Efficacy of Quinupristin-Dalfopristin in Preventing Vascular Graft Infection Due to *Staphylococcus epidermidis* with Intermediate Resistance to Glycopeptides

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A rat model was used to investigate the efficacy of quinupristin-dalfopristin (Q-D) in the prevention of vascular prosthetic graft infection due to methicillin-resistant *Staphylococcus epidermidis* with intermediate resistance to glycopeptides. The *in vitro* activity of the compound was compared to that of vancomycin by MIC determination and time-kill study. Moreover, the efficacy of collagen-sealed Q-D-soaked Dacron was evaluated in a rat model of graft infection. Graft infections were established in the subcutaneous tissue of the backs of 120 adult male Wistar rats. The *in vivo* study included a control group, one contaminated group that did not receive any antibiotic prophylaxis, two contaminated groups that received grafts soaked with 10 and 100 µg of Q-D per ml, respectively, and two contaminated groups that received grafts soaked with 10 and 100 µg of vancomycin per ml, respectively. Rats that received Dacron grafts soaked with 100 µg of Q-D per ml showed no evidence of infection (<10 CFU/ml). In contrast, for rats that received Dacron grafts soaked with 10 µg of Q-D per ml and Dacron grafts soaked with 10 or 100 µg of vancomycin per ml, the quantitative graft cultures demonstrated $2.2 \times 10^2 \pm 1.3 \times 10^2$, $2.2 \times 10^6 \pm 1.9 \times 10^5$, and $5.6 \times 10^2 \pm 0.3 \times 10^2$ CFU/ml, respectively. Taken together the results of the study demonstrate that the use of Dacron grafts soaked with Q-D can result in significant bacterial growth inhibition and show that this compound is potentially valuable for prevention of vascular prosthetic graft infection.

The increasing use of foreign material in many fields of modern surgery is associated with a definitive risk of bacterial infection. Coagulase-negative staphylococci, chiefly the skin commensal *Staphylococcus epidermidis*, are among the most common pathogens that cause biomaterial infections and the most frequent cause of late-appearing vascular graft infections in humans (1, 2, 5, 6, 10, 16, 19). Vascular prosthetic graft infection is one of the most feared complications that the vascular surgeon treats; although most cases are resolved by removal of the device and antibiotic therapy, serious complications may result, including organ failure, amputation, metastatic infection, and death. Effective strategies for the prevention of prosthetic infection vary from device to device. The centerpieces of prophylaxis are asepsis and perioperative administration of systemic antibiotics (3, 5). As adjunctive prophylaxis, in the case of vascular grafts, the use of antimicrobials that bind to prosthetic grafts at high concentrations has been proposed (4, 6, 8, 9, 15, 20, 22, 24, 26).

Since the emergence of methicillin-resistant staphylococci, glycopeptides have often been the only effective drugs. For this reason, the emergence of staphylococcal strains exhibiting reduced sensitivities to vancomycin is of particular concern. The

emergence of vancomycin resistance in coagulase-negative staphylococci was described in the 1980s. For this reason, new strategies are needed to treat infections caused by these multidrug-resistant organisms and to reduce the increasing selection pressure of antibiotics on gram-positive pathogens (11, 18, 21, 23, 25).

Various new agents have been demonstrated to have significant *in vitro* activities against staphylococci. One of these compounds is the new semisynthetic injectable streptogramin quinupristin-dalfopristin (Q-D), composed of two components which may act synergistically: quinupristin, a peptide macrolactone classified as a type B streptogramin, and dalfopristin, a polyunsaturated macrolactone classified as a type A streptogramin, in a 30:70 ratio. It has a focused spectrum of *in vitro* activity against gram-positive cocci, including multidrug-resistant isolates of staphylococci, streptococci, and *Enterococcus faecium* (7, 17, 27).

In this study we used one strain of *S. epidermidis* with intermediate resistance to vancomycin to investigate the *in vitro* activity of Q-D and its *in vivo* efficacy when it was bound to a Dacron graft for the prevention of prosthesis infection in a rat model.

