Temporin A Soaking in Combination with Intraperitoneal Linezolid Prevents Vascular Graft Infection in a Subcutaneous Rat Pouch Model of Infection with *Staphylococcus epidermidis* with Intermediate Resistance to Glycopeptides

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The efficacy of linezolid and temporin A in the prevention of prosthetic graft infection due to methicillin-resistant *Staphylococcus epidermidis* with intermediate resistance to glycopeptides was investigated in a subcutaneous rat pouch model. Linezolid and temporin A, alone or combined, greatly reduced the bacterial numbers compared to the effect with control drugs.

In recent years the effectiveness of new antimicrobial compounds to treat and prevent vascular graft infections has been evaluated in different experimental models (1, 3, 7, 14, 17). In fact, vascular graft infections remain a major surgical challenge, because prevention of risk factors and antibiotic therapy can reduce but not eradicate them (4, 8). Moreover, the continued isolation of methicillin-resistant staphylococci and the increase in the numbers of infections caused by the emergence of glycopeptide-resistant cocci among nosocomial strains have prompted the development of compounds specifically directed at the treatment of these pathogens (9, 13, 15, 16).

Few new agents have demonstrated significant in vitro activities against antibiotic-resistant staphylococci. One of these compounds is the oxazolidinone linezolid. Oxazolidinones are a new class of antimicrobials with a unique mechanism of action. They inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit; this binding prevents formation of a functional initiation complex in bacterial translation systems. Linezolid has been approved by the Food and Drug Administration for use in treating infections caused by gram-positive organisms, including multidrug-resistant isolates of staphylococci, streptococci, and enterococci (5, 12).

Temporins are a family of linear 10- to 13-residue-long peptides with a net positive charge and an amidated C-terminal antimicrobial peptide. Initially, they were isolated from the skin of the European red frog, *Rana temporaria* (17, 18). They showed activity against clinically important gram-positive cocci, including multidrug-resistant staphylococci and vancomycin-resistant *Enterococcus faecium* (3, 17, 18). Temporin A is a basic, highly hydrophobic, antimicrobial peptide amide (FLPLIGRVLSGIL-NH₂) that, like the other temporins, is active against clinically important antibiotic-resistant gram-positive cocci (17-19).

In this study we used one strain of *Staphylococcus epidermidis* with intermediate resistance to glycopeptides (GISE) to investigate the in vitro activities of temporin A and linezolid and their in vivo efficacies in preventing prosthetic infection in a rat model.

**Organisms.** A clinical isolate of methicillin-resistant *S. epidermidis* with intermediate resistance to glycopeptides (GISE), obtained from a hospitalized patient with a surgical wound infection, was studied. *S. epidermidis* ATCC 12228 was used as a control strain. In vitro investigations were performed with a laboratory strain of *S. epidermidis* (ATCC 12228) and a quality control strain in the in vitro investigations.

**Drugs.** Temporin A was synthesized manually by the solid-phase method with the Fmoc (9-fluorenylethoxycarbonyl)-Bu' procedure (Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland). Linezolid was obtained from Pharmacia & Upjohn, Kalamazoo, Mich. Vancomycin was obtained from Sigma-Aldrich, Milan, Italy. Teicoplanin was obtained from Aventis Pharma, Milan, Italy. Powders were diluted in accordance with manufacturers’ recommendations.

**Soaking Dacron in temporin A solution.** The amount of temporin A that soaked into the Dacron was estimated using UV spectroscopy. First, a 1-cm² collagen-sealed Dacron graft (Albograft; Sorin Biomedica Cardio, S.p.A., Saluggia [VC], Italy) was washed with distilled water for 10 min. Afterwards Dacron was allowed to be soaked with Fmoc-temporin A (10 mg/liter) for 20 min at room temperature. The absorption spectrum of the solution of Fmoc-temporin A was measured at λ 266 nm with a UV-visible-light spectrometer (Lambda 40P; Perkin-Elmer, Norwalk, Conn.) before soaking. Immediately after soaking Dacron was taken out of the solution, and the absorption spectrum of the solution of Fmoc-temporin A was measured again. The amount of Fmoc-temporin A soaked into the Dacron was estimated based on differences in absorption before and after the above-mentioned procedure.

**Antimicrobial susceptibility testing.** Antimicrobial susceptibilities were determined by broth microdilution as described by the National Committee for Clinical Laboratory Standards...
in tubes containing 10 ml of phosphate-buffered saline solution, and sonicated for 2 min to remove the adherent bacteria from the grafts. Quantification 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 GISE 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. MICs are presented as the modes of three separate experiments. All in vivo data were merged and referred to all 20 animals from each pair of groups. Quantitative culture results regarding the in vivo experiments are presented as means ± standard deviations of the means; for results below the lower limit of detection the value considered was 10 CFU. Data were analyzed by one-way analysis of variance; post-hoc multiple comparisons were performed by applying Bonferroni’s criterion. Significance was accepted when the P value was equal to or less than the Bonferroni critical value 0.001786 (0.05/28, where 28 is the number of the pairs of compared groups).

