Effectiveness of Steinmann Pin Coated with Gentamicin-Loaded Chitosan on *Staphylococcus epidermidis* Growth

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ABSTRACT

Introduction. Implant related infection is a serious complication and difficult to manage. Antimicrobial coating on implant surface is expected to prevent infection. Gentamicin is a broad-spectrum antibiotic that has been widely applied locally. Chitosan is a biopolymer that has bio adhesive properties and can serve as a drug carrier.

Materials and methods. This is an *in vitro* experimental study to assess the effectiveness of gentamicin-loaded chitosan-coated Steinmann pin on the growth of *Staphylococcus epidermidis*. Seven test groups were made, consist of five different concentration of gentamicin-loaded chitosan, chitosan coating only, and control without coating. Evaluation is done by assessing turbidity (qualitative measurement) and colony forming unit count (quantitative measurement). The experiment was repeated three times.

Results. In the evaluation of turbidity level and colony forming unit count, we obtained gradual decline in line with the concentration. Gentamicin concentration of 4μg inhibits the growth of *Staphylococcus epidermidis*.

Conclusions. This study found that gentamicin-loaded chitosan-coated Steinmann pin could inhibit *Staphylococcus epidermidis* growth *in vitro*. The most optimum concentration of gentamicin was 4 μg.

Keywords: Implant related infection, implant coating, Steinmann pin, chitosan, gentamicin, Staphylococcus epidermidis

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Efektivitas Pelapisan Steinmann Pin dengan Gentamicin dan Chitosan terhadap Pertumbuhan *Staphylococcus epidermidis*

ABSTRAK

Pendahuluan. Infeksi pada penggunaan implan merupakan komplikasi serius yang sulit untuk ditangani. Pendekatan dengan pemakaian lapisan antimikroba pada permukaan implan diharapkan dapat mencegah terjadinya infeksi. Gentamicin merupakan antibiotik berspektrum luas yang telah banyak diaplikasikan secara lokal. Chitosan merupakan polimer yang memiliki sifat bioadhesif dan dapat berfungsi sebagai agen pembawa obat.

Bahan dan cara kerja. Penelitian ini merupakan penelitian eksperimental *in vitro* untuk menilai efektivitas pelapisan Steinmann pin dengan gentamicin dan chitosan terhadap pertumbuhan *Staphyloccus epidermidis*. Penelitian melibatkan tujuh kelompok uji yang terdiri dari kontrol, pelapis chitosan, dan lima kelompok gentamisin dengan konsentrasi bertingkat. Evaluasi dilakukan dengan menilai kekeruhan dan penghitungan jumlah koloni. Percobaan dilakukan tiga kali pengulangan.

Hasil. Pada evaluasi tingkat kekeruhan dan penghitungan jumlah koloni kuman, didapatkan penurunan secara gradual pada tiga kali percobaan sesuai tingkat konsentrasi. Konsentrasi gentamicin 4 ug mampu menghambat pertumbuhan *Staphylococcus epidermidis*. Secara statistik didapatkan perbedaan secara bermakna antara kelompok perlakuan dengan kontrol. (p = 0,038) Kelompok gentamicin 4µg berbeda secara bermakna dengan kelompok kontrol dan kelompok chitosan.

Simpulan. Steinmann pin yang dilapisi dengan chitosan dan antibiotik menghambat pertumbuhan *Staphylococcus epidermidis* secara *in vitro*. Konsentrasi optimum tercapat pada kadar gentamicin 4 µg.

Kata kunci: *implant related infection, implant coating*, Steinmann pin, chitosan, gentamicin, *S. epidermidis*

Introduction

Musculoskeletal infection is a complication that often accompanies orthopedic surgery.[1] The incidence of infection reaches 5% in fracture fixation with appliance and installation and 2% in fixation with prosthesis. About 1.8 billion dollars each year is required for handling the implant-related infection's cases. Such infections cause morbidity to the patient in question and pose a huge burden on the health care system.[2] Pathogenesis of infection is associated with the presence of microorganism in biofilms. Biofilms are collections of cells and cellular products and form a layer attached to solid surface or substrate surface. Based on the uniqueness of this bone infection, the best management is prevention.