MATERIALS AND METHODS

***In vitro* studies. (i) Organisms.** The strain of methicillin-resistant *S. epidermidis* with intermediate resistance to vancomycin used in this study was isolated from a clinical specimen submitted for routine bacteriological investigation to the Institute of Infectious Diseases and Public Health, University of Ancona,

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Ancona, Italy. This isolate is described by use of the acronym VISE (vancomycin-intermediate *S. epidermidis*). Commercially available *S. epidermidis* ATCC 12228 was used as a quality control strain in the *in vitro* investigations.

(ii) **Drugs.** Q-D was obtained from Aventis Pharma, Centre de Recherches, Vitry-Alfortville, France. Vancomycin was obtained from Sigma-Aldrich, Milan, Italy. Laboratory powders were diluted in accordance with the recommendations of the manufacturers. Solutions of drugs were made fresh on the day of assay or were stored at -80°C in the dark for up to 20 days.

(iii) **Antimicrobial susceptibility testing.** The antimicrobial susceptibilities of the strains to Q-D and vancomycin were determined by the broth microdilution method described by the National Committee for Clinical Laboratory Standards (NCCLS) (13). In addition, the strains were tested for their susceptibilities to vancomycin by the NCCLS reference disk diffusion method with 30- μg vancomycin discs (14). Experiments were performed in triplicate.

(iv) **Time-kill studies.** To perform time-kill studies, the VISE strain was grown at 37°C in Mueller-Hinton (MH) broth. When the bacteria were in the log phase of growth the suspensions were centrifuged at $1,000 \times g$ for 15 min, the supernatants were discarded, and the bacteria were resuspended and diluted with sterile saline to achieve a concentration of 5×10^{10} CFU/ml saline. The organisms were resuspended in fresh MH broth at approximately 5×10^5 cells/ml and exposed to Q-D and vancomycin (one and four times the MIC) for up to 24 h at 37°C . Throughout the experiments, triplicate samples (0.1 ml) were withdrawn after 0, 1, 3, 6, and 24 h of incubation at 37°C . Up to seven 10-fold dilutions were made in MH broth from each sample, and, finally, the dilutions were spread onto MH agar plates and incubated for up to 72 h at 37°C to obtain viable colonies. The limit of detection for this method was approximately 10 CFU/ml. In preliminary experiments, antibiotic carryover was ruled out by plating samples of bacterial suspensions in the presence or absence of antibiotics.

In vivo studies. (i) Rat model. Adult male Wistar rats (weight range, 250 to 300 g) were studied. The study included a group with no graft contamination and no local antibiotic treatment (uncontaminated control group), one contaminated group that did not receive any local antibiotic treatment (untreated control group), two contaminated groups that received grafts soaked with 10 and 100 μg of Q-D per ml, respectively, and two contaminated groups that received grafts soaked with 10 and 100 μg of vancomycin per ml, respectively. Each group included 20 animals. Rats were anesthetized with ether, the hair on the back was shaved, and the skin was cleansed with 10% povidone-iodine solution. One subcutaneous pocket was made on each side of the median line through a 1.5-cm incision. Aseptically, 1-cm² sterile collagen-sealed Dacron grafts (Albograft; Sorin Biomedica Cardio, Saluggia VC, Italy) were implanted into the pockets. Before implantation, the Dacron graft segments were impregnated with Q-D and vancomycin, each at concentrations of 10 and 100 $\mu\text{g}/\text{ml}$. Immediately before implantation the grafts were soaked for 20 min in a sterile solution of the agents mentioned above. The pockets were closed with skin clips, and sterile saline solution (1 ml) containing the VISE strain at a concentration of 2×10^7 CFU/ml was inoculated onto the graft surface by using a tuberculin syringe to create a subcutaneous fluid-filled pocket (2). The animals were returned to individual cages and thoroughly examined daily. All grafts were explanted at 7 days following implantation. This study was approved by the Animal Research Ethics Committee of the Istituto Nazionale Riposo e Cura Anziani Istituto di Ricovero e Cura a Carattere Scientifico, University of Ancona.

(ii) **Assessment of infection.** The explanted grafts were placed in sterile tubes, washed in sterile saline solution, placed in tubes containing 10 ml of phosphate-buffered saline solution, and sonicated for 5 min to remove the adherent bacteria from the grafts. Quantitation of viable bacteria was performed by culturing serial 10-fold dilutions (0.1 ml) of the bacterial suspension onto blood agar plates. All plates were incubated at 37°C for 48 h and evaluated for the presence of the VISE strain. The organisms were quantitated by counting the number of CFU per plate. The limit of detection for this method was approximately 10 CFU/ml.

