Quinupristin/dalfopristin Bonding in Combination with Intraperitoneal Antibiotics Prevent Infection of Knitted Polyester Graft Material in a Subcutaneous Rat Pouch Model Infected with Resistant *Staphylococcus epidermidis*

R. Ghiselli[1], **A. Giacometti**[*2], **O. Cirioni**[2], **F. Mocchegiani**[1], **F. Orlando**[3], **M. S. Del Prete**[2], **G. D'Amato**[2], **G. Scalise**[2] and **V. Saba**[1]

[1]*Departments of General Surgery, I.N.R.C.A. I.R.C.C.S.,* [2]*Institute of Infectious Diseases and Public Health and* [3]*Biotechnology Centre, Research Department, I.N.R.C.A. I.R.R.C.S., University of Ancona, Ancona, Italy*

Objective: *to investigate the efficacy of quinupristin/dalfopristin in the prevention of prosthetic graft infection in a rat subcutaneous pouch model.*

Methods: *graft infections were established in the subcutaneous tissue of 140 male Wistar rats by implantation of Dacron prostheses followed by topical inoculation with* Staphylococcus epidermidis *with intermediate resistance to glycopeptides. The study included one group without contamination, one contaminated group without prophylaxis, one contaminated group that received 50 mg/l quinupristin/dalfopristin-soaked graft, one contaminated group that received 10 mg/kg intraperitoneal levofloxacin, one contaminated group that received 3 mg/kg intraperitoneal doxycycline, and two contaminated groups that received 50 mg/l quinupristin/dalfopristin-soaked plus 10 mg/kg intraperitoneal levofloxacin or 3 mg/kg intraperitoneal doxycycline. Each group included 20 animals. The grafts were removed after 7 days and evaluated by quantitative culture.*

Results: *quinupristin/dalfopristin showed a significantly higher efficacy than levofloxacin and doxycycline, even though quantitative graft cultures for rats that received only quinupristin/dalfopristin-soaked graft showed bacterial growth. Otherwise, the efficacy of levofloxacin was similar to that of doxycycline. Only the group treated with quinupristin/ dalfopristin combined with levofloxacin or doxycycline showed no evidence of staphylococcal infection.*

Conclusions: *quinupristin/dalfopristin as adjunctive topical antibiotic prophylaxis can be useful for the prevention of vascular graft infections caused by staphylococcal strains with high levels of resistance.*

Key Words: Prophylaxis; Vascular prostheses; Contamination; Bacteria; Antibiotics.

Introduction

Gram-positive infections are causing more serious infections than ever before in surgical patients, who are increasingly aged, ill, and debilitated.[1–3] Coagulase-negative staphylococci, chiefly the skin commensal *Staphylococcus epidermidis*, are among the most common pathogens that cause graft infection, a dread severe complication of vascular reconstructions frequently resulting in loss of organ function, limb, and life.[4–6]

Effective strategies for the prevention of prosthetic infection vary from device to device and are conveniently considered with respect to the period before, during and after operation. The mainstay of prophylaxis are asepsis and perioperative administration of systemic antibiotics.[1,7–9] The choice of antibiotic and length of treatment are controversial, although first and second generation cephalosporins have been the most commonly used drugs.[10,11] However, the antibiotic used should be guided by local bacterial prevalence and sensitivities, remembering that most infections are caused by staphylococci. Moreover, as adjunctive prophylaxis in the case of vascular grafts, antimicrobials bound in high concentrations to prosthetic grafts have been proposed.[12–16] Particularly, the efficacy of rifampin bonding on vascular graft to prevent prosthetic infections was demonstrated since 1990.[17–19] Nevertheless, the use of broad-spectrum antibiotics can select for the

* Please address all correspondence to: A. Giacometti, Clinica delle Malattie Infettive, c/o Ospedale Regionale, via Conca, 60020 Ancona, Italy.

emergence of resistant pathogens. Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) emerged during the 1970s and, successively in the 1980s, it has been described the emergence of vancomycin resistance in coagulase-negative staphylococci (CNS).[20–25]

