Does kanamycin have effectiveness as an antibiotic-loaded bone cement for the treatment of musculoskeletal tuberculosis?

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During my residency of the Orthopaedic surgery, Orthopaedics was rather daily working than knowledge to me. As time goes by, I've realized that it has proper mechanism and scientific evidence from my clinical experience in taking care patients. At that time, I could have delightful interest in studying Orthopaedics as my major.

Now, at 10th year after my first step in Orthopaedics, this experiment including pre-experiment planning, performing of experiment, analysis of results, completion of manuscript gave me big pleasure of study and research.

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Does kanamycin have effectiveness as an antibiotic-loaded bone cement for the treatment of musculoskeletal tuberculosis?

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Background/Purposes Antibiotic-loaded bone cement (ALBC) is used to deliver antimycobacterial agents into the focal lesion of musculoskeletal tuberculosis. However, there has been limited information about suitability of antimycobacterial drugs to be impregnated with bone cement. Kanamycin is currently used as an antimycobacterial agent for the treatment of multi-drug resistant tuberculosis. Although kanamycin is used as alternative for streptomycin which is same aminoglycoside family due to different spectrum of resistant strains, there is no information about the suitability for ALBC.

Questions/Purposes (1) the effective elution of kanamycin, (2) the duration of antimycobacterial activity of kanamycin-loaded cement, (3) mechanical strength of kanamycin compared with vancomycin in forms of ALBC.

Methods This *in vitro* experiment was conducted with three types of bone cement cylinder created by mixing 40 g bone cement with 1, 2 and 3 g kanamycin. For elution and antimycobacterial test, cement cylinders were made with a diameter of 10 ± 0.8 mm and height of 50 ± 1.2 mm. For ultimate compression test, additional 12-mm height and 6-mm diameter cement cylinders were molded to follow the standard for mechanical strength test. Five bone cement cylinders of each type were incubated in phosphate buffered saline (PBS) for 30 days with renewal of the PBS daily. Each 1 mL of eluate was extracted to measure level of elution and antimycobacterial activity on Days 1, 4, 7, 14, and 30, just before renewal the PBS. The quantity of kanamycin in eluates were evaluated by a liquid chromatography–mass spectrometry system, and the antimycobacterial activity of eluates against Mycobacterium tuberculosis H37Rv, were calculated by comparing the minimal inhibitory concentration of each eluate with that of tested drugs using broth dilution assay on microplate. The ultimate compression strength was measured with five cement cylinders per regimen using a material testing system machine before and after elution.

Results Eluates in 2 and 3g kanamycin-containing had effective antimycobacterial activity for 30 days, while elutes in 1g kanamycin has

been partially active until Day 30. The concentration of eluates at day 1 demonstrated dose-dependent tendency (Day 1, 1g kanamycin, 44.8 ug/mL ; 2g kanamycin, 64.1 ug/mL ; 3g kanamycin, 128.2 ug/mL), however it had been equalized over time. The ultimate compression strength values of original bone cement cylinder were 99.3 ± 1.04 MPa and 95.7 ± 0.83 MPa before and after elution test. The pre-eluted compression strength of kanamycin and vancomycin-loaded cement were weaker as contained larger amount of antibiotics in bone cement (1g kanamycin, 97.4 ± 2.1 MPa ; 2g kanamycin, 96.6 ± 2.1 MPa ; 3g kanamycin, 94.6 ± 1.4 MPa ; 1g vancomycin, 95.9 ± 2.3 MPa ; 2g vancomycin, 94.9 ± 2.5 MPa). There was no statistical difference of strength between all regimen of kanamycin and 1g of vancomycin in the ultimate compression test. After 30 days elution, the strength of each no-loaded, all regimen of kanamycin and vancomycin-loaded cement cylinders were significantly lowered than that of initial specimens (after elution, 1g kanamycin, 86.2 ± 3.5 MPa ; 2g kanamycin, 84.7 ± 3.7 MPa ; 3g kanamycin, 83.2 ± 3.0 MPa ; 1g vancomycin, 86.6 ± 3.6 MPa ; 2g vancomycin, 85.1 ± 3.5 MPa, $P < 0.05$).

Conclusions In forms of ALBC, above 2g kanamycin had showed effective antimycobacterial activity during a 30-day period. From 1 to 3 g

kanamycin-loaded bone cement demonstrated comparable ultimate compression strength with 1g vancomycin while maintaining effective elution until day 30. After elution test of 30 days, although the compressive strength of the kanamycin loaded bone cement decreased significantly, it was in accordance with the reference strength of the bone cement and was similar to that of vancomycin loaded bone cement.

Key words : tuberculosis, musculoskeletal tuberculosis, elution, antimycobcteroal activity, ultimate compression strength, bone cement

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I. INTRODUCTION

Musculoskeletal tuberculosis (MSTB) is the third common infection in extra-pulmonary tuberculosis which is occupying approximately 2-10% of all tuberculosis infection [1,2]. Surgical intervention including debridement, bone graft, arthrodesis or prosthetic replacement depending on the location and degree of destruction is regarded as essential treatment for MSTB [1,3,4]. In addition, MSTB requires to be treated with long - term medical treatment as well as local surgical treatment[5]. However, the proper selection of an antimycobacterial agent is important for satisfactory outcomes because of multidrug resistance (MDR) depending on the regional characteristics of tuberculosis and side effects of long-term medication [6-8].

