EXPERIMENTAL STUDY

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Investigation of mechanical strength of teicoplanin and ciprofloxacin impregnated bone cement on Day 1 and Day 15

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Objective: The aim of this study was to compare the mechanical effects of different concentrations of teicoplanin and ciprofloxacin addition in bone cement.

Methods: In an experimental design, 3 different doses of teicoplanin and ciprofloxacin (800, 1600 and 3200 mg) were added to bone cement. Mechanical tests using compression and four-point bending tests were performed on Day 1 and after antibiotic leaching in water at 37°C on Day 15. Specimens that contained no antibiotics served as controls. Mechanical strength for each antibiotic concentration on Day 1 and Day 15 were evaluated.

Results: Both teicoplanin and ciprofloxacin significantly decreased the mean strength values in compression and four-point bending tests at Days 1 and 15 ($p<0.05$). While teicoplanin significantly decreased the mean strength values at high doses in both tests at Days 1 and 15 ($p<0.05$), ciprofloxacin did not significantly change these values. When the effects of two drugs compared, there were significant differences at the 3200 mg dose at Day 1 and at 1600 and 3200 mg doses at Day 15 in the compression testing and at 3200 mg at Day 15 in the four-point bending test.

Conclusion: Teicoplanin and ciprofloxacin addition may adversely affect the biomechanical strength of bone cement. Ciprofloxacin addition seems to have less of a negative effect on strength than teicoplanin.

Key words: Bone cement; ciprofloxacin; fracture point; teicoplanin.

Acrylic bone cement, also known as polymethyl methacrylate (PMMA), has been used in orthopedics for more than 60 years.[1,2] Charnley began using bone cement, previously only used in dentistry, in hip replacement surgeries in the 1960s.[3,4] Currently, PMMA is commonly used in arthroplasty.

The most common devastating complication after arthroplasty is deep wound infection. Researchers have attempted to develop different preventative and treatment techniques in order to decrease the frequency of infection.[5] Buchholz et al.[6] recommended that antibiotics be added to bone cement in order to decrease infec-

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tion rates. They added 2 grams of gentamicin powder to 40 g of Palacos® (Smith & Nephew Inc., Memphis, TN, USA) bone cement and concluded that the rate of infection decreased from 1.2% to 0.09% in cases of primary hip arthroplasty. It has been shown that addition of antibiotics in bone cement has numerous advantages such as low systemic toxicity, achievement of high local antibiotic levels, and minimal local tissue toxicity.[7,8]

Studies focusing on the usage, oscillation, and biomechanical strength of antibiotics such as teicoplanin, gentamicin, tobramycin, vancomycin, moxifloxacin and ciprofloxacin added to bone cement have been reported in the literature.[5,9] Ciprofloxacin is a recently added bactericide antibiotic and has a broad spectrum containing staphylococcus and streptococcus in addition to gram-negative bacteriums.[9-11] However, infections caused by resistant gram-positive strains are becoming more common in arthroplasty. Nowadays, hospital infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have become a major issue. The teicoplanin-loaded cement provided equal or better antibacterial activity against MSSA, MRSA, and VISA strains compared with cements loaded with daptomycin or vancomycin in an *in vitro* assay. Teicoplanin was the better choice for the treatment of *S. aureus* infection with antibiotic-loaded PMMA cements, compared with daptomycin or vancomycin.[12] Teicoplanin-loaded bone cement did not change the biocompatibility of bone cement.[13] Therefore, adding teicoplanin, which is more effective towards resistant strains, to cement has a positive effect on the success of the treatment.

To our knowledge, no studies comparing the effects of teicoplanin and ciprofloxacin addition in bone cement have been published. The aim of this study was to compare the mechanical effects of the addition of several concentrations of the antibiotics teicoplanin and ciprofloxacin in bone cement using compression and four-point bending tests.

Materials and methods

This study was conducted by the Cumhuriyet University Medical Faculty, Department of Orthopedics and Traumatology, and the laboratories at the Department of Pharmacology and Sivas Vocational School, Department of Engineering. All samples were prepared and tested at 18±2°C.

The study included two control and twelve experimental groups (800, 1600 and 3200 mg doses of ciprofloxacin and teicoplanin tested at Day 1 and Day 15).

