Linezolid Compared with Vancomycin for the Prevention of Methicillin-Resistant *Staphylococcus aureus* or *Staphylococcus epidermidis* Vascular Graft Infection in Rats: A Randomized, Controlled, Experimental Study

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ABSTRACT

Background: Graft infections are severe complications of vascular surgery that may result in amputation or mortality. Staphylococci are the most frequent cause of vascular graft infections.

Objective: In this study we assessed the prophylactic efficacy of linezolid in comparison with vancomycin in preventing prosthetic vascular graft infection due to methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus epidermidis* (MRSE).

Methods: This randomized, controlled, experimental study using healthy adult (aged >5 months) male Wistar rats was conducted in the research laboratory of the Pamukkale University, Denizli, Turkey. The study consisted of an uncontaminated control group and 3 groups for both staphylococcal strains: a contaminated group that did not receive any antibiotic prophylaxis; a contaminated group that received preoperative intraperitoneal (IP) prophylaxis with vancomycin; and a contaminated group that received preoperative IP prophylaxis with linezolid. All rats received a vascular Dacron graft placed inside a subcutaneous pocket created on the right side of the median line. Sterile saline solution (1 mL), to which MRSA or MRSE at a concentration of 2×10^7 colony-forming units per milliliter had been added, was inoculated onto the graft surface using a tuberculin syringe to fill the pocket. The grafts were explanted 7 days after implantation and assessed by quantitative culture.

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Results: Seventy rats (mean [SD] weight, 323.7 [17.9] g; mean [SD] age, 5.98 [0.64] months) were evenly divided between the 7 groups. Statistical analysis of the quantitative graft culture suggested that both vancomycin and linezolid were effective in significantly inhibiting bacterial growth when compared with the untreated contaminated groups (all, $P < 0.001$). However, a statistically significant difference was not observed between the bacteria count in the vancomycin and linezolid prophylaxis groups. When a comparison was made between the bacterial growth in the contaminated control groups, MRSA had significantly greater affinity to the Dacron prostheses than MRSE (all, $P < 0.001$).

Conclusion: Our study found that linezolid was as effective as vancomycin in suppressing colony counts in MRSA- or MRSE-infected vascular Dacron grafts in rats. (*Curr Ther Res Clin Exp.* 2007;68:23–31) Copyright © 2007 Excerpta Medica, Inc.

Key words: vascular graft infection, linezolid, vancomycin.

INTRODUCTION

Graft infections are severe complications of vascular surgery, and management is difficult, time-consuming, costly, and can result in amputation or mortality.[1–3] Consequently, every attempt should be made to prevent vascular graft infections. Staphylococci, which are skin commensals, are known to be the most frequent cause of vascular graft infections in humans.[1,4]

We previously reported that vancomycin and teicoplanin reduced graft infection in rats after challenge with methicillin-resistant *Staphylococcus epidermidis* (MRSE).[5,6] However, the increasing prevalence of methicillin-resistant staphylococci worldwide is an important concern because of the limited number of antimicrobial agents that are effective against these organisms.[7–9] Consequently, novel therapeutic approaches are required.

Linezolid is a new synthetic antimicrobial agent that is highly active against gram-positive organisms, including methicillin-resistant organisms.[10,11] In a study by Rybak et al,[12] linezolid was comparable to vancomycin in preventing the growth of gram-positive organisms.

Because the investigations of linezolid in vascular infections have been limited and the evaluation of new drugs is essential, we decided to investigate its prophylactic efficacy in preventing vascular graft infections and to compare it with vancomycin, which is more familiar to physicians and which we recently found to be a good prophylactic agent.[5] An English language literature search of PubMed (1990–2006) was performed using the search terms *vascular graft infection*, *linezolid*, and *vancomycin*.

The aim of our study was to test the prophylactic efficacy of linezolid in preventing prosthetic vascular graft infection due to methicillin-resistant *Staphylococcus aureus* (MRSA) and MRSE and to compare it with vancomycin, which is more commonly used in vascular surgery than linezolid.