Soaking Dacron in temporin A solution. The experiments showed that, when 1 cm² of Dacron was soaked in a solution of 10 mg of temporin A/liter, 37 μg of temporin A remained on the Dacron.

In vitro susceptibility studies. According to the broth microdilution method recommended by the NCCLS, vancomycin exhibited MICs of 0.25 and 8 μg/ml for S. epidermidis ATCC 12228 and GISE, respectively, while teicoplanin exhibited MICs of 0.25 and 16 mg/liter, respectively. The two strains were similarly susceptible to linezolid, which showed MICs of 1.00 and 2.00 μg/ml for S. epidermidis ATCC 12228 and the GISE strain, respectively. Finally, temporin A showed MICs of 2 μg/ml for both strains. The differential pattern of susceptibility was confirmed by the disk diffusion test: S. epidermidis ATCC 12228 showed zone sizes of 15 and 18 mm for teicoplanin and vancomycin, respectively, while the intermediate resistance of the GISE strain to the glycopeptides was demonstrated by zone sizes of 11 mm for both vancomycin and teicoplanin. In the combination studies synergy was never observed, with the exception of the combinations between temporin A and linezolid. Actually, the strains GISE and ATCC 12228 produced FIC indexes of 0.312 and 0.187, respectively, when temporin A was combined with linezolid, while the other experiments with vancomycin and teicoplanin gave values between 0.750 and 2.0 (data not shown).

In vivo studies. None of the animals included in the uncontaminated control group had microbiological evidence of graft infection. In contrast, all 20 rats included in the untreated control group demonstrated evidence of graft infection, with quantitative culture results showing 6.9 × 10⁶ ± 2.1 × 10⁶ CFU/ml. Rats that received linezolid showed the lowest bacterial numbers (3.8 × 10⁶ ± 0.9 × 10⁶ CFU/ml). Temporin A showed also a good activity with bacterial numbers of 3.4 × 10⁵ ± 7.9 × 10⁵ CFU/ml. In contrast, for rats that received teicoplanin or vancomycin the quantitative graft cultures demonstrated 8.2 × 10⁴ ± 1.5 × 10⁵ or 6.8 × 10⁴ ± 1.3 × 10⁵ CFU/ml, respectively. All combinations showed efficacies higher than that of each single compound. In fact temporin A plus vancomycin or teicoplanin showed bacterial numbers of
The GISE clinical strain.

only temporin A and linezolid exhibited high activity against staphylococcal infections. In particular, the administration of a glycopeptides, temporin A and linezolid appear to administered alone. to inhibit completely the growth of the resistant strains when treatment, though it is important that no compound was able received topical temporin A or intraperitoneal linezolid treat-
icant differences were observed between the groups that re-
activity of temporin A and linezolid. In fact, statistically signif-
drug-related adverse effects, such as local signs of perigraft animals included in any group died or had clinical evidence of

10^2 CFU/ml per graft while the combinations between tem-
porin A and linezolid exerted the strongest antistaphylococcal efficacies (Table 1). Overall, all comparisons showed significant differences ($P < 0.0001$), except for vancomycin versus teicop-
plalin ($P = 0.0031$), linezolid versus temporin A plus teico-
plalin ($P = 0.0019$), and temporin A plus vancomycin versus temporin A plus teicoplanin ($P = 0.0251$). 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, and behavioral alterations.

The in vitro results of this study show that temporin A, linezolid, vancomycin, and teicoplanin had similar activities against the control strain *S. epidermidis* ATCC 12228 while only temporin A and linezolid exhibited high activity against the GISE clinical strain.

The in vitro results confirmed the strong antistaphylococcal activity of temporin A and linezolid. In fact, statistically significant differences were observed between the groups that received topical temporin A or intraperitoneal linezolid treatment and those that received vancomycin or teicoplanin treatment, though it is important that no compound was able to inhibit completely the growth of the resistant strains when administered alone.

Based on the observations in the present study concerning the high in vitro activity and the prophylactic in vivo efficacy shown against a staphylococcal strain with decreased susceptibility to the glycopeptides, temporin A and linezolid appear to be promising compounds for preventing multidrug-resistant staphylococcal infections. In particular, the administration of a topical peptide such as temporin A combined with a parenteral antibiotic with a strong antistaphylococcal activity may become an important future consideration for chemoprophylaxis in vascular surgery.

### REFERENCES


