ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2000, p. 2842–2844 Vol. 44, No. 10
0066-4804/00/$04.00+0

Mupirocin Prophylaxis against Methicillin-Susceptible, Methicillin-Resistant, or Vancomycin-Intermediate *Staphylococcus epidermidis* Vascular-Graft Infection

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Received 28 February 2000/Returned for modification 5 June 2000/Accepted 6 July 2000

A rat model was used to investigate the efficacy of mupirocin in the prevention of vascular prosthetic graft infection due to *Staphylococcus epidermidis* strains with different susceptibility patterns (methicillin susceptible, methicillin resistant, and with intermediate resistance to vancomycin). The effect of mupirocin-soaked Dacron was compared to that of perioperative intraperitoneal prophylaxis with vancomycin. Graft infections were established in the back subcutaneous tissue of adult male Wistar rats by implantation of Dacron prostheses (1 cm^2) followed by topical inoculation with 5×10^7 CFU of one staphylococcal strain. The study included a control group (no graft contamination), three contaminated groups that did not receive any antibiotic prophylaxis, three contaminated groups that received mupirocin-soaked grafts, three contaminated groups in which perioperative intraperitoneal vancomycin prophylaxis (10 mg/kg of body weight) was administered, and three contaminated groups that received mupirocin-soaked grafts and perioperative intraperitoneal vancomycin prophylaxis (10 mg/kg). The grafts were sterilely removed 7 days after implantation, and the infection was evaluated by using sonication and quantitative agar culture. Data analysis showed the efficacy of mupirocin against all three strains, with growth of the strains in treated rats significantly different than that in the untreated control. In addition, mupirocin was more effective than vancomycin against the strain with intermediate susceptibility to the glycopeptide. Finally, the combination of mupirocin and vancomycin produced complete suppression of the growth of all of the strains.

Vascular prosthetic graft infection is a dreaded, serious complication of vascular surgery that frequently results in prolonged hospitalization, organ failure, amputation, and death (2, 3, 23). *Staphylococcus epidermidis* is among the most common pathogens that cause biomaterial infections (2–4, 18, 23). The centerpiece of prevention is prophylactic systemic antibiotics (4, 17). In addition, in the case of vascular grafts, antimicrobials, bound in high concentrations to prosthetic grafts have been proposed as adjunctive prophylaxis (1, 5, 6, 8, 11, 12, 15). Since the emergence of methicillin-resistant (MR) staphylococci, hglycopeptides have been the only uniformly effective treatment for staphylococcal infections. Vancomycin was introduced in the 1950s, and for almost 3 decades following its introduction, resistance was reported only rarely and appeared to have little clinical significance. Nevertheless, in the 1980s the emergence of vancomycin resistance in coagulase-negative staphylococci, especially *S. epidermidis*, *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus haemolyticus*, and *Staphylococcus xylous* has been described (13, 16, 20). Mupirocin (pseudomonic acid A), produced by *Pseudomonas fluorescens*, is a topical antibiotic that is used for the treatment of superficial skin infections due to *Staphylococcus aureus* and *Streptococcus pyogenes* and for the eradication of *S. aureus* nasal colonization (9, 10, 21). It was introduced into clinical practice in the in United Kingdom in 1985, but unfortunately, resistance was described shortly after its initial use (7). Definitions of mupirocin resistance have varied, but today there is agreement about two main categories of mupirocin resistance: low level (MIC = 4 to 256 mg/liter) and high level (MIC ≥512 mg/liter) (7, 19). In this study, we investigated the in vivo efficacy of mupirocin spontaneously bound to collagen-sealed Dacron in preventing infections of the graft due to methicillin-susceptible (MS), MR, and vancomycin-intermediate (VIR) *S. epidermidis*.

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MATERIALS AND METHODS

Organisms. The commercially available MS quality control strain *S. epidermidis* ATCC 12228, one clinical isolate of MR *S. epidermidis* (Se56-99), and one clinical isolate of VIR *S. epidermidis* (Se43-98) were used. The two clinical isolates used in this study were isolated from material submitted for routine bacteriological investigation to the Institute of Infectious Diseases and Public Health, University of Ancona, Ancona, Italy.

