A Study on Prophylactic Efficacies of Daptomycin and Vancomycin in a Rat Model of MRSA Infection Secondary to Spinal Implantation

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Summary

The present study aims to investigate the prophylactic effects of daptomycin and vancomycin in an experimental spinal infection induced by spinal implantation. The study involved inoculation of a bacterial suspension prepared with the MRSA ATCC 43300 strain to the implant bed and to the peripheral tissue immediately the implantation of titanium spinal implants to the bone in adult Winstar rats. The animals were divided into three groups; seven rats in the first group received no antibiotics, while the seven rats in group two and eight rats in group three received daptomycin and vancomycin, respectively. Microbiological and histological assessments were carried out on rats with induced infections.

In the swab cultures of deep surgical areas, vancomycin demonstrated inhibition of infection of the surgical site in half of animals (50%) whereas daptomycin exhibited the same effect in more animals (71.4%), which was statistically significant compared to the control group (p<0.05). In the implant and bone culture, a statistically significant superiority was not observed in prophylactically treated with either antibiotic and nontreated control group (p>0.05).

As a result; comparing control group, preventive effects of daptomycin and vancomycin in infection induced model of spinal implantation with high bacteria inoculums were not found statistically significant whereas daptomycin showed statistically significant inhibition on deep surgical area infection.

Key words: Daptomycin, infection, spinal implantation, vancomycin

Spinal Enstrümantasyon Sonrası MRSA Enfeksiyonu Oluşturulmuş Rat Modeli Üzerinde Vankomisin ve Daptomisin'in Profilaktik Etkileri

Özet

Amaç: Bu çalışma deneysel bir enfeksiyon modelinde daptomisin ve vankomisinin profilaktik etkilerini araştırmayı amaçlamaktadır.

Yöntem ve Gereç: Bu çalışmada erişkin Winstar cinsi ratların omurgalarına titanyum spinal implantlar yerleştirildikten hemen sonra implant yatağına ve çevrede dokuya MRSA ATCC43300 serisi bakteriyel süspansiyon inoküle edildi. Hayvanlar 3 gruba ayrıldı; ilk gruptaki 7 rastantibiyotik almazken ikinci gruptaki 7 rat daptomisin, üçüncü gruptaki 8 rat ise profilaktik olarak vankomisin aldı. Mikrobiyolojik ve histolojik değerlendirmeler indüklenmiş enfeksiyonu olan ratlar üzerinde gerçekleştirilirdi.

Bulgular: Derin cerrahi alanların eküyon kültürlerinde vankomisin hayvanların yarısında (%50) cerrahi alan enfeksiyonu inhibisyonu gösterirken daptomisin kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı (p<0,05) bir şekilde daha fazla rat üzerinde
INTRODUCTION

The recent increase in intra-bone and intra-joint implantations lead to a marked increase in the incidence of infections and other associated complications. Infections secondary to implantation are difficult-to-treat complications which necessitate long-term parenteral antibiotic treatment and further surgical interventions such as implant removal (1,2,3,4,5,6).

The staphylococci and streptococci are responsible for the majority on bone and joint infections (5). Methicillin resistance of Staphylococcus aureus isolates has been reported to be up to 20% in various European countries, the incidence being above 50% in some countries including Portugal and Italy (3,4,7). In the USA, several series of studies have reported this incidence as ranging from 33 to 55%, which rises to even higher values reaching to 74% in hospital isolates (3). Spectrums of antibiotics to be used for prophylactic purposes for infections which involve the staphylococci as probable causes should therefore cover MRSA in countries with high incidence of methicillin resistance.

Vancomycin is an antibiotic approved for treatment and prophylaxis of bone and prosthesis infections caused by methicillin-resistant staphylococci which is being routinely used for this indication (5). The antibiotic has a bacteriostatic effect and its bone penetration is below the desired levels. It also has major adverse effects including bone marrow suppression, nephrotoxicity, ototoxicity and rash. Its serum levels should be monitored with long-term use (3).

Vancomycin resistance in S. aureus strains has not been described until the first report of an isolate with reduced sensitivity to vancomycin (VISA) from Japan in 1997 and the first report of vancomycin-resistant (VRSA) isolate from the USA in 2002. Today, there is an increasing number of reports of VISA and VRSA clinical isolates (3,4,7). MRSA strains are resistant to many antibiotics including ciprofloxacin and new-generation quinolones (3,6). New treatment alternatives are now needed due to the increasing antimicrobial resistance in MRSA (1,4,5,7,9,10). Antibiotic choices are limited with glycopeptides, lipopeptide, oxazolidone, streptogramin and glycycline group of agents. Daptomycin is an antibiotic belonging to the cyclic lipopeptide group which has a rapid onset of bactericidal activity and is used in the treatment of serious gram-positive infections. Daptomycin has bactericidal activity for MRSA and MSSA (7,8).

