli			Pseudomonas Pyocyanea		
	Tobramycin	Gentamicin	Cefuroxime	Tobramycin	Gentamicin	Cefuroxime	Tobramycin	Gentamicin	Cefuroxime
1	27.2	25.1	34.3	26.4	28.1	25.4	27.2	29.8	13.6
2	18	19.5	31.2	16.5	22.3	21.7	20.6	26.2	9.3
3	16.3	15.3	26.7	11.3	17.6	18.9	14.3	19.7	3.4
4	15.2	12.3	23	7.7	10.5	6.1	12.8	15.1	1.6
5	14.5	8.7	21.3	5.7	8.2	4.3	6.4	7.5	0.7
6	12.3	4.9	15.6	0.2	6.3	1.4	2.1	2.2	0.1
7	7.8	0.1	12.2	0	2.2	0	0.3	0.2	0

Table 1. Inhibition zones (mm) of various bacteria (1.5gr) with antibiotic-loaded bone cements.

Modulus of elasticity (E) was achieved by this equation: $\frac{P.Stress\ L^0}{b.c.\ \Delta L}$

Where P = Force (KgF), L^0= Original length before applying the force (cm), and b = width (cm). Therefore, the unit of E would be KgF/cm^2. Tensile strength and modulus of elasticity were calculated by mechanical devices using pressing and pulling methods in the biomechanical laboratory of Sharif University of Technology in cooperation with a biomechanical engineer.

Statistical Analysis: The analysis was accomplished by performing the independent t-test, Mann-Whitney U-Test, one-way analysis of variance (ANOVA), Chi-Square, and Fisher Exact Test. The data were analyzed with SPSS statistical software (SPSS Version 13, Chicago, IL, USA). The level of significance was set at P value < 0.05.

Results

Different tests were carried out to identify the influence, characteristics, and strength of bone cement loaded with various antibiotics. The average of inhibition zones of Staphylococcus a., E. coli, and Pseudomonas p. after adding 1.5gr of different antibiotics to 40gr of acrylic bone cement, from the first until the seventh day of the surveillance, are shown in Table 1. All the three antibiotics significantly inhibited the growth of all bacteria in our study in the first, second, third, and fourth day of the surveillance (P value < 0.001). There was a noteworthy difference between cefuroxime and the two other antibiotics, tobramycin and gentamicin, in inhibiting Staphylococcus aureus and Pseudomonas pyocyanea in which cefuroxime was the strongest and the weakest inhibitor, respectively (P value = 0.024 and 0.031). Moreover, as Table 2 shows, adding different doses of these antibiotics to 40gr of the bone cement and investigating the mean inhibition zones of Staphylococcus a. after 24 hours of the surveillance revealed no remarkable difference in inhibition zones of various doses of each antibiotic; however, there was a slight increase in inhibition zones when more doses of an antibiotic had been loaded. In comparison between manual and vacuum methods of mixing antibiotics with bone cement, there was no statistical difference; nonetheless, by the manual method, the mean inhibition zone was slightly more than the vacuum one.

Elution test of antibiotic (tobramycin)-loaded cement with normal saline was performed and resulted in slightly less inhibition zones than the results of our previous test; however, there was no significant difference in these two groups of results. Mean inhibition zone of Staphylococcus a. after elution was 21.3, 23.2, 25.5, and 26.3 while we added 0.5, 1.0, 1.5, and 2.5 grams of tobramycin to 40

Dose	Tobramycin	Gentamicin	Cefuroxime
0.5gr	23.5	24.2	34.4
1gr	24.7	24.6	35.3
1.5gr	26.4	26.1	35.9
2gr	26.8	26.2	37

Table 2. Inhibition zones (mm) of Staphylococcus aureus with different doses of antibiotics.

grams of the cement powder, respectively.