Drugs. Mupirocin (SmithKline Beecham Pharmaceuticals, Harlow, Essex, United Kingdom), oxacillin, and vancomycin (both from Sigma-Aldrich S.r.l., Milan, Italy) were diluted in accordance with the manufacturers' recommendations, yielding 1 mg/ml of stock solution. Solutions of drugs were made fresh on the day of assay or stored at −80°C in the dark for short periods. The concentration range assayed for each antibiotic was 0.25 to 512 mg/liter.

Susceptibility testing. The antimicrobial susceptibilities of the strains were determined by using the microbroth dilution method, according to the procedures outlined by the National Committee for Clinical Laboratory Standards (14). The MIC was taken to be the lowest antibiotic concentration at which observable growth was inhibited. Experiments were performed in triplicate.

Rat model. Adult male Wistar rats (weight range, 275 to 325 g) were studied. The study included a control group (no graft contamination), three contaminated groups that did not receive any antibiotic prophylaxis (MS1, MR1, and VIR1) (untreated controls), three contaminated groups that received mupirocin-soaked grafts (MS2, MR2, and VIR2), three contaminated groups in which perioperative intraperitoneal vancomycin prophylaxis (10 mg/kg of body weight) was administered (MS3, MR3, and VIR3), and three contaminated groups that received mupirocin-soaked grafts and perioperative intraperitoneal vancomycin prophylaxis (10 mg/kg) (MS4, MR4, and VIR4). Each group included 15 animals.

The rats were anesthetized with ether, the hair of 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 by a 1.5-cm-long incision. Aseptically, 1-cm^2 sterile collagen-sealed Dacron grafts (Albograft; Sorin Biomedica Cardio, S.p.A., Saluggia VC, Italy) were implanted in the pockets. Prior to implantation, the Dacron graft segments were impregnated with 100 μg of mupirocin/ml (groups MS2, MS4, MR2, MR4, VIR2, and VIR4). Antibiotic bonding was obtained immediately before implantation by soaking the grafts for 20 min in a sterile solution of mupirocin. In addition, the effect of preoperative intraperitoneal vancomycin administered 30 min before implantation at the standard dose of 10 mg/kg was evaluated in groups MS3, MS4, MR3, MR4, VIR3, and VIR4. The pockets were closed by means of skin clips, and sterile saline solution (1 ml) containing one of the above-mentioned *S. epidermidis* strains 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 (3). The animals were returned to individual cages and thoroughly examined daily. All grafts were explanted 7 days following implantation.

Serum vancomycin concentration measurement and kinetics. Preventive experiments were performed to measure serum vancomycin levels in uninfected animals receiving intraperitoneal vancomycin. Blood samples were obtained from the tail veins of six rats 1, 2, and 4 h after a single intraperitoneal dose of vancomycin (10 mg/kg). Drug levels were measured by bioassay: a spore suspension of *Bacillus subtilis* ATCC 6633 suspended in tryptic soy agar was used. The plates were read after incubation at 30°C for 18 h.

Assessment of infection. The explanted grafts were placed in sterile tubes, washed in sterile saline solution, transferred to 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 dilutions (0.1 ml) of the bacterial suspension: up to seven 10-fold dilutions from each sample were Mueller-Hinton broth and spread onto blood agar plates to obtain viable colonies. All plates were incubated at 37°C for 48 h and evaluated for the presence of the staphylococcal strains. The organisms were quantitated by counting the CFU per plate. The limit of detection for this method was approximately 10 CFU/ml.

Statistical analysis. MICs are presented as the geometric mean of three separate experiments. Quantitative culture results are presented as mean ± standard deviation of the mean. Comparisons between quantitative culture results were performed by the Student *t* test. Significance was accepted when the *P* value was ≤0.05.

RESULTS

The three strains proved to be similarly susceptible to mupirocin (MICs, 0.25, 1.26, and 1.59 mg/liter for the MS, MR, and VIR organisms, respectively), while they demonstrated different susceptibility patterns for the other antibiotics. *S. epidermidis* ATCC 12228 was susceptible to oxacillin and vancomycin (MICs, 0.39 and 0.31 mg/liter, respectively), and *S. epidermidis* Se56-99 was resistant to oxacillin (MIC, 10.08) and susceptible to vancomycin (MIC, 0.63 mg/liter), while the clinical isolate *S. epidermidis* Se43-98 showed resistance to oxacillin and intermediate resistance to vancomycin (MICs, 12.70 and 10.08 mg/liter, respectively).

