Biomechanical comparison of tigecycline loaded bone cement with vancomycin and daptomycin loaded bone cements

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ABSTRACT

Objective: The objectives of this study were “1” to analyze the compressive and tensile mechanical strength characteristics of tigecycline loaded bone cement and “2” to compare them with those of vancomycin and daptomycin loaded bone cements which are used in prosthetic joint infections complicated with resistant microorganisms.

Methods: In this study, three experimental groups, which consisted of vancomycin (subgroups containing 1 g, 2 g, and 3 g vancomycin), daptomycin (subgroups containing 0.5 g, 1 g, and 1.5 g daptomycin), and tigecycline (subgroups containing 50 mg, 100 mg, and 150 mg tigecycline) and one control group without antibiotics were used. Using a standardized protocol, all antibiotic loaded bone cements were prepared. For each antibiotic group, including the control group, 10 samples were tested. All samples were biomechanically tested in terms of compressive strength and tensile strength.

Results: Compression tests showed that all determined antibiotic concentrations resulted in a significant decrease when compared with the control group (p<0.0011). Vancomycin and daptomycin study groups demonstrated lower tensile strength than the control group (p<0.0011). However, comparison of tensile values of tigecycline study groups with the control group revealed no significant difference (p>0.0011). In addition, all statistically significant results from between groups comparisons revealed higher tensile and compressive mechanical strength values for the tigecycline groups (p<0.0011).

Conclusion: Evidence from this study has demonstrated that tigecycline loaded bone cement may have no mechanical disadvantage compared with vancomycin and daptomycin loaded bone cements in terms of mechanical strength when used at defined concentrations.

Introduction

The treatment of prosthetic joint infection (PJI) is a challenge, and it depends on many variables related to the patient and the infecting organism. Even though rarely seen, this complication still occurs in arthroplasty patients despite numerous preventive measures (1-5). In patients with PJI, the most commonly isolated infectious agents are Staphylococcus aureus and S. epidermidis. At present, the most commonly used antibiotics for these infections are aminoglycosides and glycopeptides. However, owing to the increase in antibiotic resistance, orthopedic surgeons are obliged to seek new alternatives for local treatment as well as the systemic treatment of infection.

More effective options for the use of antibiotic bone cement are being investigated particularly for organisms such as methicillin-resistant Staphylococcus aureus (MRSA). Frequently, the preferred antibiotic in MRSA infection is vancomycin. However, nowadays many studies show that vancomycin susceptibility is decreasing and the number of multiresistant strains are increasing (6-9). In recent studies, promising results have been reported regarding the efficacy of daptomycin and tigecycline in the treatment of multiresistant infection cases (6-11). Tigecycline exhibits effective intracellular activity against S. aureus. It is absorbed by bone tissue after intravenous application (12). Intracellular activity becomes particularly important as S. aureus changes its phenotype into small colony variants (SCVs) that can adapt themselves for long-term intracellular persistence. This means S. aureus can invade human osteoblast cells and survive intracellularly. Generally, these SCVs have reduced metabolism and thus have an increased antibiotic resistance, particularly against aminoglycosides (7). Tigecycline has also been shown to have activity against MRSA and biofilm associated pathogens (13, 14). Thus, to treat multiresistant or MRSA infections the intracellular activity of tigecycline is important.

There are several studies in the literature concerning the mechanical analysis of bone cements containing vancomycin either isolated or combined with other antibiotics (15-21). However, information on bone cements containing daptomycin and particularly tigecycline is extremely limited (6-9, 10, 22). The cytotoxic effect on osteoblasts is one of the major concerns in local treatment with tigecycline. However, there is only limited data available about which tigecycline...
concentrations are safe, as the cytotoxicity is dose dependent (6, 23). In our review of the literature, we did not find any publications that mechanically compare tigecycline impregnated bone cements with daptomycin or vancomycin impregnated bone cements.

The objective of this study was to mechanically compare tigecycline loaded bone cements with daptomycin or vancomycin loaded bone cements. We hypothesized that, in lower concentrations, tigecycline would not have negative effects on the mechanical properties of the bone cement when compared with vancomycin and daptomycin loaded bone cements.