Many of the experimental implant infections studies investigating therapeutic activities of antibiotics were able to markedly reduce tissue bacterial counts but bacterial eradication from the bone could not be obtained (1,4,5,11,12,13). Similar lack of treatment efficacy issues have also been noted from clinical trials, and long-term high-dose combinations were used to overcome this inefficacy issue (3). The focus, however, should be on measures to prevent development of infection in the first place.

Anahtar Kelimeler: Daptomisin, enfeksiyon, spinal implantasyon, vankomisin
Studies using different animals have reported prevention of foreign-material infection with prophylactic antibiotic treatment\(^{(6,12,13,14)}\). There are lots of studies investigating bone infection models with rats, rabbits, dogs, Guinea pigs and chickens. Rats, however, are more widely used in experimental infection models since they are a suitable species for infection induction, antibiotic use and due to their better endurance and survival characteristics\(^{(15)}\).

The present study aims to investigate prophylactic effects of vancomycin and daptomycin against high-bacteria inoculums in an instrumentally-induced experimental spinal infection model in rats, which has been studied to a lesser extent as compared to long bone infections.

**MATERIAL AND METHODS**

The study was carried out with animals from the experimental animals laboratory of Ege University in line with applicable guidelines for studies with experimental animals. Approval of the Ethics Committee of Ege University was received on the 30\(^{th}\) of July, 2010 with permission no 2010-104. Animal number was limited by the Ethics Committee.

Adult Winstar rats weighing 300-450 g were used in study. Methicillin-resistant S. aureus ATCC 43300 strain was used to induce experimental infections. Ten microliters of bacterial suspension containing \(10^6\) colony-forming units were inoculated to the surgical site for implantation. Vancomycin (Powder, from Sandoz), Daptomycin (Powder, from Novartis) were used as prophylactic antibiotics.

The rats were divided into 3 groups. Animals in group 1 were anesthetized using ketamin-xylazine (30-5mg /kg), followed by the surgical operation as described by Ofluoglu et al. as follows\(^{(11)}\). The animals were placed in prone position and thoracolumbar sections were shaved. The skin was exposed with midline incision and paravertebral muscles were shaved. Bone was decorticated from the facet joint and the lamina, followed by implantation of a titanium pin 1 mm in width and 3 mm in length through the vertebral matter. Bacterial suspension was inoculated over the decorticated lamina and the peripheral tissue and the surgical site was closed with sutures.

For group 2, 50mg/kg vancomycin was administered subcutaneously from the neck 2 hours before the surgery. The surgical procedure administered to group 1 was performed and 2 further doses of antibiotics were administered with 12-hour intervals following the first antibiotic dose.

For group 3, 50mg/kg daptomycin was administered subcutaneously from the neck 2 hours before the surgery. The surgical procedure administered to group 1 was performed and 2 further doses of antibiotics were administered with 12-hour intervals following the first antibiotic dose.

After surgical procedure, animals were returned their cages under a standard 12 h. light / dark cycle at 21 – 22 ° C. They were given food and water ad libitum and were monitored as per the standard protocol. Three animals died during the first two days without high temperature and weight loss, probably due to blood loss during surgery and anesthetic complications. Lost animals were replaced after applying the same procedures of the respective group.

The animals were sacrificed two weeks after the surgery. Surgical sites were examined macroscopically and swab samples were collected from the deep surgical site for microbiologic assessments. The implants were removed aseptically and placed in bouillon containing tioglycolate. Two more bone samples were collected from the implant site and were placed in bouillon containing tioglycolate and in formalin solution for microbiological and histological analyses, respectively.

**Microbiological Procedure:** Implant and bone tissue samples were incubated in...
bouillon containing tioglycolate at 37°C for 48 hours, followed by two separate subcultures to 5% sheep blood agar and eosin methylene blue (EMB) agar. Two swab samples from the surgical sites were seeded into separate 5% sheep blood agars. One of the two agar plates was incubated in an anaerobe vial and the other under room conditions at 37°C for 48 hours. The growing bacteria in cultures were identified using standard bacteriologic methods and the isolated S. aureus stains were tested for methicillin resistance.