After a single intraperitoneal injection, vancomycin (10 mg/kg) reached the peak level of 16.2 mg/liter. After 4 h, it had an average level of 6.4 mg/liter in serum.

None of the animals included in the uncontaminated control group had anatomic or microbiological evidence of graft infection. On the contrary, all 45 rats included in the untreated control groups (MS1, MR1, and VIR1) demonstrated evidence of graft infection, with quantitative culture results showing $5.1 \times 10^6 \pm 1.0 \times 10^6$, $6.1 \times 10^6 \pm 0.7 \times 10^6$, and $4.7 \times 10^6 \pm 0.8 \times 10^6$ CFU/ml, respectively, although there were no local signs of perigraft inflammation. For the 45 rats with mupirocin-coated Dacron grafts (groups MS2, MR2, and VIR2), the quantitative graft cultures demonstrated lower bacterial numbers ($1.2 \times 10^1 \pm 0.2 \times 10^1$, $4.4 \times 10^1 \pm 0.8 \times 10^1$, and $1.7 \times 10^2 \pm 0.3 \times 10^2$ CFU/ml, respectively). The results from groups MS3, MR3, and VIR3 (intraperitoneal vancomycin; non-antibiotic-impregnated Dacron graft) confirmed the efficacy of the perioperative glycopeptide against the MS and MR staphylococcal strains ($0.9 \times 10^1 \pm 0.1 \times 10^1$ and $2.3 \times 10^1 \pm 0.8 \times 10^1$ CFU/ml, respectively) and, on the contrary, its poor efficacy against the VIR strain ($1.3 \times 10^5 \pm 0.3 \times 10^5$ CFU/ml). Finally, the groups MS4, MR4, and VIR4 (mupirocin-coated Dacron grafts plus intraperitoneal vancomycin) showed no evidence of staphylococcal infection, with negative quantitative cultures. The results are summarized in Table 1.

TABLE 1. Quantitative microbiological results of in vivo experiments

Group[a]	Graft-bonded drug[b]	Intraperitoneal preoperative drug[c]	No. of graft segments (sterile/total)	Quantitative graft culture (CFU/ml)
Control			30/30	0.0
MS1			1/30	$5.1 \times 10^6 \pm 1.0 \times 10^6$
MS2[d]	Mupirocin		103/30	$1.2 \times 10^1 \pm 0.2 \times 10^1$
MS3[d]		Vancomycin	16/30	$0.9 \times 10^1 \pm 0.1 \times 10^1$
MS4[d]	Mupirocin	Vancomycin	30/30	0.0
MR1			2/30	$6.1 \times 10^6 \pm 0.7 \times 10^6$
MR2[e]	Mupirocin		12/30	$4.4 \times 10^1 \pm 0.8 \times 10^1$
MR3[e]		Vancomycin	16/30	$2.3 \times 10^1 \pm 0.8 \times 10^1$
MR4[e]	Mupirocin	Vancomycin	30/30	0.0
VIR1			1/30	$4.7 \times 10^6 \pm 0.8 \times 10^6$
VIR2[f]	Mupirocin		11/30	$1.7 \times 10^2 \pm 0.3 \times 10^2$
VIR3[f]		Vancomycin	4/30	$1.3 \times 10^5 \pm 0.3 \times 10^5$
VIR4[f]	Mupirocin	Vancomycin	30/30	0.0

[a] Each group contained 15 animals; MS1 to -4, groups of animals infected with MS *S. epidermidis* ATCC 12228; MR1 to -4, groups of animals infected with MR *S. epidermidis* Se 56-99; VIR1 to -4, groups of animals infected with VIR *S. epidermidis* Se 43-98.
[b] The Dacron graft segments were impregnated with 100 μg of mupirocin/ml.
[c] Vancomycin at 10 mg/kg.
[d] Statistically significant compared with group MS1.
[e] Statistically significant compared with group MR1.
[f] Statistically significant compared with group VIR1.

There were significant differences in the results from the quantitative bacterial graft cultures when the data obtained from groups MS2 to -4, MR2 to -4, and VIR2 to -4 were compared with those obtained from the respective untreated control groups ($P < 0.001$). The difference remained significant when the comparison was carried out between groups VIR1 and VIR3 ($P = 0.021$), in spite of the high bacterial numbers obtained by the quantitative cultures from group VIR3.