Statistical analysis. MICs are presented as the modes of three separate experiments. Quantitative culture results regarding the *in vivo* experiments are presented as the means $\pm$ standard deviations of the means. The results were compared by analysis of variance of the log-transformed data by the Tukey-Kramer honestly significant difference test. Significance was accepted when the *P* value was ≤ 0.05 .

RESULTS

In vitro data. According to the broth microdilution method recommended by the NCCLS, vancomycin MICs were 0.25 and 8 $\mu\text{g}/\text{ml}$ for *S. epidermidis* ATCC 12228 and the VISE strain, respectively, while Q-D MICs were 0.50 and 1 $\mu\text{g}/\text{ml}$, respec-

TABLE 1. Efficacies of Q-D and vancomycin against a glycopeptide-intermediate *S. epidermidis* strain causing graft infection in a rat model

Group ^a	Graft-bonded drug (concn [$\mu\text{g}/\text{ml}$]) ^b	Quantitative graft culture result (CFU/ml)
1		<10
2		$5.1 \times 10^6 \pm 8.8 \times 10^5$
3	Q-D ^c , (100)	<10
4	Q-D ^c (10)	$2.2 \times 10^2 \pm 1.3 \times 10^2$
5	(100) Vancomycin ^c	$5.6 \times 10^2 \pm 0.3 \times 10^2$
6	(10) Vancomycin	$2.2 \times 10^6 \pm 1.9 \times 10^5$

^a Each group consisted of 20 animals; groups 2 to 6 received saline solution (1 ml) containing the VISE strain at a concentration of 2×10^7 CFU/ml.

^b The Dacron graft segments were impregnated with 100 μg of Q-D per ml (group 3), 10 μg of Q-D per ml (group 4), 100 μg of vancomycin per ml (group 5), and 10 μg of vancomycin per ml (group 6). Groups 1 (uncontaminated control) and 2 (untreated control) received nonimpregnated Dacron grafts.

^c Statistically significant compared with group 2.

^d Statistically significant compared with groups 4 to 6.

tively. The different patterns of susceptibility were confirmed by the disk diffusion test: *S. epidermidis* ATCC 12228 showed zone sizes of 18 mm with the vancomycin disc, while the intermediate resistance of the VISE strain to vancomycin was demonstrated by a zone size of 11 mm.

Q-D at 2 $\mu\text{g}/\text{ml}$ (four times the MIC) produced reductions in bacterial counts of 0.1, 0.3, 1.1, and 1.8 log₁₀ CFU/ml after 1, 3, 6, and 24 h of incubation, respectively. This killing was comparable to that obtained with Q-D at 0.5 $\mu\text{g}/\text{ml}$ (one time the MIC). Vancomycin at 32 $\mu\text{g}/\text{ml}$ (four times the MIC) produced reductions in bacterial counts of 0.2, 0.5, 1.0, and 1.6 log₁₀ CFU/ml after 1, 3, 6, and 24 h of incubation, respectively, while vancomycin at 8 $\mu\text{g}/\text{ml}$ (one time the MIC) exhibited a low level of bactericidal activity, producing reductions in bacterial counts of 0.0, 0.1, 0.4, and 0.6 log₁₀ CFU/ml, respectively (data not shown).

In vivo studies. None of the animals included in the uncontaminated control group had microbiological evidence of graft infection. On the contrary, all 20 rats included in the untreated control group demonstrated evidence of graft infection, with quantitative culture results showing $5.1 \times 10^6 \pm 8.8 \times 10^5$ CFU/ml. Interestingly, only the group with Dacron grafts soaked in 100 μg of Q-D per ml showed no evidence of staphylococcal infection (<10 CFU/ml). In contrast, the quantitative graft cultures for the rats that received Dacron grafts soaked in 10 μg of Q-D per ml and Dacron grafts soaked in vancomycin demonstrated bacterial growth (Table 1). None of the animals included in any group died or had clinical evidence of drug-related adverse effects, such as local signs of perigraft inflammation, anorexia, vomiting, diarrhea, or behavioral alterations.