To treat infections caused by these multidrug-resistant organisms and to reduce the increasing selection pressure by glycopeptides on Gram-positive pathogens in hospitals new antibiotics are needed. One of these compounds is the new semisynthetic injectable streptogramin quinupristin-dalfopristin (Q-D). It is composed of two synergistic pristinamycin derivatives, 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. Q-D inhibits protein synthesis by irreversibly binding to the 50S ribosomal subunit. It has a focused spectrum of in vitro activity against Gram-positive cocci, including methicillin-resistant isolates of staphylococci, streptococci, and *Enterococcus faecium*.[26,27]

Levofloxacin, a fluoroquinolone with somewhat enhanced activity against Gram-positive cocci, is the L-stereoisomer of ofloxacin, which is a racemic mixture that contains equal parts of the L- and D-stereoisomers. It is more active than ofloxacin or ciprofloxacin against streptococci and staphylococci, and higher peak concentrations in serum can be achieved with the recommended doses.[28]

Doxycycline is a long-acting tetracycline that acts against susceptible organisms by inhibiting protein synthesis. It enter bacteria by an energy-dependent process and bind reversibly to the 30S ribosomal subunits of the bacteria. All tetracyclines have similar antimicrobial spectra, with activity against many Gram-positive aerobic cocci, including staphylococci and streptococci.[29]

In this study we used one strain of *S. epidermidis* with intermediate resistance to vancomycin to investigate the *in vivo* efficacy of Q-D, alone and in combination with doxycycline and levofloxacin, in preventing prosthesis infection in a rat model.

Materials and Methods

Organisms

The strain of *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,

Italy. This isolate was described using the acronym VISE (vancomycin-intermediate *S. epidermidis*).

Drugs

Q-D was obtained from Aventis Pharma, Centre de Recherches, Vitry-Alfortville, France. Vancomycin, cefazolin, cefamandole, rifampin and doxycycline (all from Sigma-Aldrich, Milan, Italy) and levofloxacin (GlaxoSmithKline, Verona, Italy) were diluted in accordance with manufacturers' recommendations. Solutions of drugs were made fresh on the day of assay or stored at $-80\,°C$ in the dark for short periods.

Antimicrobial susceptibility testing

The antimicrobial susceptibilities of the strain to the above mentioned drugs were determined by the broth microdilution method described by the National Committee for Clinical Laboratory Standards, NCCLS. In addition, the strain was tested for susceptibility to vancomycin by the NCCLS reference disk diffusion method using 30 µg vancomycin discs.[30,31] Experiments were performed in triplicate.

In vivo *rat model*

Graft infections were established in the subcutaneous tissue of 140 male Wistar rats by implantation of Dacron prostheses followed by topical inoculation of the VISE strain. The study included one group without contamination, one contaminated group without prophylaxis, one contaminated group that received 50 mg/l Q-D-soaked graft, one contaminated group that received 10 mg/kg intraperitoneal levofloxacin, one contaminated group that received 3 mg/kg intraperitoneal doxycycline, and two contaminated groups that received 50 mg/l Q-D-soaked plus 10 mg/kg intraperitoneal levofloxacin or 3 mg/kg intraperitoneal doxycycline. Each group included 20 animals. Rats were anaesthetised with ether, the hair on the back was shaved and the skin cleaned with 10% povidone–iodine solution. One subcutaneous pocket was made on each side of the median line by a 1.5 cm incision. Aseptically, 1 cm² sterile collagen-sealed double velour knitted polyethylene terephthalate (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 at concentrations of 50 mg/l. Bonding of the compound was obtained immediately

before implantation by soaking grafts for 20 min in a sterile solution of the above-mentioned agent. The pockets were closed by means of skin clips and physiological solution (1 ml) containing the VISE strain at a concentration of 2×10^7 CFU/ml was inoculated on to the graft surface using a tuberculin syringe to create a subcutaneous fluid-filled pocket. 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 I.N.R.C.A. I.R.R.C.S., University of Ancona.

Assessment of the 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 on 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 quantified by counting the number of CFU per plate. The limit of detection for this method was approximately 10 CFU/ml.

Statistical analysis

MIC values are presented as the mode of three separate experiments. Quantitative culture results regarding the *in vivo* studies were presented as mean ± standard deviation of the mean. Comparisons of the results were performed by Kruskal–Wallis 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 the isolate confirmed its intermediate resistance to vancomycin. Actually, vancomycin exhibited MICs of 8 g/ml and zone sizes of 11 mm by the disc diffusion test. Q-D, cefazolin, cefamandole, rifampin, doxycycline, and levofloxacin showed MICs of 128, 64, 32, 128, and 32 g/ml, respectively.