Gross structure, elastic modulus, and tensile strength of the bone cement were assessed with and without loaded antibiotics. Adding 0, 0.5, 1, 1.5, and 2 grams of the 3 studied antibiotics to the bone cement resulted in no dissimilarity with regard to gross structure and palpable characteristics of the cement. Mean elastic modulus of the cement was increased significantly after loading more than 1gr of all 3 types of antibiotics into 40gr of acrylic bone cement (P value = 0.034); however, manual mixing procedure reduced the elasticity significantly, rather than vacuum mixing (p value < 0.001). The pattern of increased mean elastic modulus after adding different doses of antibiotics was the same in all 3 studied antibiotics and there was no significant difference among them. Figure 1 depicts the mean elastic modulus of ALBC regarding doses of antibiotics, while 0gr stands for the control, which means the average of elastic modulus of acrylic cement without adding antibiotics.

Mean tensile strength of the cement was increased with adding 1gr of all the three antibiotics (P = 0.007), while this improved strength is markedly seen in vacuum mixing of tobramycin to 40gr of acrylic bone cement (P < 0.001). In manual mixing, 0.5gr of antibiotics could also remarkably boost the strength, but in vacuum mixing, no significant change was seen. Adding more than 1gr of antibiotics declined the increased pattern of tensile strength; nonetheless, this decreased strength did not exceed the basic control value of the non-antibiotic-loaded cement. The pattern of increased mean tensile strength after adding different doses of antibiotics was similar in all 3 studied antibiotics and there was no significant difference among them. Manual mixing procedure reduced the strength significantly, rather than vacuum mixing (P value < 0.001). Fig. 2 demonstrates the mean tensile strength of ALBC regarding doses of antibiotics, while 0gr stands for the control, which means the average tensile strength of acrylic cement without adding antibiotics.

Discussion

Local release of an antibiotic by the cement used to secure the implant is probably the most effective means of eradicating organisms located at the cement-bone and cement-implant interface. ALBC is the method of administration that produces the highest concentrations of antibiotic in the joint, neighboring bone tissue, and cement-prosthesis interface, with no risk of toxic blood levels. These concentrations are far greater than those obtained with systemic antibiotic therapy and usually exceed the minimal inhibitory concentration of relevant bacteria. In our study, it was shown that adding antibiotics to the bone cement could effectively reduce the growth of common strains which are usually seen in joint arthroplasties.

Gentamicin, tobramycin, and cefuroxime were effective in decreasing bacterial growth in-vitro; however, cefuroxime was not as efficient as the two other antibiotics in pseudomonas strains. Doses of 1gr of each of the three antibiotics could obtain the best protection against infection, among the diverse doses which were studied in our research. It was seen that protective effects of gentamicin and tobramycin lasted more than cefuroxime in-vitro and they could protect infection better than cefuroxime after several days. Despite many surgeons' viewpoint about weakening the protective effects of antibiotics which are loaded in cement by elution, it was found out that therapeutic effects of antibiotics still remain in ALBCs after elution with normal saline and are

roughly as efficient as ALBCs without elution process.

Biomechanical assessment of ALBCs loaded with different antibiotics and their diverse doses resulted in no gross structure or palpable characteristic dissimilarities, no elastic modulus declining, and no tensile strength reducing. In fact, we learned that elastic modulus of ALBCs was more than non-antibiotic-loaded bone cements; however, this increased elasticity was at its most when doses of 1 to 1.5 grams of antibiotics were applied. Moreover, tensile strength of ALBCs also was more than non-antibiotic-loaded bone cements; nevertheless, the increased strength was at its most when doses of 1 gram of antibiotics were applied, just as well as what we have seen in elastic modulus. It is noteworthy to mention that neither elastic modulus nor tensile strength were less than non-antibiotic-loaded bone cement with any doses of loaded antibiotics below 2 grams; however, the method of vacuum mixing resulted in better elasticity and strength rather than the manual mixing method.

We evaluated the efficacy of antibiotic-loaded bone cement in controlling local infection and in regard to its physical characteristics, elastic modulus, and tensile strength in-vitro. In our study, it was seen that optimal protective effects of ALBCs against bacterial strains could be seen with mixing doses of about 1gr of antibiotics in 40gr of acrylic bone cements by vacuum method, while optimal elastic modulus and tensile strength could be achieved at the same doses. Therefore, it could be recommended that mixing medium doses of antibiotics about 1gr to 1.5gr with 40gr acrylic bone cement by vacuum method may be more beneficial in inhibiting infection at the site of surgery and providing the best biomechanical structure of the cement.

