EXPERIMENTAL STUDY

Acta Orthop Traumatol Turc 2013;47(1):55-59
doi:10.3944/AOTT.2013.2806

Biomechanical properties of ciprofloxacin loaded bone cement

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Objective: The purpose of this study was to investigate the biomechanical properties of bone cement used in joint replacement surgery after the addition of ciprofloxacin.

Methods: The first group received bone cement only and served as a control for the 4 groups where 500 mg, 1000 mg, 1500 mg and 2000 mg of ciprofloxacin were added to yield 40 g of bone cement. Axial compression tests were conducted using a 50,000 Newton capacity tension-compression testing device.

Results: While axial compression strength at failure was 80.2±4.3 MPa in the control group, values in the ciprofloxacin-treated groups decreased with rising concentration of ciprofloxacin to 74.5±5.4 MPa, 70.6±4.8 MPa, 70.5±4.7 MPa, and 69.3±3.4 MPa.

Conclusion: Bone cement with addition of 500 to 1500 mg ciprofloxacin maintained mechanical axial strength values above 70.0 MPa recommended by American Society for Testing and Materials and can be safely used in joint replacement surgery.

Key words: Axial compression; bone cement; ciprofloxacin; PMMA.

Implant-related infection is a serious complication in the field of joint replacement surgery and its treatment continues to pose a challenge to this day.[1-3] In 1970, Buchholz and Engelbrecht were the first to study the effects of the addition of antibiotics to polymethylmethacrylate (PMMA) on implant-related infection rates.[4] In Scandinavian countries, bone cement is loaded with antibiotics in 95% of revision knee and hip arthroplasties.[5,6] In primary arthroplasty, antibiotics are added to bone cement to prevent infection by 48%, 85% and 69% of surgeons in Norway, Sweden and England, respectively.[5]

Adding antibiotics to bone cement may decrease the mechanical strength of PMMA when large doses are used.[7] Another problem is the limited number of antibiotics available that can be combined with bone cement. Fluoroquinolones are broad-spectrum antibacterial agents especially effective against gram-negative bacteria. They inhibit bacterial cell replication by targeting the bacterial DNA gyrase enzyme, a Type 2 topoisomerase.[8] Ciprofloxacin is the most potent fluoroquinolone against *Pseudomonas aeruginosa*. Oral and/or systemic ciprofloxacin is a good alternative to other parenteral antibiotics used in the treatment of osteomyelitis as it penetrates well into the bone. Even though ciprofloxacin has a positive effect on soft tissue and bone infections, its effects on the mechanical strength of bone cement are unknown.

The purpose of this study was to investigate whether the addition of increasing doses of

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Submitted: December 28, 2011 **Accepted:** September 13, 2012

Available online at
www.aott.org.tr
doi:10.3944/AOTT.2013.2806
QR (Quick Response) Code:

ciprofloxacin into bone cement would have an impact on its maximum axial compression strength.

Materials and methods

This multidisciplinary, controlled study was conducted at the Department of Pharmacology, the Department of Orthopedic Surgery and Traumatology, and the laboratory of the Vocational School of Higher Education of Cumhuriyet University.

Due to the lack of a powder form of ciprofloxacin, tablets containing 874.5 mg of ciprofloxacin hydrochloride monohydrate equivalent to 750 mg ciprofloxacin and titanium dioxide were used in this study. 750 mg ciprofloxacin (Ciflosin®; Deva, Istanbul, Turkey) film tablets were pulverized. The powder was then measured using a microbalance (CP 224S; Sartorius AG, Göttingen, Germany) to contain 500 mg (0.78 g), 1000 mg (1.56 g), 1500 mg (2.34 g) and 2000 mg (3.12 g) of ciprofloxacin.

Ciprofloxacin in 400 mg IV form (Ciproxin 400 mg; Bayer Türk Kimya San Tic. Ltd., Istanbul, Turkey) contains 508.9 mg ciprofloxacin lactate equivalent to 400 mg ciprofloxacin, 643.6 mg lactic acid solution, 1800 mg sodium chloride, 280 mg hydrochloric acid and 198.076 mg water for injection. In case this IV form is used, 5 boxes of ciprofloxacin (500 ml) and 40 g cement should be mixed for 2000 mg ciprofloxacin + bone cement group. Bone cement (BonOs; aap Biomaterials GmbH & Co. KG, Dieburg, Germany) was prepared using a first generation cementing technique.