Fourteen 40 g of normal viscosity orthopedic radiopaque bone cement (Biomecanica®; Biomecanica, São Paulo, Brazil) was used in this study. Ciprofloxacin obtained from Bayer HealthCare AG (Berlin, Germany) and teicoplanin (Targocid® 400 mg; Sanofi-Aventis, Anagni, Italy) were used with this cement.

A 12-mm high-cylinder mold with 43 holes and a diameter of 6 mm was prepared in accordance with ASTM (American Society for Testing and Materials) Code F 451-99a in order to conduct compression tests. The cylinder had two lids; one side sliding and the other side hinged. A plane-surfaced steel rod, with a similar diameter to that of the holes in the mold, was used to remove the prepared cement. For the four-point bending test, the 28x14x8.98 cm mold was used to produce 25 rectangular prism samples of 3.3x10x75 mm in accordance with ISO 5833.

An HTI Hounsfield® 50000 Newton capacity tension-compression testing device (Hounsfield Test Equipment Ltd., Redhill, United Kingdom) was used to conduct compression and four-point bending tests.

All samples were prepared under operating room conditions at a room temperature of 18±2°C. 80 mg, 1600 mg and 3200 mg samples were prepared using powder ciprofloxacin, a microbalance (CP 224S; Sartorius AG, Göttingen, Germany), and deducting the paper tare.

Both cement molds were oiled with a thin level of liquid Vaseline. Eighty grams of cement powder and the set amounts of antibiotic powders were homogenized by being mixed in a glass bowl for 1 minute using a spatula. Liquid monomer was added and mixed thoroughly for 30 seconds. After the prepared mixture was left for 90 seconds, both molds were filled simultaneously by hand, applying pressure to fill in all holes and gaps. A spatula was used to go over the mixture to make sure that all holes and gaps were completely filled. The lid of the mold, prepared for the compression test, was placed, and powergrip was used to compress the cement mold. Powergrip was loosened 15 minutes later, and the lids were removed. The cylinder samples were removed from the cement mold with the help of the plane-surfaced steel rod, with a diameter of 5.5 mm.

The bars between the molds prepared for the four-point bending test were removed and the rectangular prism samples were removed from the mold with the help of a spatula. Both procedures were conducted simultaneously.

The samples prepared for all groups were reviewed macroscopically, and their radiographs were taken using a digital X-ray device. Samples that did not have suitable

measurements, plane surfaces, or had cracks and gaps exceeding 10% of the section surface were excluded from the study. For each group, 10 samples were selected from the remaining samples using a randomized method and prepared.

On Day 15, the samples were placed in a 37°C water-bath, and in separate plastic boxes for each group. Compression test samples were given a 100 ml isotonic serum, and four-point bending test samples were given a 200 ml isotonic serum. The isotonic serum was changed every 24 hours. The antibiotic oscillated into the serum due to diffusion was distanced from the environment and in vivo conditions were simulated. The rate of antibiotic oscillation was assumed to be high; therefore, the compression test and the four-point bending test were repeated at the end of Day 15.

The compression test and the four-point bending test were conducted one day after the samples were prepared and on Day 15.

The compression test was conducted in accordance with the method stated in ASTM Code F 451-99a, 'standard specification for acrylic bone cement'. The compression tests of all samples were conducted using an HTI Hounsfield® tension-compression testing device in accordance with section F 451-99a of the ASTM. The mechanical strength was identified as the point where strain increased (upper yield point) with no increase in stress, created by the 2% permanent deformation (2% proof stress) caused by a 25 mm/min load applied to the cylinders, or the point where the sample broke, whichever occurred first. Tables were created in MPa (N/mm^2) after dividing the Newton value obtained for every sample by the surface area ($A=2\pi r$).

The four-point bending test was conducted in accordance with ISO 5833 (implants for surgery – acrylic resin cements) standards. The force for the bending test was determined in accordance with uniaxial bending stress calculation guidelines and bending stress values were calculated in MPa (N/mm^2). The 25 rectangular prism samples of 3.3x10x75 mm were prepared for each group using molds in accordance with ISO 5833 standards. The upper mold was magnetic and 60-mm long, while the lower mold was 100-mm long. Both molds were 30-mm thick and the rods that established the four points were 10.5-mm long and had a radius of 3 mm. The distance between the two rods in the upper mold was 20 mm and the gaps outside the rods were equal. The distance between two rods in the lower mold was 60 mm, and the gaps outside the rods were equal. Force was applied at a speed of 5 mm/min to prepared samples between both molds. The force recorded at the fracture point was accepted as the mechanical strength limit.

