Suppression of Drug-Resistant Staphylococcal Infections by the Quorum-Sensing Inhibitor RNAIII-Inhibiting Peptide

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Staphylococcus aureus and S. epidermidis are major causes of infection related to biofilm formed on indwelling medical devices. Such infections are common causes of morbidity and mortality and, because of biofilm resistance to antibiotics, are difficult to treat. The RNAIII-inhibiting peptide (RIP) (YSPWTFN-NH2) inhibits the pathogenesis of staphylococci by disrupting bacterial cell-cell communication (known as “quorum sensing”). Using a vascular-graft rat model, we show that RIP, applied locally and systemically, can completely inhibit drug-resistant S. aureus and S. epidermidis biofilms. The present study provides the first direct demonstration that interfering with cell-cell communication by use of a quorum-sensing inhibitor can eliminate medical device–associated staphylococcal infections. We suggest that medical devices could be coated with RIP to prevent infections, including those by antibiotic-resistant staphylococcal strains.

Pathogenic mechanisms of infection related to biofilm resistance to antibiotics, are difficult to treat because of biofilm resistance to antibiotics, are difficult to treat. The RNAIII-inhibiting peptide (RIP) (YSPWTFN-NH2) inhibits the pathogenesis of staphylococci by disrupting bacterial cell-cell communication (known as “quorum sensing”). Using a vascular-graft rat model, we show that RIP, applied locally and systemically, can completely inhibit drug-resistant S. aureus and S. epidermidis biofilms. The present study provides the first direct demonstration that interfering with cell-cell communication by use of a quorum-sensing inhibitor can eliminate medical device–associated staphylococcal infections. We suggest that medical devices could be coated with RIP to prevent infections, including those by antibiotic-resistant staphylococcal strains.
incubated at 37°C for 48 h. The organisms were quantitated by counting the number of colony-forming units per plate; the threshold of detection was ∼10 cfu/mL [10, 11]. No residual vegetation from explanted grafts was observed. Quantitative culture results are presented as mean ± SD; comparison of the results was by analysis of variance performed on the log-transformed data. Significance was defined as \( P = .05 \). The present study was approved by the Animal Research Ethics Committee of the National Institute for Research and Therapy in the Elderly, University of Ancona (Ancona, Italy).

Staphylococcal strains used for the in vivo studies were as follows: MSSA ATCC 29213, MRSA ATCC 43300, GISA clinical isolate AG1 (University of Ancona; Ancona, Italy), MSSE ATCC 12228, and MRSE and GISE AG2 clinical isolates (University of Ancona; Ancona, Italy). The clinical isolates’ antimicrobial susceptibilities to methicillin, vancomycin, and teicoplanin were determined by the broth-microdilution method described by the National Committee for Clinical Laboratory Standards (NCCLS); in addition, the strains were tested for susceptibility to vancomycin and teicoplanin by the NCCLS reference disk–diffusion method, with 30-mg vancomycin and teicoplanin disks [10, 11]. The following strains were used as controls for in vitro susceptibility tests: MSSE strain ATCC 12228, MSSA strain ATCC 29213, and MRSA strain ATCC 38591.

Results and discussion. The results presented in table 1 indicate that all rats in the infected control groups (i.e., rats that had received either saline-soaked grafts or inactive RIP analogue–soaked grafts) demonstrated evidence of graft infection, with culture results showing either \( S. aureus \) or \( S. epidermidis \) at concentrations of ∼10^2–10^7 cfu/mL. In contrast, all rats in the RIP-soaked–graft group (local treatment) and the RIP-injected group (parenteral treatment) experienced reduced bacterial load, with culture results showing ∼10^2–10^4 cfu/mL. All rats in the RIP-soaked–graft group that were also administered RIP intraperitoneally (local-plus-parenteral treatment) demonstrated no evidence of graft infection and had no quantifiable culture results, indicating 100% protection. It is noteworthy that none of the rats showed clinical evidence of drug-related adverse effects. Inhibitory effects of RIP were specific, because rats that received grafts coated with the inactive RIP analogue demonstrated evidence of graft infection, which was similar to what was seen in untreated rats.

We suggest that RIP could be used to coat medical devices to prevent bacterial colonization and consequent infection. In addition, RIP has been shown to function synergistically with antibiotics and thus to recommend itself in combination therapy [10–12]. To date, RIP has been shown to inhibit any strain or species of staphylococci tested (MSSA, MRSA, GISA, VISA, MSSE, MRSE, GISE, and VISE) [10–12] and no resistance to RIP has been observed (e.g., when cells [\( S. aureus 8325-4 \)] were grown in vitro in the presence of RIP for 5 consecutive days, they did not form a biofilm [8]). This apparent lack of resistance to or specificity for any strain may be because RIP exerts its effect via TRAP [9], which is a protein that is highly conserved among staphylococci [13].

In the present study, it has been shown that RIP, applied locally and systemically, completely inhibits graft-associated infection; ours is the first direct demonstration that interfering with cell–cell communication by use of a quorum-sensing inhibitor can prevent medical device–associated staphylococcal infections. Our data, together with the recent identification of new compounds that act on quorum-sensing mechanisms in other bacteria [14], support the possibility that a new class of antibacterial drugs can be developed as a viable alternative to current antibiotics.

### References


### Table 1. Prevention of infection, in rats, by strains of \( \text{Staphylococcus epidermidis} \) and \( S. aureus \), by use of RNAIII-inhibiting peptide (RIP)–coated Dacron grafts, by use of RIP administered parenterally, and by use of both in combination.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>( S. epidermidis ) strain</th>
<th>( S. aureus ) strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methicillin susceptible</td>
<td>Methicillin resistant</td>
</tr>
<tr>
<td>Control</td>
<td>710 ± 150</td>
<td>680 ± 110</td>
</tr>
<tr>
<td>Untreated</td>
<td>540 ± 80</td>
<td>708 ± 210</td>
</tr>
<tr>
<td>Inactive RIP analogue</td>
<td>0.08 ± 0.024</td>
<td>0.052 ± 0.017</td>
</tr>
<tr>
<td>RIP</td>
<td>0.067 ± 0.019</td>
<td>0.045 ± 0.009</td>
</tr>
<tr>
<td>Local only (^a)</td>
<td>0.08 ± 0.023</td>
<td>0.052 ± 0.017</td>
</tr>
<tr>
<td>Parenteral only (^a)</td>
<td>0.055 ± 0.019</td>
<td>0.045 ± 0.009</td>
</tr>
<tr>
<td>Local plus parenteral (^a)</td>
<td>0.067 ± 0.019</td>
<td>0.045 ± 0.009</td>
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\(^a\) \( P < .05 \) compared with both the untreated control group and the inactive-RIP-analogue control group; threshold of detection, 10 cfu/mL.