Antibiotic-loaded bone cement (ALBC), which is used in debridement or arthroplasty, is a feasible method to control the recurrence of local infection [9]. Although there are a lot of studies about biomechanical properties and clinical outcomes on ALBC associated with bacterial infection, relatively few studies

have been conducted on MSTB. In the production of an ALBC, the range and selection of antimycobacterial drugs is limited because antibiotics made as a powdered form is required to provide effective elution and antimycobacterial activity [10,11]. We have already investigated the elution and antimycobacterial activity of first and second-line antimycobacterial drugs by *in vitro* experiments. In those experiments, isoniazid and streptomycin proved to have effective antimycobacterial activity.

Among the antimycobacterial agents of aminoglycoside family, kanamycin, capreomycin and streptomycin are available in powder forms. We previously performed an *in vitro* study of streptomycin - loaded bone cement to investigate its elution characteristics and antimycobacterial activity[11]. Kanamycin, capreomycin and amikacin were selected as second-line agents for the treatment of multidrug-resistant tuberculosis at the starting point of this experiment. Among aminoglycoside agents for tuberculosis treatment, except for streptomycin, only kanamycin has a powder form with dual effects on both bacterial and tuberculosis infection. Furthermore, kanamycin is relatively inexpensive and easily available in various countries. In a clinical result, a combination of antibiotics to control a broad spectrum of bacteria and tuberculosis demonstrated satisfactory results [5]. An antimycobacterial agent which is able to control both bacterial and tuberculosis infection might be more beneficial due to the unclear diagnosis until confirmation of the tissue or specimen obtained during operation. However, there has been limited

information about the suitability of kanamycin when mixed with bone cement. Therefore, we investigated : (1) the effective elution of kanamycin as an antibiotic-loaded cement (2) the antibacterial activity of kanamycin-loaded cement, and (3) the mechanical strength of kanamycin compared with vancomycin-loaded cement.

II. MATERIALS AND METHODS

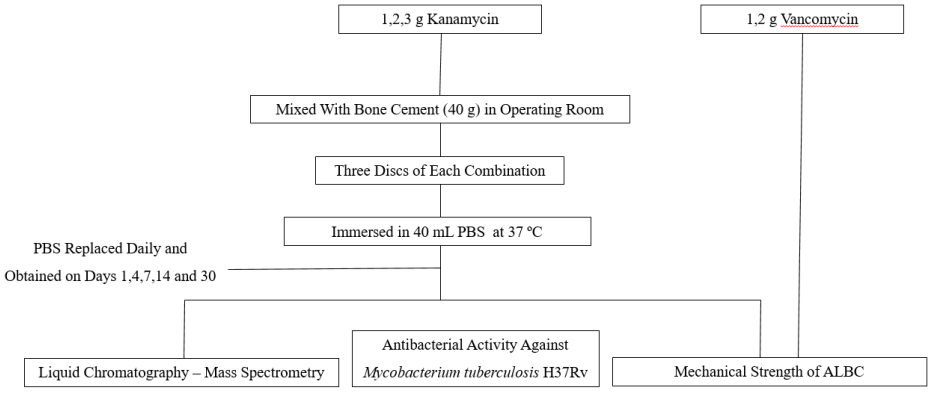

Fig. 1. The schematic flow of experiment.

1. Preparation of ALBC

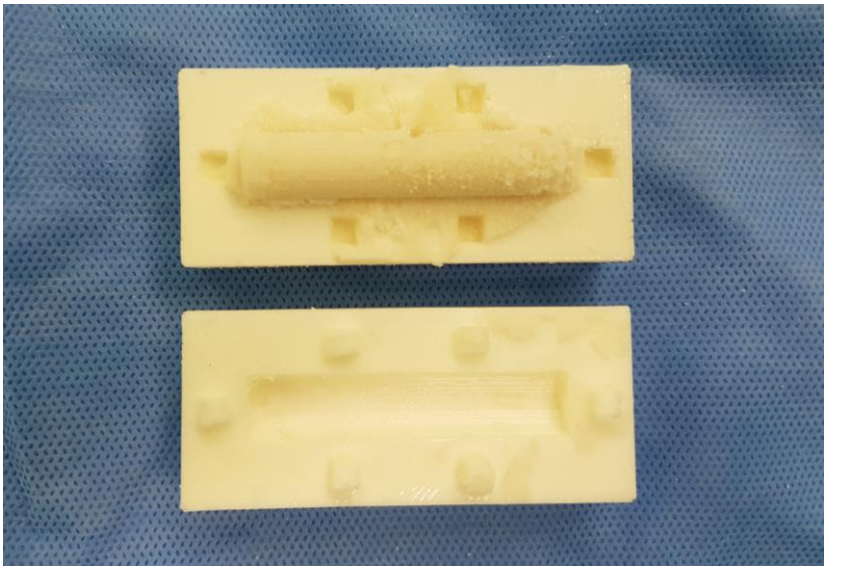

Fig. 2. The gross shape of test specimen for elution and antimycobacterial activity test.