Data was analyzed using SPSS v14.0 (SPSS Inc., Chicago, IL, USA) software. Variance analysis (one-way

Table 1. Comparison between the mean strength results in MPa (N/mm^2) of compression test and four-point bending test conducted on Day 1 and Day 15 for different doses of ciprofloxacin.

Ciprofloxacin	Compression test (Mean±SD)		Four-point bending test (Mean±SD)	
	Day 1	Day 15	Day 1	Day 15
Control	94.73±7.43	103.56±12.25	70.08 ±6.68	66.90 ±3.46
800 mg	80.33±8.55*	88.69±10.07*	54.81±14.96*	52.77±13.01*
1600 mg	79.91±7.38*	88.15±6.72*	53.88±14.91*	46.39±14.91*
3200 mg	74.67±2.31*	81.10±11.43*	51.45±3.77*	44.91±15.39*

*p<0.05; statistically different from the control group.

Table 2. Comparison between the mean strength results in MPa (N/mm^2) of compression test and four-point bending test conducted on Day 1 and Day 15 for different doses of Teicoplanin.

Teicoplanin	Compression test (Mean±SD)		Four-point bending test (Mean±SD)	
	Day 1	Day 15	Day 1	Day 15
Control	94.73±7.43	103.56±12.25	70.08 ±6.68	66.90 ±3.46
800 mg	85.70±7.42	89.18±12.55*	54.44±11.02*	49.33±5.51*
1600 mg	84.30±6.10*	78.65±5.82*	51.16±14.98*	36.54±8.19*
3200 mg	82.73±10.74*	65.48±7.27*	47.11±10.62*	30.80±8.13*

*p<0.05; statistically different from the control group.

ANOVA) was used to compare the different doses of antibiotics within the same group and the Tukey test was used to identify the groups that caused the difference in analysis result. The Mann-Whitney U-test was used to compare Day 1 and Day 15 for each medication group and compare the compression test results and the four-point bending test results for 800 mg, 1600 mg, and 3200 mg on Day 1 and Day 15 for each medication. The error level referred to in all tables as arithmetic mean±standard deviation was accepted as 0.05.

Results

There was a significant difference between the control group and the 800 mg, 1600 mg, and 3200 mg ciprofloxacin-added bone cement groups on Day 1 and Day 15 in both of the compression and four-point bending tests ($p<0.05$) (Table 1). Additionally, there was a significant difference between the control group and the 1600 mg and 3200 mg teicoplanin-added bone cement groups on Day 1 in the compression test ($p<0.05$). On Day 15, there was a significant difference between the control group and all other teicoplanin-added groups in compression testing ($p<0.05$) (Table 2). There was a significant difference between the four-point bending test results of the control group and all other ciprofloxacin- and teicoplanin-added groups on Days 1 and 15 ($p<0.05$) (Table 1 and 2).

There were no significant differences in mean breaking point results of both compression and four-point bonding tests between Day 1 and Day 15 groups for all ciprofloxacin doses. On the other hand, there was a significant difference in mean breaking point results at Days 1 and 15 in the 1600 mg and 3200 mg teicoplanin groups ($p<0.05$) (Fig. 1).

When the two drugs were compared with the compression test, there were significant differences between teicoplanin and ciprofloxacin at the 1600 mg dose at Day 1 and at 1600 and 3200 mg doses at Day 15 ($p<0.05$) (Figs. 2a and b). In the four-point bending test, there were no difference between drugs for all doses at Day 1, but there was a significant difference in the 3200 mg dose at Day 15 ($p<0.05$) (Figs. 2c and d).