DISCUSSION

The success of surgical prophylaxis in the prevention of graft infections is dependent on the pharmacokinetics of antibiotic tissue penetrance with maintenance of adequate tissue levels for the duration of the vascular surgical procedure and on the in vivo efficacy of the drug against the etiologic agent. Actually, clinical experience with polymer-related staphylococcal infections clearly shows that host defense mechanisms and antibacterial chemotherapy are often unable to prevent and cure these infections, despite the use of antibiotics with proven in vitro activity (23). Moreover, the recent emergence of glycopeptide resistance in MR staphylococcal isolates heightens concern about the need for other antibiotics in prophylactic regimens (13, 16, 20). In the case of vascular surgery, several antimicrobials have been proposed as adjunctive prophylaxis after binding in high concentrations to prosthetic grafts (1, 8, 18, 22, 24). Nevertheless, the selection of appropriate antibiotics for this adjunctive prophylaxis is unfortunately hampered by the fact that most agents are already used in systemic treatments and resistant strains might have been previously selected for. Mupirocin, developed for topical use exclusively, has excellent in vivo and in vitro activity against a wide range of staphylococcal isolates: for this reason, in this study we investigated the in vivo efficacy of mupirocin, spontaneously bound to collagen-sealed

Dacron grafts, in preventing *S. epidermidis* infection of the graft in a rat model.

Mupirocin demonstrated high in vitro activity against the three staphylococcal strains tested, with slight reduction of its activity against the MR and VIR strains, and a good in vivo efficacy, with significant reduction of bacterial growth, when tested as an agent applied to the Dacron grafts. Similar in vivo results were observed when vancomycin was administered as perioperative antibiotic prophylaxis, with the exception of those obtained from group VIR3. Furthermore, a positive interaction was observed between mupirocin and vancomycin when the two agents were tested together (groups MS4, MR4, and VIR4), with complete suppression of bacterial growth.

Taken together, the results of this study demonstrate that the use of mupirocin, as an antimicrobial agent applied to a Dacron graft can result in significant staphylococcal growth inhibition even if multiresistant organisms are topically inoculated on the Dacron prostheses. Furthermore, it is important to note that mupirocin did not show toxicity. None of the animals included in the MS2, MR2, and VIR2 groups died or had clinical evidence of drug-related adverse effects, such as local signs of perigraft inflammation, anorexia, vomiting, diarrhea, or behavioral alterations.

The widespread use of several antimicrobial agents in both therapeutic and prophylactic regimens has resulted in a dramatic increase in the prevalence of multiresistant organisms. However, the antistaphylococcal in vitro activity and the prophylactic in vivo efficacy demonstrated in the present study make substances such as mupirocin potentially useful for antimicrobial perioperative chemoprophylaxis. Future research is needed to elucidate its utility in surgical practice.