PMMA cylinders of 12 mm height and 6 mm in diameter were molded according to the American Society for Testing and Materials F451-99a Standard Specification for Acrylic Bone Cement. Molds had a sliding lid on one side and a hinged lid on the other side. A plane-surfaced steel rod with a diameter matching the mold was used to remove the prepared PMMA. All samples were prepared under operating room conditions at 18±2°C room temperature.

Samples in the first group contained bone cement only, whereas samples in Group 2, 3, 4 and 5 contained bone cement plus 500 mg, 1000 mg, 1500 mg and 2000 mg ciprofloxacin, respectively.

A total mass of 40 grams of cement powder and antibiotic powder were mixed for one minute. After the addition of liquid monomer, the preparation was mixed for an additional 30 seconds, then left for 90 seconds at room temperature. The molds were filled applying manual pressure. Lids were closed and each mold was compressed using a power grip. After 15 minutes, the power grip was unscrewed and lids were opened. A plane-surfaced steel rod with an approximate diameter of 5.5 mm was used to remove the cylindrical samples from the molds. Surfaces were corrected using number 0 sandpaper. Five groups of 43 samples were obtained.

In each group, 43 cylindrical samples were examined both macroscopically and using digital X-ray. Samples with cracks and spaces that made up more than 10% of their cross-section were excluded from the study (Fig. 1). A randomized method was used to choose 20 of the remaining samples.

Compression tests were conducted as stated in the "F451-99a: Standard Specifications for Acrylic Bone Cement" section of the ASTM after a five-day period in which samples were kept in closed jars at a temperature of 20±2°C. Axial compression tests were conducted at 18±2°C using a 50,000 Newton capacity tension-compression testing device (HTI Hounsfield®; Hounsfield Test Equipment, Redhill, UK). Testing took place at the laboratory of the Department of Engineering at Sivas Vocational School of Higher Education. Cross-head speed was set at 5 mm/min and samples were loaded up to failure.

SPSS v.17.0 software was used for statistical analysis. Descriptive data was expressed as mean±SD. Groups were compared statistically using general linear models of ANOVA followed by Tukey's test. P values less than 0.05 were considered significant.

Results

Failure points were at a compressive strength of 80.21±4.3 MPa in the control group and 74.51±5.4 MPa, 70.55±4.8 MPa, 70.49±4.7 MPa and 69.34±3.4 MPa in the groups where bone cement contained 500, 1000, 1500 and 2000 mg of ciprofloxacin, respectively.

Groups where the antibiotic was added to the cement had a significantly lower failure point than the control group ($p<0.05$). In addition, the failure strength of the 500 mg ciprofloxacin + bone cement group was significantly higher in comparison to the other experimental groups ($p=0.001$). There were no statistically significant differences between the 1000, 1500 and 2000 mg ciprofloxacin + bone cement groups (Table 1 and Fig. 1).

All groups, apart from the 2000 mg ciprofloxacin + bone cement group had failure strength values above the 70 MPa required by ASTM F451-99a standards.

Discussion

It has been reported that 22% of revisions in total joint replacement are due to implant-related infections.[9]

Infection continues to be among the most feared complications of total joint replacement even though preventive measures and techniques have been developed in recent years. Different antibiotic agents, routes of administration and concentrations have been studied in an attempt to lower infection rates.

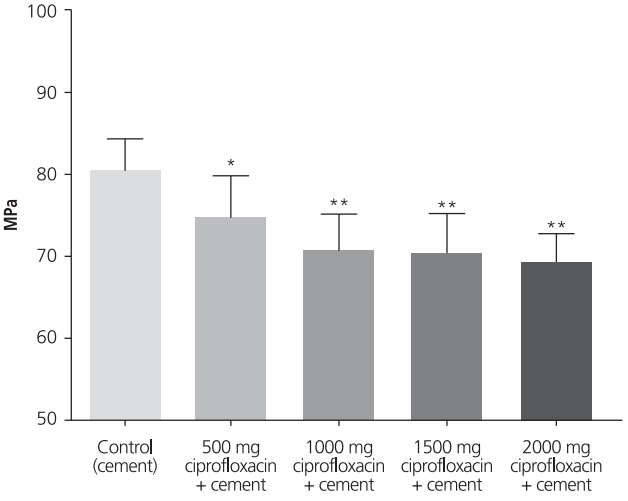
In a broad randomized study conducted in Norway between 1987 and 2001, 22,170 primary total hip replacements were analyzed.[10] Infection rates were 1.8 times higher in individuals given systemic antibiotic treatment (5,960 patients) than in patients who had received antibiotic-loaded cement (15,676).