The overall flow of this study is described in Fig. 1. For the elution and the antimycobacterial activity tests, three doses of antibiotics were mixed with 40 g of bone cement powder not containing antibiotics (CMW^{TM} 3; DePuy-Synthes, Warsaw, IN, USA). A total of 1, 2, or 3 g of kanamycin (Kanamycin monosulfate, Sigma-Aldrich, Saint Louis, MO, USA) were mixed with bone cement under a sterile environment in the operating room. After adding the liquid monomer, manual mixing of cement powder and kanamycin was performed for 2 minutes and then the doughy mixture was molded in a customized frame to obtain a cylindrical specimen (Fig 2). Five specimens of

each of the three different doses of kanamycin-loaded bone cement were made to measure the elution characteristics and the antibacterial activity. The specification of specimens for the elution and antimycobacterial activity tests were 10 ± 0.8 mm in diameter and 50 ± 1.2 mm in height, with a weight of 4.4 ± 0.26 g.

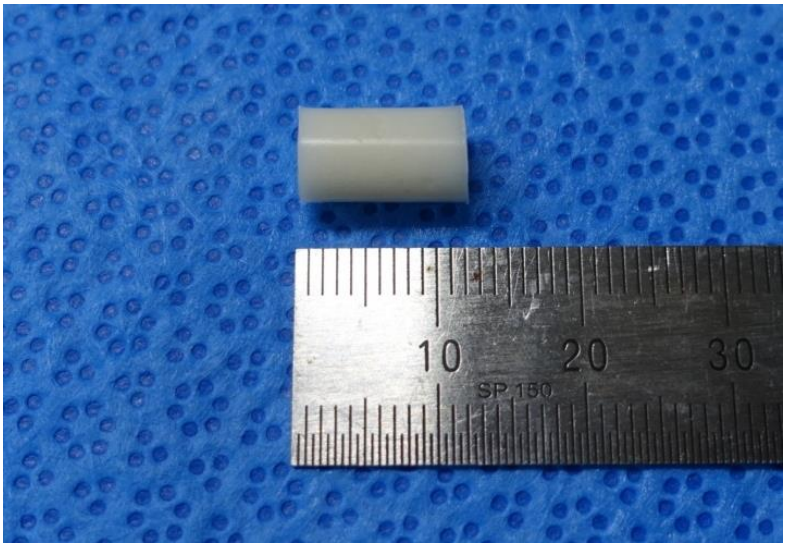

Fig. 3. The gross shape of test specimen for ultimate compression test.

For UCS test, three different doses of kanamycin and two different doses of vancomycin (Vancorin®, CJ HealthCare, Seoul, South Korea) were mixed with 40 g bone cement powder. Bone cement mixed with no antibiotic was produced as a control group. Finally, 11 groups of specimens for each dose of kanamycin and vancomycin-loaded bone cement were prepared to measure the UCS. All specimens has been washed daily and immersed with 30 mL of phosphate

buffered saline (PBS) to determine the UCS after 30 days of elution. The test specimens were made in regulated laboratory conditions (air, at 22 ± 1 ℃), in accordance with ASTM F451 and ISO 5833[12,13]. The bone cement and related tools to be used in the dough were stored for at least 2 hours at a relative humidity of 40% or more and tested under the temperature and humidity conditions. An appropriate molding was made with a stainless-steel frame to produce a bone cement cylinder with a length of 12 ± 0.1 mm and a diameter of 6 ± 0.1 mm. The duration of polymerization which had been proceeded after dough time and finished by complete hardening of ALBC was determined (kanamycin 1g ; 8.6 ± 0.8 min, 2g ; 8.9 ± 0.8 min, 3g ; 9.3 ± 1.1 min, vancomycin 1g ; 8.2 ± 0.6 min, 2g ; 8.8 ± 0.9 min, respectively). The ends of the specimens were made flat and smooth to be parallel to each other and at right angles to the long axis of the cylinder (Fig 3). The specimens were visually inspected for surface defects and accepted when appearance was uniform and meet the dimensional requirements.

2. Elution test

Each dose of kanamycin-loaded cement has been immersed at 37℃ with 40 mL PBS in a 50-mL test tube and protected from light during the experimental period. At every 24 hours, PBS was aseptically replaced with fresh PBS. Each 1 ml of PBS sample was extracted to determine elution characteristics and

antimycobacterial activity at days 0, 1, 4, 7, 14, and 30, just before renewal of the PBS. A total of 180 samples, which included 90 samples to determine elution characteristics and 90 to determine antimycobacterial activity were obtained. All samples had been stored at -20° C until the final measurements for elution and antimycobacterial activity. Analysis for the quantification of kanamycin in the eluate was performed by a liquid chromatography–mass spectrometry system. After the mass spectrometer (API3200™; AB SCIEX, Framingham, MA, USA) detected compounds, they were segregated using high-performance liquid chromatography (Agilent 1100 series; Agilent Technologies, Santa Clara, CA, USA). For high-performance liquid chromatography, 0.1% formic acid: acetonitrile (80:20 [v/v]) was used as the mobile phase, and Kinetex® C18 (2.6 μm, 4.6 mm × 50 mm; Phenomenex, Torrance, CA, USA) was used as the column. The multiple reaction monitoring and positive ion mode were used for kanamycin.