The use of antimicrobial coatings on the surface properties of biodegradable implants is the current strategy developed in order to prevent the occurrence of infection in orthopedic implant use. The use of antimicrobial coating is expected to prevent the attachment of bacteria on the surface of the implant so it does not form biofilms.[3] Various materials and types of antibiotics have been tested as an implant coating. Chitosan is a biopolymer considered to inhibit the growth of bacteria on implants.[4]

Gentamicin is a prototype of aminoglycoside with a broad spectrum. The main indication of the antibiotic is a general severe infection with gram-negative bacteria. Gentamicin has a bactericidal function. Glycoside antibiotics inhibit bacterial protein biosynthesis. The accumulation of antibiotic on the bacterial ribosome cause misreading in translation, resulting in the formation of incorrect enzyme protein and structure of one protein, causing an irreversible cell damage.[5]

The purpose of this study was to determine the antibacterial power of coating the stainless steel implant using a mixed solution of chitosan and gentamicin.

Materials and methods

This was an experimental study. *Staphylococcus epidermidis* bacteria used in this study was obtained from microbiology laboratory of Faculty of Medicine, University of Gadjah Mada. Gentamicin in five different concentration levels was used as independent variable in this study. The evaluation covered turbidity level of isolates of *Staphylococcus epidermidis* and number of colony forming units (CFU) observed with naked eye.

Preparation of gentamicin-loaded chitosan. The concentration of chitosan used in this study was 3% and 2% in 1% H_3COOH solution. To create a 3% and 2% solution of chitosan, a total of 75 and 50 µg of chitosan powder was dissolved into 250 ml of solvent H3COOH 1% and then stirred using a magnetic Stirrer for one hour to become homogeneous. Gentamicin in 5 levels of concentration (4, 2, 1, 0.5, 0.25 µg) was mixed with the solution. Concentration of 4 µg is determined by maximum inhibitory concentration and validation tests performed previously, where at 4 µg concentration, no colony growth was found.

Pin coating procedure. Pin coating was done in two stages. The first stage used 3% chitosan 3and after it had been dried at room temperature for 24 hours, the second stages followed by using 2% chitosan.

Preparation of Staphylococcus epidermidis. Staphylococcus epidermidis was cultured in Trypticase Soy Broth (TSB) media and cultured in Muller Hinton agar of 100µl. They were incubated at 37 ºC for 24 hours. Several colonies were taken from the culture and suspended in 10 ml 0.9% NaCl to obtain turbidity equal to Mc. Farland standard solution.

Bacterial concentration obtained was 108 CFU/ml. Concentration was then used as a standard in doing this research.

Incubation of Staphylococcus epidermidis to Steinmann pin. Steinmann pin was introduced into a tube containing 200 ml *Staphylococcus epidermidis* solution. They were incubated for twenty four hours at 37 °C. The procedure was conducted in *triplo* in different time.

Measurement of outcome. The evaluations of the antimicrobial effect against *Staphylococcus epidermidis* and gentamicin chitosan coating were done qualitatively and quantitatively. Qualitative test was done by comparing turbidity level of the treated group with a visual control. As a control, the same bacterial isolates are used regardless of the treatment (positive control) as a benchmark of turbidity level. Assessment of turbidity level was done visually in a bright room and with a clean and homogeneous screen. Blinding examination was done by two persons to reduce bias and subjectivity. Quantitative evaluation to calculate CFU was carried out by growing isolates in Muller Hinton medium that was incubated for 24 hours at temperature of 37 ˚C. A total of 0.1 ml isolate of gentamicin at each level and concentration of chitosan were extracted using a pipette and flattened on the surface of agar media. Each media was labeled with corresponding gentamicin level and chitosan's concentration. Evaluation was done by counting the number of colony that was visually formed. Counting was performed by two blinded examiner to reduce bias and subjectivity. Data obtained from the research results were statistically analyzed using Kruskal-Wallis test and Mann-Whitney test. Statistical analysis was performed using IBM SPSS version 17.

Results

Based on observations, a difference in turbidity level was found between isolates in the negative control with pin treated using chitosan coating mixture and gentamicin. Optimum results were obtained in pin coated with 2 and 4 µg gentamicin. Pin coated with chitosan alone also results in relatively lower turbidity compared with uncoated pin and pin coated with 0.25, 0.5, and 1 µg gentamicin. There was a decreasing gradation of turbidity starting from the noncoated pin and pin coated with gentamicin level ranging from 0.25 to 4 µg. It was found that the higher the level of gentamicin, the lower the turbidity level. The tube was appeared clear on the pin coated with gentamicin 0.25 µg on the first and third experiment and also on the pin with chitosan alone, while in the second experiment, the tube appeared clear at a concentration of 0.5 µg gentamicin and the tube coated with chitosan alone was appeared not clear.