In vivo *data*

All 20 rats included in the untreated control group demonstrated evidence of graft infection, with quantitative culture data showing $6.0 \times 10^6 \pm 3.3 \times 10^6$ CFU/ml while none of the animals included in the uncontaminated control group had microbiological evidence of graft infection. Q-D showed a significantly higher efficacy than levofloxacin and doxycycline ($p < 0.001$), even though quantitative graft cultures for rats that received only Q-D-soaked graft showed bacterial growth. Otherwise, intraperitoneal levofloxacin showed to be slightly more effective than doxycycline intraperitoneal, even though there were not significant differences in the results from the quantitative bacterial graft cultures for data obtained from the two groups ($p = 0.12$). In detail, for the group that received Q-D-soaked graft and the groups that received intraperitoneal levofloxacin and doxycycline the quantitative graft cultures showed $2.5 \times 10^2 \pm 1.8 \times 10^2$, $5.8 \times 10^5 \pm 2.0 \times 10^5$ and $6.7 \times 10^5 \pm 8.9 \times 10^4$ CFU/ml, respectively. Only the groups with combined treatment showed no evidence of staphylococcal infection (<10 CFU/ml). The results are summarized in Table 1. Overall, there were

Table 1. Quantitative microbiologic results of the *in vivo* experiments.

Group [a]	Graft-soaked drug [b]	Intraperitoneal preoperative drug [c]	Quantitative graft culture (CFU/ml)
Uncontaminated group	–	–	<10.0
Contaminated group	–	–	$6.0 \times 10^6 \pm 3.3 \times 10^6$
Group 1	Q-D	–	$2.5 \times 10^2 \pm 1.8 \times 10^2$ [de]
Group 2	–	Levofloxacin	$5.8 \times 10^5 \pm 2.0 \times 10^5$
Group 3	–	Doxycycline	$6.7 \times 10^5 \pm 8.9 \times 10^4$
Group 4	Q-D	Levofloxacin	<10.0 [de]
Group 5	Q-D	Doxycycline	<10.0 [de]

[a] Each group was formed by 20 animals.
[b] The Dacron graft segments were impregnated 50 mg/l Quinupristin-Dalfopristin (Q-D).
[c] Levofloxacin 10 mg/kg; doxycycline 3 mg/kg.
[d] Statistically significant when compared with contaminated control group ($p < 0.001$).
[e] Statistically significant when compared with groups 2 and 3 ($p < 0.001$).

significant differences only when the data obtained from the contaminated control group were compared with those obtained from the group that received Q-D-soaked and the groups that received combined treatment ($p < 0.001$).

Discussion

S. epidermidis and other coagulase-negative staphylococci isolated from hospitalised patients were often considered contaminants. Now, they are among the leading causes of nosocomial infection *S. epidermidis* is the most important pathogen causing infections related to implanted foreign bodies. It is well known that vascular surgery patient's own endogenous flora is the most likely source of infection and that it can occur by contamination with only a few bacteria during the implantation of the device.[4] Most important in the pathogenesis of foreign body-associated infection due to staphylococci is the colonisation of the polymer surface. Bacteria that adhere to implanted medical devices can encase themselves in a hydrated matrix of polysaccharide and protein, and form a slimy layer known as a biofilm. Formation of these sessile communities and their inherent resistance to antimicrobial agents are the root of many persistent and chronic bacterial infections.[6] In addition, the use of a broad-spectrum antibiotics selects for the emergence of resistant pathogens. Potential sites of nosocomial Gram-positive infections include the urinary tract, intravascular loci, lung and pleural space, peritoneal cavity, and finally surgical sites including prosthetic devices. The emergence of methicillin-resistant staphylococci and glycopeptide-intermediate and glycopeptide-resistant Gram-positive cocci created a pressing need for effective alternative antibiotics.[17–20,25,32] In the recent years, the new streptogramin combination Q-D demonstrated a selective spectrum of antibacterial activity, primarily against Gram-positive aerobic bacteria. Therefore, it has been assessed mainly in emergency-use protocols, in hospitalised patients with skin and skin-structure infections and in patients with glycopeptide-resistant Gram-positive infections.[20]