3. Measurement of antimycobacterial activity

Antimycobacterial activity of kanamycin was evaluated with a microplate assay of *Mycobacterium tuberculosis* (Mtb) H37Rv [14]. After Mtb was stained with Alamar Blue dye, red fluorescence was read after 3 and 6 hours to indicate viability. Mtb H37Rv was grown in 10 mL of Middlebrook 7H9 broth (Difco Laboratories Inc, Sparks, MD, USA) supplemented with 0.2% (v/v) glycerol

(Sigma-Aldrich), 1.0 g/L casitone (Difco Laboratories Inc), 10% (v/v) oleic acid, albumin, dextrose, and catalase (OADC, Difco Laboratories Inc), and 0.05% (v/v) Tween® 80 (Sigma-Aldrich) until optical density at 600 nm reached 0.4. The minimal inhibitory concentration (MIC) of eluent was determined using a broth dilution assay on a microplate. The initial dilution in the microplate wells were 2.5%, 5%, 10%, 25%, 50%, and 75%, of the eluent, and then twofold serial dilutions of such initial dilutions were made with 7H9 broth in the microplate. Each plate well was inoculated with H37Rv at 2×10^5 CFU/mL. Plates containing eluate dilutions and Mtb were incubated at 37℃ for 7 days, after which Alamar Blue dye was added for 3 and 6 hours, and then fluorescence was measured in a FLUOstar® Optima (BMG Labtech GmbH, Ortenberg, Germany) microplate fluorometer using a bottom-reading mode with excitation at 560 nm and emission at 590 nm.

The MIC was defined as the lowest concentration of antibiotic in the eluate that inhibited fluorescence by 90% compared with the fluorescence of bacteria-only wells. The MICs of kanamycin, of which stock was prepared at 10 mg/mL in dimethyl sulfoxide, were measured at the same time. The mean MIC value that was experimentally measured for kanamycin was 1.0 μg/mL. The antimycobacterial concentration of kanamycin in each eluate was calculated by comparing the MIC of each eluate with that of kanamycin, assuming that the obtained MICs of eluent should include the MIC of the tested antibiotic:

Antimycobacterial concentration = (MIC of tested drug x 100)/concentration (%) of eluent at MIC

Antimycobacterial activity against Mtb was considered effective when the antimycobacterial concentration was greater than the mean MIC value of the tested drug [11].

Considering the mean MIC values and initial dilution, the antimycobacterial activity of kanamycin was calculated and described using the method as follows:

Antimycobacterial activity of kanamycin = 1.0 μg/mL × $2^{(\text{number of dilution} + 1)}$.

4. Ultimate compression strength test

The UCS test of pre- and post-elution bone cement made by original product without antibiotics and each doses of kanamycin and vancomycin was performed to evaluate the mechanical strength of ALBC using the material testing system machine (Instron 3366, Instron, Norwood, MA, USA) at a loading rate of 21 mm/min. The compression strength of post-elution specimens was mechanically tested at the end of the elution period. The specimen was positioned on a flat supporter clamped to the machine lower wedge grip, and then a stiffened plate was placed on the top surface of the cement. The experimental setup ensured the full surface contact of the specimen to achieve a uniform pressure. During testing, parameters including force, displacement, and

time were recorded simultaneously by material testing system software (series IX/s, Instron, Norwood, MA). The peak force divided by the cross section surface of the specimen was defined as the UCS [15]. Average and standard deviation values for the UCS was calculated for each group.

5. Statistical analysis

SPSS Statistics for Windows Version 23.0 (IBM Corporation, Armonk, NY, USA) and a probability less than 0.05 indicated statistical significance, with 95% CI. A two-way analysis of variance was used to compare the value of samples from five discs for each elution, antimycobacterial, ultimate compression tests. If a significant difference was determined, pair-wise comparison was performed with Holm-Sidek post hoc analysis.