Bone cement is most frequently combined with vancomycin and/or aminoglycosides in the treatment or prevention of orthopedic infections.[11-13] Anguita-Alonso et al. found that cefazolin, ciprofloxacin, gatifloxacin, levofloxacin, linezolid, and rifampin could be used together with PMMA.[14] In another study, teicoplanin, vancomycin and gentamicin were preferred.[15] The addition of 2 g of gentamicin to 40 g Palacos® (Smith&Nephew Orthopedics, Memphis, TN, USA) bone cement lowered infection rates from 1.2% to 0.009% in primary hip arthroplasty cases.[4,16] Other *in vitro* and *in vivo* studies have proved that other thermostable antibiotics beside gentamicin can attain local antibiotic levels above the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in bone cement.[17,10] Among antibiotics being studied, linezolid is the most thermostable after PMMA polymerization.

Only a few types of antibiotic-loaded bone cement are commercially available. When prepared in-house, a suitable antibiotic needs to chosen by the surgical team. The antibiotic combined with bone cement must attain adequate local concentrations in order to be effective against the targeted microorganism.[18] Antibiotics that are not effective when mixed with cement are rifampicin, chloramphenicol, and some penicillin types.

Numerous studies have proven that ciprofloxacin is effective within PMMA.[9,14,19] We preferred ciprofloxacin as it is thermostable and is commonly used parenterally for infection prophylaxis. Ciprofloxacin-loaded bone cement has been used in several studies, however, axial compression was not assessed.[20,21]

Tunney et al.[9] isolated 49 factors from orthopedic implants and analyzed their susceptibility to 7 antimicrobial agents: gentamicin sulfate, erythromycin, fusidic acid, cefamandole, cefotaxime, ciprofloxacin, and vancomycin. Ciprofloxacin and vancomycin proved to be more effective than gentamicin and more active perioperatively than cefamandole and erythromycin. Compared to teicoplanin and vancomycin, ciprofloxacin is more economical.



*The difference in comparison to both the control group and the other experimental groups is statistically significant (p<0.05).
**The difference in comparison to the control group is statistically significant (p<0.05).

Fig. 1. Intergroup mean fracture points.

Ciprofloxacin is effective in the treatment of infections caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*.[20]

However, Huddleston et al.[22] observed that ciprofloxacin delayed the healing of fractures, decreased callus endurance, reduced the number and diameter of chondrocytes in enchondral ossification sites and caused abnormalities in trabecular bone formation. Histopathological examinations revealed mature and uniform chondrocytes in the control group, while the limited number of chondrocytes in the treatment group displayed pleomorphism and signs of immaturity. In their *in vitro* study, Antoci et al. report-

Table 1. The mean of compression endurance (MPa) of all five groups.

	N	Mean	SD	Result
Control (Cement)	20	80.2100	4.30742	
500 mg ciproflaxacin added cement	20	74.5145	5.40370	F=18.91 p=0.001*
1000 mg ciprofloxacin added cement	20	70.5500	4.86482	
1500 mg ciprofloxacin added cement	20	70.4970	4.73548	
2000 mg ciprofloxacin+ cement	20	69.3445	3.42788	
Total	100	73.0232	6.03393	

*p<0.05

ed that ciprofloxacin concentrations greater than 100 µg/mL and vancomycin and tobramycin concentrations greater than 2000 µg/mL severely decreased cellular proliferation.[23] In an *in vitro* ciprofloxacin elution study, Tsourvakas et al.[24] added 150 mg ciprofloxacin to 6 g PMMA and obtained maximum values of 80.80±9 µg/ml for only two days throughout the 1-year test duration. This value is lower than the toxic value defined by Antoci. Tsourvakas et al. also reported that a ciprofloxacin concentration greater than 2 µg/ml was obtained up to the 100th day and these values are above MIC for various pathogens.