Materials and Methods

Sample preparation; antibiotics, bone cement, and groups
Three antibiotic loaded bone cement groups, vancomycin (subgroups containing 1 g, 2 g, and 3 g vancomycin), daptomycin (subgroups containing 0.5 g, 1 g, and 1.5 g daptomycin), and tigecycline (subgroups containing 50 mg, 100 mg, and 150 mg tigecycline), and a control group without antibiotics were used in the study. Antibiotics were first blended with the standard 40 g Polymethylmethacrylate powder (Osteobond copolymer bone cement, Zimmer, Warshaw, IN, USA) by manually using a stainless-steel bowl and spatula for 5 minutes. During the preparation of samples, all bone cement and antibiotic powders were weighed separately with a precision scale before mixing to avoid weight differences that could occur in the fabricated production. The mixture of antibiotic and bone cement powder was then added to a standard 20 mL monomer liquid and mixed until a homogenous dough was obtained. The doughs were then transferred to aluminum alloy molds, which were specially prepared for the samples’ compression and tension tests and kept under pressure with a metal plate clamped to the mold for 30 minutes to provide solidification therein.

Cylinders with a diameter of 6 mm and a height of 12 mm were prepared in accordance with ASTM F451 standard for compression tests (6, 19, 20, 24). Half-length specimens of ISO527 standard were prepared for tensile tests at 10 mm width and 75 mm length (test area 5 mm width, 25 mm length) (Figure 1) [25]. All prepared samples were evaluated radiologically with a standard digital mammographic X-ray and samples with any visible amount of air bubbles were excluded from the study. In addition, all samples included in the study were weighed on a precision scale and their average weights were confirmed.

All samples were prepared at standard 25°C ambient temperature. Commercial forms of antibiotics were purchased and used (Vancomycin: Edicin® 1 g vial, Sandoz, Holzkirchen, Germany; Daptomycin: Cubicin® 500 mg vial, Novartis, Basel, Switzerland; Tigecycline: Tygacil® 50 mg vial, Pfizer, New York, NY, USA). For each antibiotic group, including the control group, 10 samples were tested. A total of 100 compression and 100 tensile tests were completed.

### HIGHLIGHTS

- Tigecycline exhibits effective intracellular activity against S. aureus and have activity against MRSA and biofilm associated pathogens.
- Due to the cytotoxic effect on osteoblasts, in the local treatment of infections, higher concentrations of Tigecycline must be avoided.
- Lower concentrations of Tigecycline does not have detrimental mechanical effects on antibiotic loaded bone cement.

Mechanical testing/evaluation

All samples prepared for compression tests were subjected to a load-to-failure test at a constant loading rate of 5 mm/minute with a universal test instrument (Autograph AG-IS 5kN, Shimadzu Co., Kyoto, Japan). All specimens were prepared at least 24 hours before the test and stored at 23±1 °C in the test environment. The data was recorded with the instrument’s software (Trapezium X, Shimadzu Co., Kyoto, Japan) at a data rate of 50 ms (200 Hz).

The samples prepared for the tensile tests were subjected to a load-to-failure test at a constant loading rate of 5 mm/minute with the same universal test instrument, 10 in each group, such as compression test samples (Data rate: 50 ms or 200 Hz).

Statistical analysis

The data obtained from the Trapezium software were grouped and transferred to the Statistical Package for Social Sciences version 22, (IBM SPSS Corp., Armonk, NY, USA). Statistical analyses were done using Kruskal-Wallis analysis of variance and Bonferroni-corrected Mann-Whitney U tests. Because the study was an in vitro controlled study with small “n” numbers, no tests were done for assessing the normality of data distribution. As Bonferroni-corrected Mann-Whitney tests were used the value of p was accepted as alpha=0.0011 (For 95% confidence interval: p/number of comparisons).

Results

In the evaluation of compression tests, statistically significant difference was found between all compression values of control group and antibiotic loaded bone cement groups (p<0.0011) (Table 1). For the tension tests, pairwise comparison between the control group and vancomycin and daptomycin loaded bone cement groups were significant (p<0.0011), but tigecycline groups showed statistically insignificant results (p>0.0011) in Bonferroni-corrected Mann-Whitney tests (Table 1).