For histological analysis the samples were fixed in neutral buffered formalin solution, decalcified in 4 N formic acid sodium citrate (10%), embedded in paraffin, and cut into 6µm. longitudinal sections. The sections were stained with hematoxylin and eosin and Masson's stain. The following alterations characteristic of osteomyelitis, were assessed: bone marrow inflammation, cortical alterations, subperiosteal, medullary and intracortical abscesses, sequester and cell characteristics.

Statistical analysis: The results were evaluated using chi-square test using SPSS version 16.

RESULTS

Pronounced evidences of infection were observed macroscopically in all animals of the control group (group 1) and growth was detected in cultures of the deep surgical site soft tissue, implant and the bone.

Macroscopic assessment of the surgical site demonstrated no evidence of infection in five out of eight animals (62.5%) in the vancomycin group and in six out of seven animals (85.7%) in the daptomycin group. Pronounced findings of infection including abscess and purulent inflammation were observed in three animals in the vancomycin group and in one animal in the daptomycin group.

There was no growth in the cultures of swab samples obtained from the surgical site in four animals (50%) in the vancomycin group and in five animals (71.4%) in the daptomycin group.

There was no growth in implant cultures of the three animals (37.5%) in the vancomycin group and two animals (28.8%) in the daptomycin group.

There was no growth in cultures of bone samples of the four animals (50%) in the vancomycin group and two animals (28.8%) in the daptomycin group and the histological analysis of the animals with growth demonstrated findings of osteomyelitis (Figure 1).

In histological analysis, varying degrees granulation tissues which included new endothelial capillary and inflammation cells were seen 18 of 22 animals whereas necrosis was detected 6 of 22 rats; but these factors were associated with surgical procedure more than infection state.

Microabscess were seen in two rats in vancomycin group which cultures also were positive in deep tissue, implant and bone materials.

Cell type was not show uniformity in three groups; histiocyte that is present with foreign substances in chronic process, was detected three rats in vancomycin group and only one rat in daptomycin group but polymorphonuclear leukocyte as an indicator of early stage inflammation were found 10 of 22 rats regardless of group with or without antibiotic. The results of both analysis of microbiological and pathological were given in Table 1.
**Table 1:** Results of bacteriological and pathological analysis

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<th>Deep tissue</th>
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<th>Bone</th>
<th>Bone marrow inflammation</th>
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C: Control group, V: Vancomycin group, D: Daptomycin group
Cell type: 1: Lymphocyst, 2: PNL, 3: Histiocyt
Granulation tissue; +1: mild, +2: moderate, +3: dense

**Figure 1:** Granulation tissue showing several residual bony structures due to the microorganisms on the left side and relatively normal trabecular osseous structures on right side of the image were seen. (HE-x40)
DISCUSSION

Incidence of post-elective surgery infections in spinal surgery practice is approximately 1% \(^{(10)}\). The incidence of infections rises 2.1 to 8.5% in surgeries involving metal implants \(^{(11)}\). There is a wide range of contributing factors for infection development such as the experience of the surgery team, operation duration, conditions of the surgery room and post-operative care conditions alongside the patient-specific factors. Despite all measures aimed at infection control, it is currently not possible to control the incidence of infection below a certain level.

A method to decrease the incidence of infection, in addition to the standard methods, is the use of prophylactic antibiotics. This approach, however, varies according to the type and duration of surgery, patient characteristics and their antimicrobial resistance.

A large study by Garey et al. investigated the effect of prophylactic vancomycin on surgical site infections in 2048 subjects who had undergone cardiothoracic surgery. The study concluded that the incidence of infections may be reduced from 26.7 to 3.4% if prophylactic vancomycin is administered 16-60 minutes instead of 0-15 minutes prior to surgical incision \(^{(4)}\).

As a part of a national initiative to prevent surgical infections, Bratzler et al. recommended vancomycin prophylaxis for individuals allergic to beta-lactam in cardiovascular surgeries and knee and hip implantations and suggested that the antibiotic should be started during the hour preceding the surgery and should be continued for no more than 24 hours \(^{(1)}\).