It is well known that the success of surgical prophylaxis can be dependent on the pharmacokinetics of antibiotic tissue penetrance with its maintenance of adequate tissue levels for the duration of the vascular surgical procedure. As adjunctive prophylaxis in the case of vascular grafts, antimicrobials bound in high concentrations to prosthetic grafts gave encouraging results. In particular several studies demonstrated the efficacy of rifampin bonding to prevent vascular graft infections.[12–19]

Since Q-D has been introduced into clinical practice as a consequence of the emergence of multidrug-resistant staphylococci, we investigated its ability to prevent graft infections due to a strain with intermediate resistance to vancomycin. Our results were similar to those reported by the above-mentioned studies, who found that the use of antibiotic-soaked Dacron graft alone or with parenteral administration of other drugs can result in significant bacterial growth inhibition even though high concentrations of organisms are topically inoculated on the Dacron prostheses. However, it is important to note that only Q-D combined with intraperitoneal antibiotics was able to inhibit completely the growth of the resistant strains.

The clinical experience clearly shows that the prevention of serious staphylococcal infections, such as vascular graft infection, typically requires the use of antimicrobial agents with bactericidal activity. For this reason, a prevention through an effective antibiotic prophylaxis plays an important role on the control of these infections with a great impact on patient mortality and the cost-effectiveness of hospital care. In view of our results, further studies are needed to investigate the potential usefulness of Q-D in perioperative chemoprophylaxis of prosthetic surgery.

References

1 HENKE PK, BERGAMINI TM, ROSE SM, RICHARDSON JD. Current opinion in prosthetic vascular graft infection. *Am Surg* 1998; **64**: 39–45.

2 VON EIFF C, HEILMANN C, PETERS G. New aspects in the molecular basis of polymer-associated infections due to staphylococci. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 853–856.

3 BARIE PS. antibiotic-resistant gram-positive cocci: implications for surgical practice. *World J Surg* 1998; **22**: 118–126.

4 BERGAMINI TM, CORPUS RA JR, BRITTIAN KR, PEYTON JC, CHEADLE WG. The natural history of bacterial biofilm graft infection. *J Surg Res* 1994; **56**: 393–396.

5 BERGAMINI TM, CORPUS RA JR, McCURRY TM et al. Immunosuppression augments growth of graft-adherent *Staphylococcus epidermidis*. *Arch Surg* 1995; **130**: 1345–1350.

6 COSTERTON JW, STEWART PS, GREENBERG EP. Bacterial biofilm: a common cause of persistent infections. *Science* 1999; **284**: 1318–1322.

7 BERGAMINI TM, PEYTON JC, CHEADLE WG. Prophylactic antibiotics prevent bacterial biofilm graft infection. *J Surg Res* 1992; **52**: 101–105.

8 CITAK MS, CUÉ JI, PEYTON JC, MALANGONI MA. The critical relationship of antibiotic dose and bacterial contamination in experimental infection. *J Surg Res* 1992; **52**: 127–130.

9 MONZON M, OTEIZA C, LEIVA J, AMORENA B. Synergy of different antibiotic combinations in biofilms of *Staphylococcus epidermidis*. *J Antimicrob Chemother* 2001; **48**: 793–801.

10 MARRONI M, CAO P, FIORIO M et al. Prospective, randomized, double-blind trial comparing teicoplanin and cefazolin as

antibiotic prophylaxis in prosthetic vascular surgery. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 175–178.

11 MAKI DG, BOHN MJ, STOLZ SM *et al*. Comparative study of cefazolin, cefamandole, and vancomycin for surgical prophylaxis in cardiac and vascular operations. A double-blind randomized trial. *J Thorac Cardiovasc Surg* 1992; **104**: 1423–1434.

12 SARDELIC F, AO PY, TAYLOR DA, FLETCHER JP. Prophylaxis against *Staphylococcus epidermidis* vascular graft infection with rifampicin-soaked, gelatin-sealed Dacron. *Cardiovasc Surg* 1996; **4**: 389–392.

13 CHERVU A, MOORE WS, GELABERT HA, COLBURN MD, CHVAPIL M. Prevention of graft infection by use of prostheses bonded with a rifampin collagen release system. *J Vasc Surg* 1991; **14**: 521–524.