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MATERIALS AND METHODS

The study was approved by the Pamukkale University Animal Research Ethics Committee (Denizli, Turkey). All animals received humane care in compliance with the principles of laboratory animal care developed by the National Academy of Sciences.[13]

Organisms and Susceptibility Testing

The MRSA and MRSE strains were isolated from a clinical specimen (graft infection) submitted for routine bacteriologic investigation to the Pamukkale University Department of Microbiology. The clinical isolates were identified by Gram staining, catalase reaction, tube coagulation test, and API-Staph test (bioMérieux, Lyon, France). Methicillin sensitivity was investigated using an oxacillin disk diffusion test.[14]

Drugs

Linezolid (Pfizer, Halden, Norway) and vancomycin (Abbott, Saint-Remy sur Avre, France) were diluted as described in previous studies.[15,16] The doses of vancomycin and linezolid (10 mg/kg) were based on dose regimens that had been found to be effective in the prevention of vascular graft infections in animal studies.[15–17] The drug solutions were prepared on the day of assay. The antimicrobial susceptibilities of the strains to linezolid and vancomycin were determined using the Kirby-Bauer disk diffusion method, according to the procedure outlined by the Clinical and Laboratory Standards Institute,[18] and the strains were found to be susceptible to vancomycin and linezolid.

In Vivo Rat Model

In this randomized, controlled, experimental study, healthy adult (aged >5 months) male Wistar rats were equally and randomly assigned to 7 groups: group I (uncontaminated control group: implanted graft, no contamination, no antibiotic prophylaxis); group II (untreated control group: implanted graft, local contamination with MRSA, no antibiotic prophylaxis); group III (implanted graft, local contamination with MRSE, no antibiotic prophylaxis); group IV (implanted graft, local contamination with MRSA, prophylaxis with preoperative intraperitoneal [IP] vancomycin 10 mg/kg); group V (implanted graft, local contamination with MRSE, prophylaxis with preoperative IP vancomycin 10 mg/kg); group VI (implanted graft, local contamination with MRSA, prophylaxis with preoperative IP linezolid 10 mg/kg); and group VII (implanted graft, local contamination with MRSE, prophylaxis with preoperative IP linezolid 10 mg/kg).

All rats were anesthetized with a 2:1 mixture of ketamine hydrochloride 100 mg/mL (Pfizer, Luleburgaz, Turkey):xylazine hydrochloride 20 mg/mL (Bayer AG, Leverkusen, Germany) 0.75 mL/kg IM. The hair on each rat's back was shaved and the skin was disinfected with 10% povidone-iodine solution. A 1.5-cm incision was made to create a subcutaneous pocket on the right side of the median line. Under sterile conditions, a 1-cm^2 sterile, woven, gelatin-impregnated

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Dacron graft (Gelwave, Sulzer Vascutek Ltd., Inchinnan, United Kingdom) was implanted in the pocket. In addition, the effect of preoperative intraperitoneal vancomycin (groups IV and V) and linezolid (groups VI and VII) administered 30 minutes before implantation at the standard dose of 10 mg/kg was assessed. The pocket was closed using skin clips. Sterile saline solution (1 mL), to which MRSA or MRSE at a concentration of 2×10^7 colony-forming units (CFUs) per milliliter had been added, was inoculated onto the graft surface using a tuberculin syringe to fill the pocket. The animals were returned to their individual cages and checked daily for progress in healing and signs of wound infection. During the entire study, the animals were kept at the Animal Research Laboratory (Pamukkale University, Denizli, Turkey) under veterinary supervision. The rats were kept at a room temperature of 25°C ± 1.9°C and humidity of 52% ± 6%, and received a standard diet as well as water ad libitum. All grafts were explanted under sterile conditions 7 days after implantation (**Figure**). All animals were euthanized following explantation.