References

1. Passuti N, Gouin F. Antibiotic-loaded bone cement in orthopedic surgery. Joint Bone Spine 2003;70 (3):169-74.

2.Espehaug B, Engesaeter LB, Vollset SE, et al. Antibiotic prophylaxis in total hip arthroplasty. Review of 10,905 primary cemented total hip replacements reported to the Norwegian arthroplasty register, 1987 to 1995. J Bone Joint Surg Br 1997; 79: 590–595.

3. Josefsson G, Kolmert L. Prophylaxis with systematic antibiotics versus gentamicin bone cement in total hip arthroplasty. A ten-year survey of 1,688 hips. Clin Orthop 1993; 292: 210 –214.

4. Buchholz HW, Engelbrecht H. -ber die Depotwirkung einiger Antibiotika bei Vennischung mit dem Kunstharz Palacos. Chirurg 1970; 41:511–5.

5. Buchholz HW, Elson RA, Heinert K. Antibiotic-loaded acrylic cement: current concepts. Clin Orthop 1984;190: 96–108.

6. Buchholz HW. Tiefen Infektionen nach aallohplastischern Hüftgelenkersatz. Langenb Arch Chir 1973; 334: 547.

7. Buchholz HW. Results of exchange operations for infection. In: Elson RA, Caldwell ADS, editors. Revision arthroplasty. Proceedings of a Symposium held at Sheffield University. Oxford: Medical Education Service; 1979. pp. 103–10.

8. Baker AS, Greenham LW. Release of gentamicin from acrylic bone cement. Elution and diffusion studies. J Bone Joint Surg 1998; 70A:1551–7.

9. Wahlig AH, Dingeldein E, Buchholz HW, Buchholz M, Bachmann F. Pharmacokinetic study of gentamicin-loaded cement in total hip replacements. Comparative study of varying dosages. J Bone Joint Surg 1984;66B:175–9.

10. Elson RA, Jephcott AE, Mcgechie DB, Verettas. Antibiotic-loaded acrylic cement. J Bone Joint Surg 1977; 59B: 200–5.

11. Walenkamp GHIM. Gentamicin-PMMA-beads. A clinical, pharmacokinetic and toxicological study. Amsterdam: Cliteur; 1983. pp. 164.

12. Davies JP. Comparison of the mechanical properties of Simplex P, Zimmer Regular and LVC bone cements. J Biomed Mater Res 1987; 21:719–30.

13. Davies JP, O'Connor DO, Burke DW, Harris WH. Influence of antibiotic impregnation on the fatigue life of Simplex P and Palacos R acrylic bone cements, with and without centrifugation. J Biomed Mater Res 1989; 23: 379–97.

14. Lautenschlager EP,Marshall GW,Marks KE, Schwartz J, Nelson CL. Mechanical strength of acrylic

bone cements impregnated with antibiotics. J Biomed Mater Res 1976; 10:837–45.

15. Lee AJC. The mechanical properties of bone cements. J Med Eng Technol 1977; 1:137–40.

16. Lindberg L. Antibiothérapie prophylactique en chirurgie orthopédique. Cahier d'Enseignement de la SOFCO7, vol. 37. Paris: Expansion Scientifique Française; 1990. pp. 66-75.

17. Joseffson G, Lindberg L, Wiklander B. Systemic antibiotics and gentamicin-containing bone cement in the prophylaxis of postoperative infections in total hip arthroplasty. Clin Orthop 1981; 159: 194-200.

18. Maderazo EG, Judson S, Pasternak H. Late infections of total joint prostheses. A review and recommendations for prevention. Clin Orthop Relat Res 1988; 229: 131-42.

19. Poss R, Thornhill TS, Ewald FC, Thomas WH, Batte NJ, Sledge CB. Factors influencing the incidence and outcome of infection following total joint arthroplasty. Clin Orthop Relat Res 1984; (182):117-26.

20. Fitzgerald RH Jr. Infected total hip arthroplasty: diagnosis and treatment. J Am Acad Orthop Surg 1995; 3:249-262.

21. Garvin KL, Hinrichs SH, Urban JA. Emerging antibiotic-resistant bacteria. Their treatment in total joint arthroplasty. Clin Orthop 1999; 369:110-123.