III. RESULTS

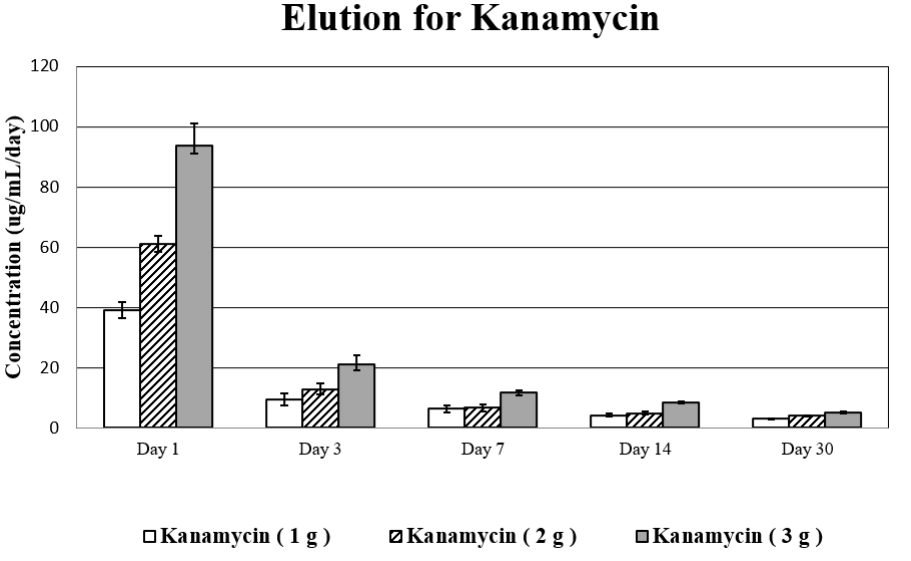

Fig 4. The trend in eluting of kanamycin. The graph shows elution of 1, 2 and 3 g Kanamycin mixed with 40 g customized bone cement using a liquid chromatography–mass spectrometry system. The white bar indicates elution of 1 g kanamycin, the oblique lined bar indicates elution of 2 g kanamycin and the gray bar indicates elution of 3 g kanamycin.

Kanamycin has been detected in eluates of all regimen during the 30 days eluting period (Fig. 4). Concentrations of all doses had been decreased with time. However, regardless of initial dose mixed, there was no difference in the amount of elution at day 30 (1 g kanamycin, 3.07 ± 0.42 μg/mL; 2 g kanamycin, 4.04 ± 1.14 μg/mL; 3 g kanamycin, 5.11 ± 2.27 μg/mL; 95% CI, p = 0.372).

Table 1. Median values of estimated concentration in the antimycobacterial activity of eluents (ug/mL)†

	Day 1	Day 4	Day 7	Day 14	Day 30
Kanamycin 1 g	32.0*	8.0*	8.0*	NA	NA
Kanamycin 2 g	64.1*	8.0*	8.0*	4.0*	4.0*
Kanamycin 3 g	128.2*	16.0*	16.0*	8.0*	8.0*

†Effluent could be decided effective when concentration is Kanamycin 1.0 ug/ml.

*Effective when the concentration of antibiotics was greater than the mean MIC

value.

Abbreviation: NA (no activity : indicates that one or more of the five specimen demonstrate the concentration below the mean MIC value.)

Based on the mean MIC values obtained from the experiment (mean MIC value for kanamycin was 1.0 μg/mL). Eluates in 2 and 3 g kanamycin-containing cylinders had effective antimycobacterial activity for 30 days, while elutes in 1 g kanamycin cylinders has been active only until day 7. The antimycobacterial activity of eluates at day 1 demonstrated a dose-dependent tendency (Day 1, 1 g kanamycin, 44.8 μg/mL; 2 g kanamycin, 64.1 μg/mL; 3 g kanamycin, 128.2 μg/mL), however, it gradually equalized by day 30. The median values of calculated concentration are described in the Table 1.

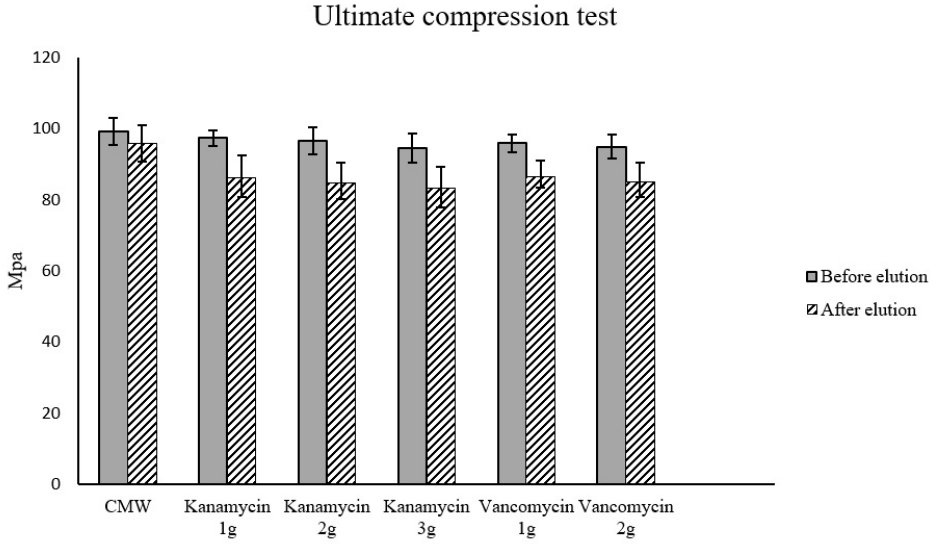

Fig 5. The comparison of ultimate compression strength in pre - and post -

elution test.