We found decreasing gradation level on the growth of isolates *Staphylococcus epidermidis* in line with the decreasing level of gentamicin, with less or no growth at higher level of gentamicin. In the first experiment, the bacteria completely did not grow at concentrations of gentamicin 4 µg. At concentrations below 4 µg, gentamicin was seen only able to inhibit the growth of *Staphylococcus epidermidis*.

In the group given only chitosan without gentamicin, the growth of *Staphylococcus epidermidis* isolates were also relatively low compared to the control and quantity was lower than the group with pin coated with gentamicin 0.5 µg. The first, second, and third experiments showed different results at the same level of concentration. Nevertheless, it showed significant gradations of colony growth rate in line to the concentration of gentamicin in all experiments.

Based on the Kruskal-Wallis test, the number of

colonies in each experimental group differed significantly. ($p = 0.038$) Mann-Whitney test resulted in significant mean difference between the control group with groups of 2 μg and 4 μg gentamicin. Comparing only groups with gentamicin-loaded chitosan, there were significant differences only in the group of gentamicin 4 μg. Group treated with gentamicin 4 μg differed significantly with all test groups except with gentamicin 2 μg and 1 μg.

Discussions

Systemic antibiotic was given before orthopedic surgery to prevent infection in the use of implants. Due to disturbances in bone structure and local vascularization, adequate doses of antibiotics cannot be achieved at the site of implant. Implant-bone surface are prone to infection, therefore the use of local antibiotics is very reasonable. Side effects of systemic antibiotics can be avoided and the dose of higher local antibiotic can be given without risk of systemic toxicity.[6]

The use of gentamicin as a local antibiotic which is shown to be effective in a variety of orthopedic surgery, with bactericidal properties, broad spectrum and stable to high temperatures has become the basis for selection of gentamicin in this study.[3] Gentamicin is stable to heat which may arise from the drilling or screw insertion. Furthermore, the pattern of infection-related bacteria in Sardjito General Hospital was mostly class of Staphylococcus.[7] Although most of the activity of gentamicin is gram-negative bacteria, it also works on gram-positive bacteria such as *Staphylococcus*.

We found barriers to the growth of bacterial isolates of *Staphylococcus epidermidis* after treated with gentamicin-coated pin, indicated by the decrease in turbidity and number of CFU. Coating with chitosan and gentamicin concentration of 2 μg and 4 μg gives real effect as anti-bacterial than the other. These results were supported by statistical analysis which shows real differences between treatment groups. The effectiveness of gentamicin and chitosan as a coating implant are consistent with the experiment of Greene in 2007 which showed that gentamicin would start with real decays in 72-96 hours and to eliminate *Staphylococcus epidermidis* in the incubation period of 16-20 hours.

Chitosan as a cation have adhesive properties. It is biocompatible and not toxic to tissue. In this study, it was shown that chitosan itself had antibacterial properties in both qualitative and quantitative test. On the qualitative tests, it appeared that group of isolates given pin coated only with chitosan demonstrated to inhibit the growth of bacteria. Similarly, on the quantitative test, relatively low numbers of colony was found.

Antibacterial effects of chitosan itself was relatively strong, but at the time of mixing with gentamicin, the antibacterial effect of chitosan was decreasing as seen in the levels of 0.25 μg and 0.5 μg in all the repetitions both qualitatively and quantitatively. That happened because of the possibility of antagonistic effects between chitosan and gentamicin, for mechanical or because of other reasons that still requires further investigation.

This study still has several shortcomings, including limited sample and using only one kind of antibiotic. Furthermore, measuring duration of gentamicin and chitosan coating stays on the pin was not done. This study implies local approach to the manipulation of surface of implant for treatment of infection may give effective results. However, further research about this should be done.

Conclusions

This study found that gentamicin-loaded chitosan-coated Steinmann pin could inhibit *Staphylococcus epidermidis* growth *in vitro*. The most optimum concentration of gentamicin was 4 μg.

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