The present study investigates the effect of prophylactic antibiotic treatment only on infection prevention in an experimental model of infection in rats. Among the available antibiotics, vancomycin is recommended for prophylactic approaches before surgeries in hospital with high incidences of MRSA and MRSE. There is, however, no consensus on the definition of high incidence. Daptomycin, on the other hand, is an antibiotic with rapid onset of bactericidal activity which is effective against the bacteria during the stationary phase and its side effects is lower compared to vancomycin. Due to these characteristics, we considered daptomycin as an appropriate antibiotic to be investigated in terms of prophylactic antibiotic treatment.

AUC-MIC and Cmax-MIC ratios and levels of antibiotics at infection sites have been described as the key parameters for treatment success in pharmacodynamic studies with antibiotics \(^{(19,20,22)}\). Several studies have demonstrated that 50-60 mg/kg subcutaneous daptomycin is sufficient to mimic 6 mg/kg/qid daptomycin administration in humans. The treatment failure with daptomycin in a chronic osteomyelitis model reported from the study by Luu et al. with 10 mg/kg daptomycin was described to be due to the low dose of the antibiotic \(^{(7)}\). In a study by Safter et al., AUC/MIC was determined as 1061 following the sc administration of the 50 mg/kg dose of daptomycin and the authors described that this dose level is required to achieve a 2 log 10 bacteria reduction \(^{(14)}\).

Another study found an AUC 0-24 value of 855 with 50 mg/kg daptomycin and 88 µg h/ml for 50 mg/kg vancomycin and reported that these doses were adequate \(^{(13)}\). Several other studies have also used similar doses of vancomycin and daptomycin \(^{(3,6,8,9,13,15,21,22)}\).

The recommended treatment for surgical prophylaxis in humans is to administer the antibiotic one hour before the surgery and continue the treatment for one day at maximum \(^{(1)}\). The present study adopts an approach similar to that used in humans. Since the antibiotics are administered subcutaneously, 50 mg/kg sc daptomycin and 50 mg/kg sc vancomycin were
administered to respective groups 2 hours before the surgical intervention.

Both antibiotics were repeated for two doses with 12-hour intervals.

Experimental studies within the field document the use of bacterial inoculum between $10^2$ - $10^8$ cfu (3,6,8,11,20). In a similar model, we have used $10^6$ cfu / 10 microliters inoculum which was shown infective by Ofluğlu et al (11).

No bacterial count per g of tissue was performed since the success of prophylactic antibiotic was to be measured based on prevention of infection (<1 cfu in culture).

Consequently, in the vancomycin group, the surgical site had normal findings of growth when examined macroscopically in five out of eight animals (62.5%) and growth was not detected in four out of eight animals in peripheral tissue culture (50%), in implant cultures of three out of eight animals (37.5%) and in bone cultures of four out of eight animals (50%).

In the daptomycin group, macroscopic findings were normal in six out of seven animals (85.7%) and growth was not observed in five out of seven animals in peripheral tissue culture (71.4%), in implant cultures of two out of seven animals (28.6%) and in bone cultures of two out of seven animals (28.6%).

Preventive effect on bone infection of vancomycin in 3 of 8 (%37.5%) and daptomycin in 2 of 7 case (28.6%) has been observed, despite high bacterial inoculum and spinal instrumentation and low levels due to long diffusion time of antibiotics in this tissue.

In this study, we could not measure bone and tissue levels of antibiotics in daptomycin and vancomycin treatment groups due to technical inabilities. As a result, we do not know whether antibiotics in bone and tissues reached to effective levels after the given treatment doses or not. On the other, surgical prophylaxis in humans was simulated with this study. Daptomycin achieved a statistically significant success rate of 71.4% in preventing deep surgical area infection (p<0.05). There was growth in implant culture of one animal in the vancomycin group whilst no growth was observed in bone cultures. This difference was attributed to the bone culturing failure or biofilm formation, leading to delayed surgical infection. Vancomycin was 50% and daptomycin was 28.5% successful in bone infection prevention, which is the primary target of surgical prophylaxis. The difference was statistically insignificant (p>0.05). We, however, believe that this is associated with the limited number of animals available for the study due to ethical considerations. In addition, since it is very difficult to compare the high-dose bacterial inoculums with the conditions of routine surgical procedures, we are in the opinion that more successful results could have been achieved in humans.

In conclusion, we believe that studies with higher number of animals through which more meaningful results may be obtained to contribute to efficacy assessments of antibiotics, as well as studies using higher doses (15) or antibiotic combinations for example with rifampin addition (6) where tissue levels of antibiotics are assayed are needed to enable better evaluation of the results of the present study.

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REFERENCES