Discussion

Local antibiotic oscillation is achieved by adding antibiotics to bone cement. This application is a lifesaving option in prosthesis surgery where infection is a major problem. Antibiotic-added bone cement is obtained by

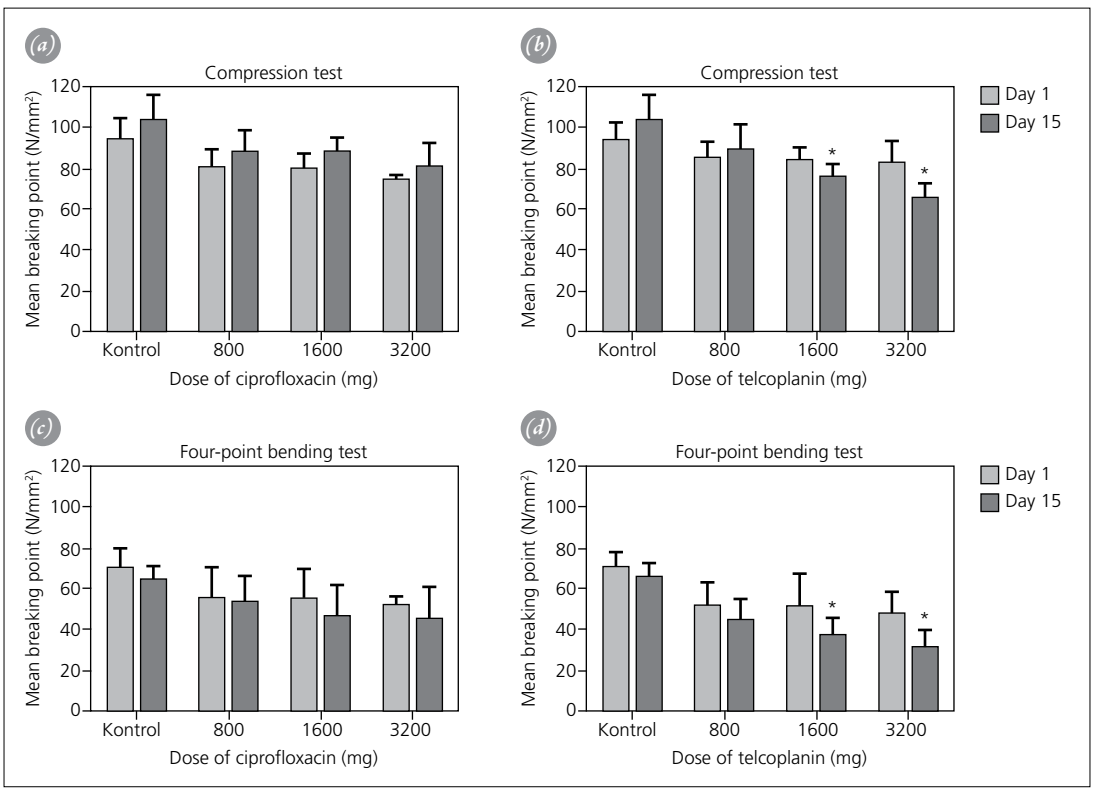

Fig. 1. Comparison between the mean strength results in MPa (N/mm²) of compression test and four-point bending test conducted on Day 1 and Day 15 for different doses of ciprofloxacin and teicoplanin. *$p<0.05$; statistically different from Day 1 group.