There were significant differences in the results of the quantitative bacterial graft cultures when the data obtained for the antibiotic-treated groups were compared with those obtained for the contaminated control group ($P < 0.05$), with the exception of the group treated with grafts soaked in 10 μg of vancomycin per ml. When data obtained for each group with grafts soaked in an antibiotic were compared with those obtained for any other group with a graft soaked in an antibiotic, the differences were always statistically significant for grafts soaked in 100 μg of Q-D per ml ($P < 0.05$). Finally, no statistically significant differences were observed between the group

with grafts soaked in 10 µg of Q-D per ml and the group with grafts soaked in 100 µg of vancomycin per ml.

DISCUSSION

Debate continues over the best treatment for prosthetic vascular graft infections. *S. epidermidis* is the most important pathogen causing infections related to implanted foreign bodies. It has been recovered from the skin, subcutaneous fat, lymph nodes, and arterial walls of more than one-third of individuals undergoing vascular reconstruction, despite the use of aseptic vascular surgical technique and prophylactic antibiotics. Most important in the pathogenesis of foreign body-associated infection due to this organism is the colonization of the polymer surface by formation of a biofilm (2). For this reason, prevention through effective antibiotic prophylaxis plays a pivotal role in the control of these infections and has an important impact on patient mortality and the cost-effectiveness of hospital care.

The success of prophylactic antibiotics during surgery is dependent on the pharmacokinetics of the antibiotic in tissue and the maintenance of adequate levels of the antibiotic in tissue for the duration of the vascular surgical procedure. Nevertheless, errors in the sterilization procedures and the increases in the incidence and the levels of resistance of *S. epidermidis* can predispose individuals to prosthesis infections (2, 3, 10). Glycopeptides such as vancomycin are used parenterally to treat infections caused by gram-positive bacteria, especially staphylococcal infections after the emergence of methicillin-resistant staphylococci. Recently, they have been administered as perioperative antibiotic prophylaxis (25). Nevertheless, the recent emergence of glycopeptide resistance in coagulase-negative staphylococci heightens concern about the need for other antistaphylococcal agents (12, 18, 21).

Analysis of the data from in vitro studies shows that Q-D and vancomycin had similar activities against the control strain, *S. epidermidis* ATCC 12228, while Q-D exhibited a higher level of activity than vancomycin against the VISE clinical strain. In fact, the broth microdilution method and the disk diffusion method recommended by the NCCLS showed that vancomycin exerted intermediate activity against the VISE strain, although there are still differences between the current NCCLS interpretive standards and the recommendations made to define categories of susceptibility to glycopeptides in some other countries (25).

The in vivo results were similar to those reported by other investigators (4, 6, 8, 9, 15, 22, 24, 26), who found that the use of Dacron grafts soaked in an antibiotic can result in significant bacterial growth inhibition, even though high concentrations of organisms were topically inoculated on the Dacron prostheses. Actually, statistical analysis demonstrated that any prophylactic antibiotic treatment except vancomycin at 10 µg/ml was useful. Nevertheless only Q-D at the highest concentration tested inhibited bacterial growth within the limits of detection. Finally, no statistically significant differences were observed between the groups that received grafts soaked with 10 µg of Q-D per ml and those that received grafts soaked with 100 µg of vancomycin per ml. However, it is noteworthy that Q-D and vancomycin are physically different compounds and the quan-

titative results could be affected by the lack of equivalent abilities of the two antibiotics to coat the artificial materials.

It has long been recognized that the treatment of serious bacteremia caused by staphylococci, such as vascular graft infection, typically requires the use of agents with bactericidal activities against the organism. This fact is likely emphasized by the recent emergence in clinical settings of multidrug-resistant bacteria that belong, for the most part, to the staphylococcal species. New therapeutic options are needed, although experience demonstrates that every new antimicrobial agent introduced into clinical practice can be plagued by the emergence of organisms resistant to its effect. The strong in vitro activity and the prophylactic in vivo efficacy demonstrated by Q-D against a staphylococcal strain with decreased susceptibility to the glycopeptides used in the present study make Q-D potentially useful for future topical antimicrobial treatments, such as perioperative chemoprophylaxis in prosthetic surgery.

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