Pairwise comparisons between tigecycline and vancomycin groups demonstrated significantly higher mechanical strength values for tigecycline 50 mg group than for vancomycin 2 g and 3 g groups and for tigecycline 100 mg group than for vancomycin 3 g group in compression tests (p<0.0011) (Table 2).
The tensile strength of tigecycline 100 mg and 150 mg groups were significantly higher than that of the vancomycin 2 g group (p<0.0011) (Table 2). In addition, all tigecycline groups showed significantly higher values than the vancomycin 3 g group in tension tests (p<0.0011) (Table 2).

Pairwise comparisons between tigecycline and daptomycin groups revealed significantly higher mechanical strength values for tigecycline 50 mg group than for daptomycin 0.5 g, 1 g, and 1.5 g groups and for tigecycline 100 mg group than for daptomycin 1 g and 1.5 g groups in compression tests (p<0.0011) (Table 3). Comparison of tension tests showed that tigecycline 100 mg and 150 mg groups showed significantly higher values than the daptomycin 1.5 g group (p<0.0011) (Table 3).

In compression tests, the average mechanical strength values of all antibiotic loaded groups were above the minimum acceptable threshold of 70 MPa according to ISO (5833) standards. Although some of the pairwise comparisons were not statistically significant, all tigecycline groups showed higher values than all vancomycin and all daptomycin groups in both compression and tension tests (Table 2, 3) (Figure 2, 3). The tigecycline study group showed a slight directly proportional increase in the mean tensile strength as the antibiotic concentration increased (Figure 3). However, this was not statistically significant (p>0.0011). In vancomycin and daptomycin study groups a gradual decrease (inversely proportional) in the mean tensile strength was observed as the antibiotic concentration increased (Figure 3).

Discussion

One of the parameters affecting the mechanical strength of antibiotic loaded bone cement is the concentration of antibiotic powder along with others such as type of the antibiotic, elution time and characteristics, hydration, environment containing fat, temperature, and stress levels [15, 16, 27]. Our findings confirm that the addition of tigecycline...
In our study, we obtained significantly lower mechanical strength in all vancomycin groups than the control group including the 1 g (2.5% w/w) vancomycin group. However, the values were above the minimum acceptable threshold according to ISO requirements. The results we obtained with vancomycin loaded groups are mostly consistent with the compression test results from the literature, but there are also some differences. These differences may be due to the application of different test methods and/or use of materials with slightly different material properties (i.e., different brands).

Hsu et al., reported that antibiotic loaded bone cements containing 0.5 g, 1 g, and 2 g of daptomycin achieved over 100 MPa in all groups in compressive tests (8). These values are even higher than the control group values in our study. Our daptomycin loaded bone cement groups showed inversely proportional compressive strength values relative to the daptomycin concentration, all of which were lower than the control group. But all daptomycin study groups were above the minimum acceptable value according to the ISO standards (Figure 2).

To date, there are only two published studies concerning tigecycline loaded bone cement (6, 7). Kreis et al., found that the mean compressive strength values of bone cements containing 1.225% (w/w) tigecycline (76.4 MPa) were higher than the ISO standards (70 MPa), but mean bending strength values (44.3 MPa) were lower than the ISO standards (50 MPa) (7). The authors concluded that tigecycline showed effective intracellular antimicrobial activity with negative influence on the biomechanical stability of bone cement. Compared with our study, the values obtained in this study were slightly lower than those of our values of 150 mg tigecycline group (81.06±3.73 MPa). This can be explained by comparing the groups between the studies; the amount of antibiotic powder mixed with the unit quantity of bone cement is slightly less in our 150 mg tigecycline group (a 50 mg vial contains an average of 145 mg antibiotic powder according to our measurements, which corresponds to 1.087% (w/w) in our tigecycline 150 mg group).