The each UCS values of the cement cylinder not loaded with antibiotics were 99.3 ± 1.04 MPa and 95.7 ± 0.83 MPa before and after the elution test, respectively. The pre-eluted compression strength of kanamycin and vancomycin-loaded cement were weaker with larger amount of antibiotics contained in the cement (1 g kanamycin, 97.4 ± 2.1 MPa; 2 g kanamycin, 96.6 ± 2.1 MPa; 3 g kanamycin, 94.6 ± 1.4 MPa; 1 g vancomycin, 95.9 ± 2.3 MPa; 2 g vancomycin, 94.9 ± 2.5 MPa) (Fig. 5). However, there was no statistical difference of strength between all kanamycin-loaded cylinders and that of cylinders loaded with 1 g vancomycin in the compression test. After 30 days elution, the strength of each non-loaded, kanamycin-loaded, and vancomycin-loaded cement cylinders were significantly lowered than that of the initial specimens (after elution, 1 g kanamycin, 86.2 ± 3.5 MPa; 2 g kanamycin, 84.7 ± 3.7 MPa; 3 g kanamycin, 83.2 ± 3.0 MPa; 1 g vancomycin, 86.6 ± 3.6 MPa; 2 g vancomycin, 85.1 ± 3.5 MPa, $P < 0.05$).

IV. DISCUSSION

In the elution test, all doses of kanamycin has been detected during 30 days of elution. The amount of detected kanamycin at the first day of the eluting period was proportional to the amount of antibiotics contained within the bone cement. However, there was no significant difference in the amount measured after 30

days according to the dose. In terms of antimycobacterial activity, eluates from 2 and 3 g kanamycin-containing cylinders had maintained effective antimycobacterial activity for 30 days, while elutes from 1g kanamycin-containing cylinders had demonstrated an effect until day 7. Compared to vancomycin-loaded bone cement in the UCS test, all doses of kanamycin-loaded cement demonstrated comparable compression strength before the elution test. After 30 days of eluting, although there was a significant weakening of strength compared with pre-elution, there was no significant difference between kanamycin and vancomycin and it also had sufficient strength to meet the standard of bone cement.

There are multiple factors which are known to determine the release of antibiotics from ALBC [16-20]. The amount of antibiotics contained in the bone cement had been regarded as an important factor affecting the elution or mechanical strength of antibiotics in an experimental setting[11,20]. In the *in vitro* experiments, bone cement loaded with a greater amount of antibiotics usually showed higher concentrations in the eluate during the early phase, however the gap between concentrations was narrowed toward the late phase[10,11,16,20,21]. In most of the experimental studies, a similar pattern of elution was observed, initially, large amounts were released and this then decreased sharply [16,20-23]. The combination of drugs, mixing techniques or choice of product also make a significant difference in the elution kinetics of antibiotics from ALBC. Experimental studies have demonstrated that the delayed addition of antibiotic

or the dough-phase mixing method enhanced the elution of vancomycin compared with the standard preparation[22,24]. Furthermore, the selection of bone cement or antibiotic brand also affected the elution efficacy of ALBC[16]. Because this study was designed to produce multiple doses of single drug loaded bone cement with conventional techniques for mixing and bone cement used commonly in the clinical fields, these results seem to provide reliable data in the usual setting of ALBC.

Under the same strain and setting of experiment, 2g or more of kanamycin showed similar antimycobacterial effect compared to streptomycin, even 1g of kanamycin maintained antimycobacterial activity until day 7[11]. Despite the equivalent antimycobacterial activities of streptomycin and kanamycin between our former and this experiment, there have been differences in clinical indication between streptomycin and kanamycin depending on the regional characteristics of resistance[25-27]. Based on the 2014 WHO guidelines for the programmatic management of drug-resistant tuberculosis, kanamycin was included in the group 2, which are injectable agents. Even, streptomycin, amikacin, and capreomycin were also included in the group 2, streptomycin has not been recommended as a second-line anti-tuberculosis injectable agent due to high rates of streptomycin resistance in strains of MDR-TB[28]. Furthermore, among the antibiotics made as a powder form in the group 2, kanamycin is only used to treat both bacterial and tuberculosis infections while capreomycin has only anti-tuberculosis activity[28]. In the clinical outcomes of two-stage revision

arthroplasty for tuberculosis periprosthetic joint infection, the impregnation of streptomycin in a cement spacer resulted in successful outcomes [5]. In their data, because five patients who were diagnosed with tuberculosis periprosthetic joint infection had coinfection with Gram-positive bacterial species, streptomycin was impregnated in combination with vancomycin to cover both strains[5]. When considering personal or regional conditions of the infection, kanamycin might be chosen instead of streptomycin because it has different susceptibility and resistance against tuberculosis and bacterial strains. Recently, WHO has updated the treatment guidelines for multidrug- and rifampicin-resistant tuberculosis which excluded kanamycin due to the increased rate of resistance and side effects in the recent time, and it included streptomycin, which was not recommended in previous version of treatment guideline[28,29]. However, this is a guideline in systemic use, it may be considered to be different from the utility of kanamycin in ALBC.