The chondrotoxicity of quinolones can affect articular cartilage and/or the epiphyseal growth plate depending on its developmental stage. The pathogenesis of chondrotoxicity is explained by the magnesium-chelating properties of quinolones.[25] Toxic effects of quinolones on connective tissue structures can also result in tendinopathies.[25,26] However, fluoroquinolone-induced arthropathy is most frequently benign and heals without sequelae.[26] *In vivo* studies confirm that ciprofloxacin is non-genotoxic and non-carcinogenic; therefore, ciprofloxacin is considered safe for therapeutic use.[27]

A major problem of antibiotic-loaded bone cement is its decreased mechanical strength. During preparation, a low percentage in the porosity of bone cement can be achieved through vacuum mixing.[28] A porosity of 5% is considered the lower limit for successful fixation.

In our study, mechanical tests were conducted *in vitro* five days after the bone cement was prepared. *In vivo*, however, once antibiotic delivery from the bone cement into the body starts, the mechanical strength of antibiotic-added bone cement may decrease as pores form within the cement. As a result, it is recommended that mechanical strength of the bone cement should be assessed after the release of antibiotics in future studies. Another well-known fact is the aging characteristic of bone cement. Other leading factors that increase the porosity and decrease the mechanical endurance of bone cement are the blood, fat and other fluids that mix with the cement.[17]

Mechanical strength depends on the type of cement used, preparation technique and the type and amount of antibiotic added. When used as a spacer during two-stage revisions in the treatment of implant-related infections or as part of the antibiotic chain used in chronic osteomyelitis treatment, the mechanical strength of antibiotic-loaded bone cement plays only a minor role. However, when used in cement replacement fixations, mechanical endurance gains importance and it is advisable to conduct compression tests in order to determine the amount of antibiotic that can be safely added to the cement.

Several studies have investigated the effect adding antibiotic to bone cement has on its mechanical strength.[9,14,15,19] In one of these studies using third generation cement application and preparations, Göğüş et al. proved that the highest teicoplanin dosage that could safely be added to 40 g of Surgical Simplex P bone cement was 1600 mg.[17]

The main limitations of our study were that we were unable to obtain the original ciprofloxacin molecule for our study and we used Ciflosin® 750 mg film tablets. The coloring agent in the tablet, titanium dioxide, could have affected the mechanical strength of the bone cement. However, we believe that since the same preparation of antibiotic was used in all experimental groups, its effect on the study is minimal. Another limitation was that the clinical use of tablet forms is limited.

In conclusion, ciprofloxacin-added bone cement can be used for prophylactic purposes in primary joint replacements in high-risk patients. More advanced studies are required regarding the *in vivo* use of ciprofloxacin and vancomycin.

Acknowledgement

The authors would like to thank Feza Korkusuz for his contributions in study design.

Conflicts of Interest: No conflicts declared.

References

1. Yonekura Y, Miyamoto H, Shimazaki T, Ando Y, Noda I, Mawatari M, et al. Osteoconductivity of thermal-sprayed silver-containing hydroxyapatite coating in the rat tibia. J Bone Joint Surg Br 2011;93:644-9.
2. Vercillo M, Patzakis MJ, Holtom P, Zalavras CG. Linezolid in the treatment of implant-related chronic osteomyelitis. Clin Orthop Relat Res 2007;461:40-3.
3. Senthi S, Munro JT, Pitto RP. Infection in total hip replacement: meta-analysis. Int Orthop 2011;35:253-60.
4. Buchholz HW, Engelbrecht H. Depot effects of various antibiotics mixed with Palacos resins. [Article in German] Chirurg 1970;41:511-5.
5. Jiranek WA, Hanssen AD, Greenwald AS. Antibiotic-loaded bone cement for infection prophylaxis in total joint replacement. J Bone Joint Surg Am 2006;88:2487-500.
6. Mader JT, Calhoun J, Cobos J. *In vitro* evaluation of antibiotic diffusion from antibiotic-impregnated biodegradable beads and polymethylmethacrylate beads. Antimicrob Agents Chemother 1997;41:415-8.
7. Mohd Fuad D, Masbah O, Shahril Y, Jamari S, Norhamdan MY, Sahrim SH. Biomechanical properties of bone cement with addition of cefuroxime antibiotic. Med J Malaysia 2006; 61 Suppl A:27-9.