Infection Assessment

The explanted grafts were placed in sterile tubes and washed in sterile saline solution. They were then placed in tubes containing 10 mL of phosphate-buffered saline solution and sonicated for 5 minutes to remove the adherent bacteria from the grafts. Quantitation of viable bacteria was performed by cul-
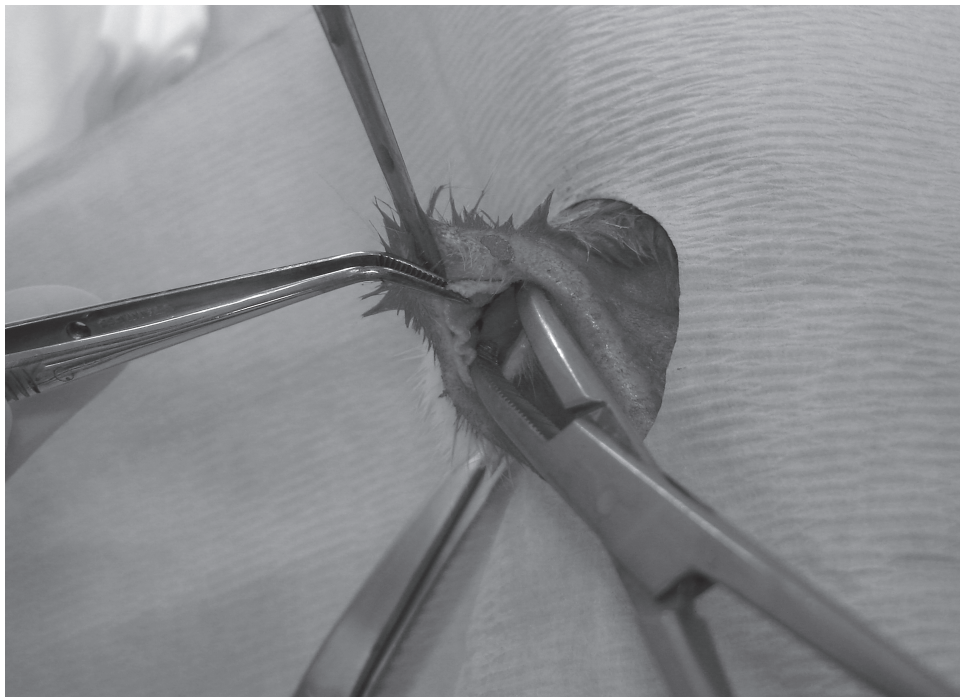

Figure. Removal of an implanted Dacron vascular graft from an adult male Wistar rat.

turing consecutive 10-fold dilutions (0.1 mL) of the bacterial suspension on blood agar plates. All plates were incubated at 37°C for 48 hours and assessed for the presence of the staphylococcal strains. The organisms were quantitated by counting the number of CFUs per plate.[18]

Statistical Analysis

Quantitative culture results were presented as mean (SD). Differences among the groups were assessed using 1-way analysis of variance, and multiple comparisons between the groups were performed with the Tukey post hoc honestly significant difference test. Differences were considered statistically significant when $P < 0.05$. Statistical analysis and animal randomization were performed using SPSS version 11.0 (SPSS Inc., Chicago, Illinois).

RESULTS

Seventy rats (mean [SD] weight, 323.7 [17.9] g; mean [SD] age, 5.98 [0.64] months) were equally divided among the 7 groups. Evidence of graft infection was present in all rats included in the untreated control groups (groups II and III). In contrast, none of the animals included in the uncontaminated control group (group I) had anatomic or microbiological evidence of graft infection. All the groups that received preoperative intraperitoneal antibiotic prophylaxis suggested evidence of infection, although with reduced quantitative bacterial graft culture results in comparison with the untreated control groups.

The results of the quantitative graft culture are summarized in the **table**. Statistically significant differences were observed between the results of the quantitative bacterial graft cultures when the data obtained from all prophylaxis-treated groups (groups IV–VII) were compared with those obtained from the untreated contaminated groups (all, $P < 0.001$). However, no statistically sig-

Table. Quantitative microbiologic results from in vivo experiments on methicillin-resistant staphylococci in vascular grafts implanted in rats for 1 week.

Group	Microorganism	Preoperative Intraperitoneal Drug	Quantitative Graft Culture, Mean (SD), CFUs/mL
I	–	–	$0^{*\dagger}$
II	MRSA	–	2.6×10^7 (6.1×10^6)
III	MRSE	–	6.6×10^6 (2.6×10^6)*
IV	MRSA	Vancomycin	6.7×10^2 (3.7×10^2)*†
V	MRSE	Vancomycin	5.6×10^2 (3.8×10^2)*†
VI	MRSA	Linezolid	8.2×10^2 (4.5×10^2)*†
VII	MRSE	Linezolid	7.0×10^2 (5.6×10^2)*†

CFU = colony-forming unit; MRSA = methicillin-resistant *Staphylococcus aureus*; MRSE = methicillin-resistant *Staphylococcus epidermidis*.
*$P < 0.001$ versus group II.
$^{\dagger}P < 0.001$ versus group III.