In the measurement of concentration of kanamycin from elution and antimycobacterial activity, there was a difference in absolute value even though it was the same sample. This difference might be caused by the differences of measurement methods and additives to incubate and control the Mtb H37Rv for MIC measurement. Currently, there are various assay methods including liquid chromatography-mass spectrometry and fluorescence polarization immunoassay which are used to measure the amount of eluting of antibiotics from ALBC[16,20,21,30]. In this experiment, liquid chromatography-mass spectrometry

has been utilized for the elution test because it had been one of preferred technology to detect and quantify the level of antibiotics in the therapeutic drug monitoring [31-35]. Meanwhile, the concentration of kanamycin in the MIC measurement was determined by the broth dilution assay on the microplate using fluorometer. In addition, as described in method part of this study, dilution was necessary to obtain satisfactory values of MIC during pilot study. Even differences in absolute values of concentration of kanamycin were noted depending on the measurement methods, overall trends of concentration seemed similar. Another possibility of such observational difference was that the measured values of MIC for the Mtb H37Rv in this study were relatively lower than those of other strains[36-39].

In this experiment, the estimated values in mechanical properties of the pure kanamycin and vancomycin, were similar to those of previous vancomycin-loaded bone cement studies [16,40-42]. Following the international industrial standards for bone cement, the UCS used for definite fixation of prosthesis should be maintained beyond the minimum International Organization for Standardization (ASTM F451, ISO 5833)[12,13]. In our study, the ultimate compression strength of all doses of kanamycin and vancomycin-loaded bone cements after elution exceeds 70MPa. Several experiments have assessed whether the addition of antibiotics to bone cement reduces the mechanical properties depending on the dose of antibiotics[41,43-45]. Generally, a decrease in the mechanical properties with the addition of

antibiotics is supported by the finding that a greater porosity of bone cement causes a loss in load-bearing structural area and concentrates mechanical stress on specific areas [20]. However, a significant increase of the mechanical properties has also been reported due to the superior stiffness of antibiotics compared to that of the polymer matrix [46]. In terms of the releasing kinetics of antibiotics from bone cement, the porosity of bone cement is known to be associated with the long-term release of antibiotics [47,48]. Therefore, due to the association of mechanical properties and elution kinetics from porosity and other factors, there is a clinical limitation in using liquid antibiotics for bone cement. Although liquid antibiotics significantly increase the antibiotic elution, they are not used in the clinical practice because of their negative influence on the mechanical properties as much as weakened below the strength for bone cement standard [48,49]. Also, several additional factors including methods of ALBC preparation and mixing, dose and molecular weight of antibiotics, products of bone cement contribute to produce differences of porosity [24,30,43,50,51]. Therefore, comparison of kanamycin with vancomycin in this study might provide reliable information, because vancomycin has been already investigated in various settings for experiments and clinical uses furthermore there is no study about mechanical strength of kanamycin-loaded bone cement [16,19,20,22,40,45,51,52].

Based on the previous studies, the mechanical strength of bone cement after eluting exhibits inconsistent characteristics [16,17,21,41,53]. Our study demonstrated the reduced mechanical property of bone cement after elution, which is similar

with results of other studies [16,49,54]. Penetration of water into the cement, which leads to an increased formation of pores, voids, microcracks and hydrolysis of the outermost layers of polymethyl methacrylate cement were suggested as the causes of this phenomenon[21,54]. However, in case of product without impregnation of antibiotics, the opposite results were also reported [55]. In addition, a brand of gentamicin-loaded bone cement demonstrated increased mechanical property after elution[56]. Another study showed complex and contradictory results by different viscosity of bone cement and loaded doses of antibiotics [43]. Also, several additional factors including methods of ALBC preparation and mixing, dose and molecular weight of antibiotics, products of bone cement contribute to produce differences of porosity[24,30,43].

There were several limitations in this study. First, this experiments did not include various antibiotics. Elution and antimycobacterial activity tests of the first and second-line drugs were previously performed [10,11]. The present study with the same settings as in previous experiments allows indirect comparison. Therefore, this study was aimed to evaluate the effectiveness of kanamycin in the same setting. Second, we could not investigated various strains of Mtb. In fact, in areas where there is a high incidence of MSTB, the proportion of MDR strains is relatively high. However, due to the technical difficulties of controlling Mtb strains and quantification of MIC in laboratory environments, MDR strains were not included in this study. Third, the mechanical strength test has been performed in only the UCS. The satisfaction of full modes in the

mechanical test could ensure complete stability for permanent fixation such as arthroplasty[12,13]. Although this study included only UCS test, it seems considerable information for clinical use because we analyzed the elution and antimycobacterial activity of kanamycin-loaded cement in a situation where there is lack of study about kanamycin loaded bone cement. Fourth, bone cement of various brands and options were not regarded. Previous studies have shown that there are significant differences in the mechanical properties of cement products [16,49,55]. Finally, microscopic investigations to analyze the surface of bone cement including micro-computed tomography or scanning electron micrographs were not performed.

V. CONCLUSION

Of the different forms of ALBC, those containing kanamycin above 2 g showed antimycobacterial effects fully during a 30-day period. From 1 to 3 g kanamycin-loaded cement demonstrated comparable compression strength with 1 g vancomycin while maintaining effective elution activity until day 30. After an elution test of 30 days, although there was a significant weakening of strength, the absolute value after elution was confirmed to be maintained above the strength required for implantation. Furthermore, our study noted that the mechanical strength of kanamycin was excellent and as good as vancomycin, which is commonly used in forms of ALBC. These results might provide useful

information for selecting anti-tuberculosis drugs and doses to mix with bone cement for the surgical treatment of MSTB.