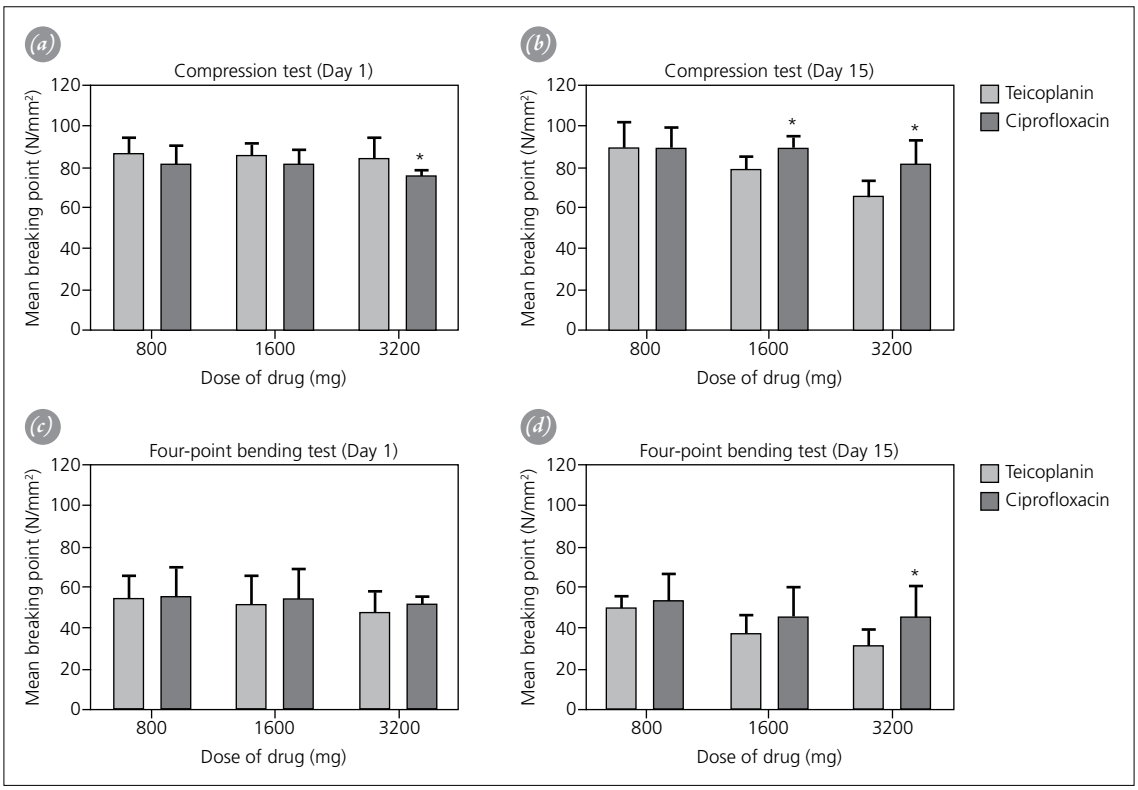

Fig. 2. Comparison between the mean strength results in MPa (N/mm²) of different doses ciprofloxacin and teicoplanin in compression test and four-point bending test conducted on Day 1 and Day 15. *p<0.05; statistically different from the teicoplanin group.

adding antibiotics to the powder form of bone cement prior to mixing the powder with liquid. The antibiotic is distributed more homogenously in ready-made, antibiotic-added bone cement in comparison to antibiotic-added bone cement where the antibiotic is added by hand.[14] It has been proven that the cement mixing procedure has a significant effect on the compression force and shear strength.[15,16]

In this study, both teicoplanin and ciprofloxacin significantly decreased the mean strength values in compression and four-point bending tests at Day 1 and 15. While teicoplanin significantly decreased the mean strength values at high doses in both tests at Day 1 and 15, ciprofloxacin did not significantly change these values. When the effects of the two drugs were compared, there was a significant difference at the 3200 mg dose at Day 1 in the compression test. In contrast, there were significant differences at the 1600 and 3200 mg doses at Day 15 in the compression test and at the 3200 mg dose at Day 15 in the four-point bending test.

Göğüş et al.[5] conducted compression and four-point bending tests on teicoplanin-added bone cement by adding different doses of teicoplanin to 40 g of Surgical Simplex P bone cement. They concluded that there was no significant difference between results for Day 0 while there was a significant decrease in strength in the 800 g teicoplanin-added group on Day 15. The mechanical strength obtained for all concentrations in both groups was above 70 MPa, the base limit stated by the ASTM. Four-point bending test results concluded that there was a significant decrease in strength after 1200 mg for the Day 0 group and with a dose of 400 mg for the Day 15 group. While values for 4000 mg in the Day 15 group were below 50 MPa, the base limit stated by ISO, results close to 50 MPa were obtained for the 3200 mg-added group and the 2000 mg-added group. The authors concluded that 1600 mg of teicoplanin was the highest dose that could be added to 40 grams of Surgical Simplex P bone cement under third generation cementation and preparation conditions. Contrary to the method used by Göğüş et al.,[5] in this study, the antibiotics were added to bone cement and mixed by hand, a method more commonly used under operating room conditions. Compression test results for all concentrations of both antibiotics in the Day 0 and Day 15 groups were above 70 MPa, the base value set forth by the F 451-99a standard of the ASTM.[17] In terms of the four-point bending test, values below 50 MPa,[18] the base limit set by ISO 5833,

were obtained for 3200 mg teicoplanin-added cement in the Day 0 group, for 800 mg, 1600 mg, and 3200 mg teicoplanin-added cement in the Day 15 group, and 1600 mg and 3200 mg ciprofloxacin-added cement in the Day 15 group.

In conclusion, teicoplanin and ciprofloxacin addition may adversely affect the biomechanical strength of bone cement. Ciprofloxacin addition seems to have less of a negative effect on strength than teicoplanin.

Conflicts of Interest: No conflicts declared.

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