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nificant between-group differences were observed when the bacteria counts were compared between the vancomycin and linezolid prophylaxis groups contaminated with MRSA and MRSE. In the contaminated control groups (groups II and III), MRSA had greater affinity to Dacron prostheses than MRSE ($P < 0.001$). However, no significant difference was found between the affinity of MRSA and MRSE to Dacron grafts when all the contaminated groups were compared. Finally, none of the study animals died or had clinical evidence of drug-related adverse events (eg, signs of perigraft inflammation, anorexia, vomiting, diarrhea, or altered behavior).

DISCUSSION

In this prosthetic vascular graft infection study, prophylaxis with linezolid (10 mg/kg) or vancomycin (10 mg/kg) was associated with significant decreases in the quantitative bacterial graft cultures compared with those of the controls. The prophylactic efficacy of linezolid in terms of bacteria counts was similar to that of vancomycin.

Staphylococci are the most commonly isolated microorganisms in vascular surgical procedures,[19] and the emergence of resistant gram-positive bacteria is of particular concern.[20,21]

Because of the existence of methicillin-resistant staphylococci, vancomycin has been the drug of last resort in many cases. Studies have reported vancomycin-resistant clinical isolates.[22,23] These are warning signs that emphasize the need for new prophylactic approaches with alternative antistaphylococcic compounds in vascular surgery. Linezolid is the first licensed drug in the oxazolidinone class of antibiotics. Consistent in vitro antimicrobial activity against methicillin-resistant staphylococci is an important attribute of linezolid.[24–26]

In this study, we found that both vancomycin and linezolid, when used for preventing vascular graft infection, resulted in significant inhibition of bacterial growth. However, the question arises as to whether the antibiotics used in this study can be recommended for clinical use. These antibiotics should be used as prophylactic agents in surgical centers where active surveillance has suggested that methicillin-resistant staphylococci are often the cause of device-related infections.[27–29] The unnecessary use of these antibiotics may result in selection for resistant organisms that may be carried to other patients and produce more serious infections.[30] Infections due to glycopeptide-resistant gram-positive organisms are difficult to treat, and there have been increased morbidity and mortality associated with these infections.[31] As linezolid is a new antibiotic that does not possess inherent cross-resistance to other antibiotic classes nor rapid in vitro resistance,[32,33] it may be included as a new prophylactic agent to delay the emergence of resistant staphylococcal strains. Unfortunately, linezolid resistance in *S aureus* has been reported.[34,35]

To prevent the emergence of resistant microorganisms, programs to educate health care workers about infection-control precautions against *S aureus* with

glycopeptide and linezolid resistance should be developed and infection-control specialists should monitor compliance with these precautions. Additionally, health care workers should be educated periodically about the indications for glycopeptides and linezolid to reduce the overuse and misuse of these drugs.

In our model, the explanted grafts were sonicated to remove the adherent bacteria. Therefore, we were able to obtain quantitative cultures of both the bacteria included on the graft material and those grown on biofilm surrounding the Dacron prostheses.

This experimental study has several limitations and caution regarding the application of these results is needed. Our in vivo model used a direct method of MRSA and MRSE colonization on the graft. Thus, the animal model in our study is not directly comparable with graft implantation into a blood vessel. Additionally, the antibiotics were administered intraperitoneally instead of intravenously, and antibiotic binding to the Dacron grafts was not assessed. These results should be compared with the findings in the clinical setting of grafts implanted in the arteries of human patients. Our report suggests that the use of linezolid as a prophylaxis for vascular graft infection is worthy of further research, especially in humans.

CONCLUSION

In this experimental study, prophylactic use of linezolid was found to be efficacious in significantly reducing colony counts in MRSA- or MRSE-infected vascular Dacron grafts in rats, and its prophylactic efficacy was comparable to that of an established vancomycin regimen.

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