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.
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±2ºC 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±2ºC. Axial compression tests were conducted at 18±2ºC 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. 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. Anguita-Alonso et al. found that cefazolin, ciprofloxacin, gatifloxacin, levofloxacin, linezolid, and rifampin could be used together with PMMA. In another study, teicoplanin, vancomycin and gentamicin were preferred. 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. 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. 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 be 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. 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. 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.

Tunney et al. 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.

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

However, Huddleston et al. 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-

| N  | Mean  | SD  | Result       |
|----|-------|-----|--------------|
| Control (Cement) | 20    | 80.2100 | 4.30742 | F=18.91 p=0.001* |
| 500 mg ciprofloxacin added cement | 20    | 74.5145 | 5.40370 | |
| 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.\textsuperscript{[23]} In an \textit{in vitro} ciprofloxacin elution study, Tsourvakas et al.\textsuperscript{[24]} added 150 mg ciprofloxacin to 6 g PMMA and obtained maximum values of 80.8 ± 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.\textsuperscript{[25]} Toxic effects of quinolones on connective tissue structures can also result in tendinopathies.\textsuperscript{[21,24]} However, fluoroquinolone-induced arthropathy is most frequently benign and heals without sequelae.\textsuperscript{[26]} In \textit{in vivo} studies confirm that ciprofloxacin is non-genotoxic and non-carcinogenic; therefore, ciprofloxacin is considered safe for therapeutic use.\textsuperscript{[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.\textsuperscript{[28]} A porosity of 5% is considered the lower limit for successful fixation.

In our study, mechanical tests were conducted \textit{in vitro} five days after the bone cement was prepared. \textit{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.\textsuperscript{[27]}

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.\textsuperscript{[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.\textsuperscript{[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 \textit{in vivo} use of ciprofloxacin and vancomycin.

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