14 OSADA T, YAMAMURA K, FUJIMOTO K *et al*. Prophylaxis of local vascular graft infection with levofloxacin incorporated into albumin-sealed dacron graft. *Microbiol Immunol* 1999; **43**: 317–321.

15 GIACOMETTI A, CIRIONI O, GHISELLI R *et al*. Polycationic peptides as prophylactic agents against methicillin-susceptible and methicillin-resistant *Staphylococcus epidermidis* vascular graft infection. *Antimicrob Agents Chemother* 2000; **44**: 3306–3309.

16 GHISELLI R, GIACOMETTI A, GOFFI L *et al*. Efficacy of rifampin-levofloxacin as a prophylactic agent in preventing *Staphylococcus epidermidis* graft infection. *Eur J Vasc Endovasc Surg* 2000, 20: 508–511.

17 COGGIA M, Goeau-Brissonniere O, LEFLON V, NICOLAS MH, PECHERE JC. Experimental treatment of vascular graft infection due to *Staphylococcus epidermidis* by in situ replacement with a rifampin-bonded polyester graft. *Ann Vasc Surg* 2001; **15**: 421–429.

18 GOEAU-BRISSONNIERE O, MERCIER F, NICOLAS MH *et al*. Treatment of vascular graft infection by in situ replacement with a rifampin-bonded gelatin-sealed Dacron graft. *J Vasc Surg* 1994; **19**: 739–741.

19 GOEAU-BRISSONNIERE O, LEPORT C, LEBRAULT C *et al*. Antibiotic prophylaxis of late bacteremic vascular graft infection in a dog model. *Ann Vasc Surg* 1990; **4**: 528–532.

20 RAAD I, ALRAHWAN A, ROLSTON K. *Staphylococcus epidermidis*: emerging resistance and need for alternative agents. *Clin Infect Dis* 1998; **26**: 1182–1187.

21 BIAVASCO F, VIGNAROLI C, VARALDO PE. Glycopeptide resistance in coagulase-negative staphylococci. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 403–417.

22 HIRAMATSU K, HANAKI H, INO T *et al*. Methicillin-resistant *Staphylococcus aureus*clinical strain with reduced vancomycin. *J Antimicrob Chemother* 1997; **40**: 135–136.

23 McMANUS AT, GOODWIN CW, PRUITT BA JR. Observations on the risk of resistance with the extended use of vancomycin. *Arch Surg* 1998; **133**: 1207–1211.

24 JARVIS WR. Epidemiology, appropriateness, and cost of vancomycin use. *Clin Infect Dis* 1998; **26**: 1200–1203.

25 SCHWALBE RS, STAPLETON JT, GILLIGAN PH. Emergence of vancomycin resistance in coagulase-negative staphylococci. *N Engl J Med* 1987; **316**: 927–931.

26 FUCHS PC, BARRY AL, BROWN SD. Interactions of quinupristin-dalfopristin with eight other antibiotics as measured by time-kill studies with 10 strains of *Staphylococcus aureus* for which quinupristin-dalfopristin alone was not bactericidal. *Antimicrob Agents Chemother* 2001; **45**: 2662–2665.

27 SAHGAL VS, URBAN C, MARIANO N *et al*. Quinupristin/dalfopristin (RP 59500) therapy for vancomycin resistant *Enterococcus faecium* aortic graft infection: case report. *Microb Drug Resist* 1995; **1**: 245–247.

28 CHAMBERS HF, XIANG LIU Q, LIUXIN CHOW L, HACKBARTH C. Efficacy of levofloxacin for experimental aortic-valve endocarditis in rabbits infected with viridans group streptococcus or *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1999; **43**: 2742–2746.

29 CHOPRA I, HAWKEY PM, HINTON M. Tetracyclines, molecular and clinical aspects. *J Antimicrob Chemother* 1992; **29**: 245–277.

30 NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS. 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard. M7-A4. National Committee for Clinical Laboratory Standards, Wayne, PA, U.S.A.

31 NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS. 1997. Performance standards for antimicrobial disk susceptibility test. Approved Standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, PA, U.S.A.

32 SIERADZKI K, VILLARI P, TOMASZ A. Decreased susceptibilities to teicoplanin and vancomycin among coagulase-negative methicillin-resistant clinical isolates of staphylococci. *Antimicrob Agents Chemother* 1998; 42: 100–107.