Nichol et al., investigated the results of wear and impact testing of bone cements loaded with varying concentrations of linezolid and tigecycline (6). The impact tests showed that the group containing 10% (w/w) tigecycline had slightly lower values, but this was not statistically significant. The authors noted that the concentration of tigecycline may need to be controlled owing to the possible cytotoxicity of the released antibiotic toward osteoblast cells. As the mechanical test methods are different, a direct comparison to this study is impossible. However, it may suggest that the results of mechanical tests are not contrasting.

In this study, when compared with the control group, we determined that mechanical strength values were significantly lower in all antibiotic loaded bone cement groups. Whereas these results are consistent with most studies in the literature, surprisingly some studies suggest otherwise. It has also been reported that the compressive strength of the antibiotic loaded bone cement did not change after elution or increase relative to the control group (10, 15).

Within this information and the review of the literature, we have not found any other study comparing the mechanical properties of tigecycline loaded bone cement with vancomycin and/or daptomycin loaded bone cements.

In our study, tigecycline loaded bone cement groups were found to have higher compressive and tensile strength values than vancomycin and daptomycin loaded bone cement groups. This is possibly be-
cause of the lower amount of antibiotic powder added to the unit amount of bone cement. This situation may change by increasing the amount of tigecycline concentration in bone cement. However, Nichol et al., stated that the addition of higher concentrations of tigecycline resulted in a marked reduction in cell activity, so the use of high concentrations of tigecycline will also have the risk of cytotoxic effects on osteoblasts (6). Ignjatović et al., in an in vitro study for the evaluation of a multifunctional nano drug delivery system based on tigecycline-loaded calcium-phosphate/poly-DL-lactide-co-glycolide, reported that the nanoparticulate multifunctional drug delivery system showed good compatibility and antibacterial effect and added that at low concentrations (0.6% wt) it did not reveal cytotoxic effects when compared with the corresponding controls (23). However at higher concentrations (5% wt), a significant deleterious effect on osteoblasts was detected. There are no other studies defining which concentrations are safe for the local treatment with tigecycline. Therefore, to be practical, we decided to test lower concentrations for tigecycline and selected 50 mg (1 vial: 0.362% w/w), 100 mg (2 vials: 0.725% w/w) and 150 mg (3 vials: 1.087% w/w) groups, and compared these with low to moderate concentrations of vancomycin and daptomycin loaded bone cements (as used in routine clinical practice). As further lowering the concentrations of vancomycin and daptomycin to equalize with tigecycline concentration would be insignificant, we did not choose to make a weight-to-weight comparison.

When the mechanical strength values of the compression tests were examined, a decrease in the values was observed as the antibiotic concentration increased (Figure 2). However, taking into consideration both of the statistic analysis of the data and the differences in antibiotic concentrations, it cannot be stated that one of the antibiotic cement groups is superior to the others (Table 2, 3). To make such a statement, antibiotic concentrations must be matched with a weight-to-weight method.

Our study also has some limitations. The absence of four-point bending, wear, and fatigue tests as well as testing before and after elution in liquid (phosphate buffered or protein-containing) medium may be considered as weaknesses of this study. Liquid medium and the elution of antibiotic from bone cement may lead to alterations in the mechanical evaluation of antibiotic loaded bone cements (16, 17, 20). In studies analyzing the mechanical properties of bone cements mostly one or two test methods are employed (6-8, 10, 18-20, 26). Likewise, to keep the test methods as simple as possible, we included two test methods. Further studies including alterations of the mechanical properties with elution, quantification studies, antimicrobial activity studies, and more importantly studies aimed for determining the safe concentration of tigecycline are required.

In conclusion, this study demonstrated that, tigecycline loaded bone cement has no disadvantage compared with vancomycin and daptomycin loaded bone cements in terms of mechanical strength when used at the indicated concentrations. Local treatment with tigecycline loaded bone cement may be an option or a salvage therapy in selected patients with highly resistant strains. This study is an in vitro mechanical study and does not reflect in vivo conditions. Further in vitro/in vivo studies should be encouraged (as mentioned above) in addition to clinical and microbiological studies to investigate the validity of local treatment of infection with tigecycline loaded bone cement. Nevertheless, considering the intracellular activity and treatment efficiency in highly resistant cases along with our findings, tigecycline loaded bone cement may be a promising alternative.

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