REFERENCES

1. **Bandyk, D. F., E. V. Kinney, T. I. Riefsnyder, H. Kelly, and J. B. Towne.** 1993. Treatment of bacteria-biofilm graft infection by in situ replacement in normal and immune-deficient states. J. Vasc. Surg. **18:**398–405.
2. **Bergamini, T. M., R. A. Corpus, Jr., K. R. Brittian, J. C. Peyton, and W. G. Cheadle.** 1994. The natural history of bacterial biofilm graft infection. J. Surg. Res. **56:**393–396.
3. **Bergamini, T. M., R. A. Corpus, Jr., T. M. McCurry, J. C. Peyton, K. R. Brittian, and W. G. Cheadle.** 1995. Immunosuppression augments growth of graft-adherent *Staphylococcus epidermidis*. Arch. Surg. **130:**1345–1350.
4. **Bergamini, T. M., J. C., Peyton, and W. G. Cheadle.** 1992. Prophylactic antibiotics prevent bacterial biofilm graft infection. J. Surg. Res. **52:**101–105.
5. **Chervu, A., W. S. Moore, H. A. Gelabert, M. D. Colburn, and M. Chvapil.** 1991. Prevention of graft infection by use of prostheses bonded with a rifampin collagen release system. J. Vasc. Surg. **14:**521–524.
6. **Citak, M. S., J. I. Cué, J. C. Peyton, and M. A. Malangoni.** 1992. The critical relationship of antibiotic dose and bacterial contamination in experimental infection. J. Surg. Res. **52:**127–130.
7. **Cookson, B. D.** 1998. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. J. Antimicrob. Chemother. **41:**1–8.
8. **Dhir, A., A. J. Miller, G. C. Landon, I. I. Raad, and D. M. Musher.** 1994. Vancomycin penetration into biofilm covering infected prostheses and effect on bacteria. J. Infect. Dis. **170:**720–723.
9. **Finlay, J. E., L. A. Miller, and J. A. Poupard.** 1997. Interpretative criteria for testing susceptibility of staphylococci to mupirocin. Antimicrob. Agents Chemother. **41:**1137–1139.
10. **Fuller, A. T., G. Mellows, M. Woolford, G. T. Banks, K. D. Barrow, and E. B. Chain.** 1971. Pseudomonic acid: an antibiotic produced by *Pseudomonas fluorescens*. Nature **234:**416–417.
11. **Goeau-Brissoniere, O., C. Leport, F. Bacourt, C. Lebrault, R. Comte, and J. C. Pechere.** 1991. Prevention of vascular graft infection by rifampin bonding to a gelatin-sealed Dacron graft. Ann. Vasc. Surg. **5:**408–412.
12. **Harvey, R. A., D. V. Alcid, and R. S. Greco.** 1982. Antibiotic bonding to polytetrafluoroethylene with tridodecylmethylammonium chloride. Surgery **92:**504–512.
13. **McManus, A. T., C. W. Goodwin, and B. A. Pruitt, Jr.** 1998. Observations on the risk of resistance with the extended use of vancomycin. Arch. Surg. **133:**1207–1211.
14. **National Committee for Clinical Laboratory Standards.** 1993. Approved standards M7-A3. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **Osada, T., K. Yamamura, K. Fujimoto, K. Mizuno, T. Sakurai, M. Ohta, and T. Nabeshima.** 1999. Prophylaxis of local vascular graft infection with levofloxacin incorporated into albumin-sealed dacron graft. Microbiol. Immunol. **43:**317–321.
16. **Sanyal, D., A. P. Johnson, R. C. George, B. D. Cookson, and A. J. Williams.** 1991. Peritonitis due to vancomycin-resistant *Staphylococcus epidermidis*. Lancet **337:**54.
17. **Sardelic, F., P. Y. Ao, D. A. Taylor, and J. P. Fletcher.** 1996. Prophylaxis against *Staphylococcus epidermidis* vascular graft infection with rifampicin-soaked, gelatin- sealed Dacron. Cardiovasc. Surg. **4:**389–392.
18. **Sardelic, F., and J. P. Fletcher.** 1995. Rifampicin impregnated Dacron grafts: no development of rifampicin resistance in an animal model. Eur. J. Vasc. Endovasc. Surg. **9:**314–318.
19. **Schmitz F. J., E. Lindenlauf, B. Hofman, A. C. Fluit, J. Verhoef, H. P. Heinz, and M. E. Jones.** 1998. The prevalence of low- and high-level mupirocin resistance in staphylococci from 19 European hospitals. J. Antimicrob. Chemother. **42:**489–495.
20. **Schwalbe, R. S., J. T. Stapleton, and P. H. Gilligan.** 1987. Emergence of vancomycin resistance in coagulase-negative staphylococci. N. Engl. J. Med. **316:**927–931.
21. **Sutherland, R., R. J. Boon, K. E. Griffin, P. J. Masters, B. Slocombe, and A. R. White.** 1985. Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. Antimicrob. Agents Chemother. **27:**495–498.
22. **Vicaretti, M., W. J. Hawthorne, P. Y. Ao, and J. P. Fletcher.** 1998. An increased concentration of rifampicin bonded to gelatin-sealed Dacron reduces the incidence of subsequent graft infections following a staphylococcal challenge. Cardiovasc. Surg. **6:**268–273.
23. **Von Eiff, C., and G. P. Heilmann.** 1999. New aspects in the molecular basis of polymer-associated infections due to staphylococci. Eur. J. Clin. Microbiol. Infect. Dis. **18:**843–846.
24. **Yamamura, K., T. Sakurai, K. Yano, T. Osada, and T. Nabeshima.** 1995. Prevention of vascular graft infection by sisomicin incorporated into fibrin glue. Microbiol. Immunol. **39:**895–896.