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ABSTRACT(IN KOREAN)

카나마이신 함유 골시멘트는 근골격계 결핵 치료에 효과적으로 사용될 수 있는가?

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이 재 후

배경: 항생제 함유 골시멘트는 근골격계 결핵 병소의 병변에 항결핵 제제를 효과적으로 전달할 수 있는 방법이다. 그러나 골시멘트로 사용 될 수 있는 항결핵제제의 적합성에 대한 정보는 제한적이다. 카나마이신은 현재 다중 약물 내성 결핵 치료를 위한 항결핵제로 사용됩니다. 카나마이신은 내성 균주의 스펙트럼이 다르기 때문에 동일한 아미노 글리코사이드 계통 인 스트렙토마이신에 대한 대안으로 사용되고 있지만 항생제 함유 골시멘트로서의 적합성에 대한 정보는 없다.

목적: (1) 카나마이신의 용출력, (2) 카나마이신이 충진 된 시멘트의 유효 항균 기간, (3) 반코마이신 함유 골시멘트와 비교 한 카나마이신 함유 골시멘트의 기계적 강도.

대상 및 방법: 40 g의 골시멘트와 1, 2, 3 g의 카나마이신을 혼합하여 만든 3 가지 조합으로 원기둥 형태의 골시멘트 시편을 제작하여 용출 및 항결핵 향균력 시험이 수행되었다. 골시멘트 시편는 직경 10 ± 0.8 ㎜, 높이 50 ± 1.2 ㎜로 제작되었다. 최종 압축 시험을 위해 기

계적 강도 시험의 표준을 따르기 위해 높이 12㎜ 및 직경 6㎜의 시멘트 실린더를 추가로 제작하였다. 각 유형의 5 개의 골 시멘트 실린더를 30일 동안 매일 새로운 인산 완충 식염수로 교체하면서 배양하였다. 각 1 mL의 용출액을 추출하여 PBS를 교체하기 직전의 1 일, 4 일, 7 일, 14 일 및 30 일에 용출 수준 및 항결핵 향균력을 측정했습니다. 용출액 중의 카나마이신 양을 액체 크로마토 그래피 질량 분석기로 측정하고, 용출액의 항균 균 효능을 마이코 박테리아 결핵 H37Rv에 대하여 마이크로 플레이트에서의 브로스 희석 분석법을 사용하여 시험 약물의 최소 용출 농도와 비교함으로써 계산하였다. 최종 압축 강도는 용출 전후의 재료 시험 시스템 기계를 사용하여 항생제 용량당 5 개의 골시멘트 시편로 수행되었다.

결과: 30일간의 용출 기간 동안 2 및 3g의 카나마이신 함유 골시멘트는 유효한 항결핵 향균력을 보였으나, 1g 카나마이신은 14 일까지 향균력을 보였다. 1일째 추출한 용출액의 농도는 함유 항생제의 용량에 비례하였으나 (1 일, 카나마이신 1g, 44.8㎍ / mL, 카나마이신 2g, 64.1㎍ / mL, 3g 카나마이신, 128.2㎍ / mL), 시간이 지남에 따라 균등화 되었다. 항생제가 함유되지 않은 고유의 골시멘트 최종 압축 강도 값은 용출 시험 전후에 99.3 ± 1.04 MPa 및 95.7 ± 0.83 MPa였다. 카나마이신과 반코마이신이 함유 된 골시멘트의 용출전 압축 강도는 시멘트에 함유 된 항생제의 양이 많을수록 낮았다 (카나마이신 1g, 카나마이신 2g, 96.6 ± 2.1 MPa, 카나마이신 3g, 94.6 ± 1.4 MPa, 1 g 반코마이신 , 95.9 ± 2.3 MPa, 2 g 반코마이신, 94.9 ± 2.5 MPa). 궁극적 압축 검사에서 모든 용량의 카나마이신과 반코마이신 1g 사이에 통계적으로 유의 한 차이는 없었다. 30 일간 용출 후, 카나마이신 및 반코마이신이 함유 된 골시멘트의 압축 강도는 용출 전보다 유의하게 낮았다 (용출 후, 카나마이신 1g, 86.2 ± 3.5MPa, 카나마이신

2g, 84.7 ± 3.7MPa 3 g 카나마이신, 83.2 ± 3.0 MPa, 1 g 반코마이신, 86.6 ± 3.6 MPa, 2 g 반코마이신, 85.1 ± 3.5 MPa, P <0.05).
결론: 항생제 함유 골시멘트의 형태에서 2g 이상의 카나마이신은 30일 동안 효과적인 항균력을 보였다. 1g 내지 3g의 카나마이신이 함유된 골시멘트는 30 일까지 효과적인 용출을 유지하면서 1g의 반코마이신과 동등한 압축 강도를 나타냈다. 30 일의 용출 시험 후, 카나마이신 함유 골시멘트의 압축 강도는 유의한 감소가 있었음에도 골시멘트의 기준 강도에 부합하였으며 반코마이신 함유 골시멘트와 유사 하였다.

핵심되는 말 : 결핵, 근골격 결핵, 용출, 향균력, 골시멘트, 압축강도