8. Tuncay I, Ozbek H, Köşem M, Unal O. A comparison of effects of fluoroquinolones on fracture healing (an experimental study in rats). Ulus Travma Acil Cerrahi Derg 2005;11:17-22.
9. Tunney MM, Ramage G, Patrick S, Nixon JR, Murphy PG, Gorman SP. Antimicrobial susceptibility of bacteria isolated from orthopedic implants following revision hip surgery. Antimicrob Agents Chemother 1998;42:3002-5.
10. Engesæter LB, Lie SA, Espehaug B, Furnes O, Vollset SE, Havelin LI. Antibiotic prophylaxis in total hip arthroplasty: effects of antibiotic prophylaxis systemically and in bone cement on the revision rate of 22,170 primary hip replacements followed 0-14 years in the Norwegian Arthroplasty Register. Acta Orthop Scand 2003;74:644-51.
11. Baleani M, Persson C, Zolezzi C, Andollina A, Borrelli AM, Tigani D. Biological and biomechanical effects of vancomycin and meropenem in acrylic bone cement. J Arthroplasty 2008; 23:1232-8.
12. Walenkamp GH. Gentamicin PMMA beads and other local antibiotic carriers in two-stage revision of total knee infection: a review. J Chemother 2001;13 Spec No 1:66-72.
13. Senthi S, Munro JT, Pitto RP. Infection in total hip replacement: meta-analysis. Int Orthop 2011;35:253-60.
14. Anguita-Alonso P, Rouse MS, Piper KE, Jacofsky DJ, Osmon DR, Patel R. Comparative study of antimicrobial release kinetics from polymethylmethacrylate. Clin Orthop Relat Res 2006;445:239-44.
15. Ismael F, Bléton R, Saleh-Mghir A, Dautrey S, Massias L, Crémieux AC. Teicoplanin-containing cement spacers for treatment of experimental *Staphylococcus aureus* joint prosthesis infection. Antimicrob Agents Chemother 2003;47: 3365-7.
16. Buchholz HW, Elson RA, Heinert K. Antibiotic-loaded acrylic cement: current concepts. Clin Orthop Relat Res 1984; (190):96-108.
17. Göğüş A, Akman S, Göksan SB, Bozdağ E. Mechanical strength of antibiotic-impregnated bone cement on Day 0 and Day 15: a biomechanical study with Surgical Simplex P and teicoplanin. [Article in Turkish] Acta Orthop Traumatol Turc 2002;36:63-71.
18. DiMarcantonio T. Aggressive debridement recommended for acute infections after knee replacement. Orthopedics Today 2007;27:10-11.
19. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA 1998;279: 1537-41.
20. Adams K, Couch L, Cierny G, Caloun J, Mader JT. *In vitro* and *in vivo* evaluation of antibiotic diffusion from antibiotic-impregnated polymethylmethacrylate beads. Clin Orthop Relat Res 1992:(278):244-52.
21. DiMaio FR, O'Halloran JJ, Quale JM. *In vitro* elution of ciprofloxacin from polymethylmethacrylate cement beads. J Orthop Res 1994;(12):79-82.
22. Huddleston PM, Steckelberg JM, Hanssen AD, Rouse MS, Bolander ME, Patel R. Ciprofloxacin inhibition of experimental fracture healing. J Bone Joint Surg Am 2000;82:161-73.
23. Antoci V Jr, Adams CS, Hickok NJ, Shapiro IM, Parvizi J. Antibiotics for local delivery systems cause skeletal cell toxicity *in vitro*. Clin Orthop Relat Res 2007;462:200-6.
24. Tsourvakas S, Alexandropoulos C, Karatzios C, Egnatiadis N, Kampagiannis N. Elution of ciprofloxacin from acrylic bone cement and fibrin clot: an *in vitro* study. Acta Orthop Belg 2009;75:537-42.
25. Stahlmann R, Lode H. Toxicity of quinolones. Drugs 1999;58 Suppl 2:37-42.
26. Hayem G, Carbon C. A reappraisal of quinolone tolerability. The experience of their musculoskeletal adverse effects. Drug Saf 1995;13:338-42.
27. Herbold BA, Brendler-Schwaab SY, Ahr HJ. Ciprofloxacin: *in vivo* genotoxicity studies. Mutat Res 2001;498:193-205.
28. Demirel H. Mechanical endurance of bone cement with antibiotics prepared with a different method: biomechanical study performed with teicoplanin. Master's thesis, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey: 2004.