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

Giorgio Dell’Acqua,1 Andrea Giacometti,4 Oscar Cirioni,1 Roberto Ghiselli,1 Vittorio Saba,1 Giorgio Scalise,1 Yael Gov,3 and Naomi Balaban2,5

1Balapharm International, Grafton, and 2Department of Biomedical Sciences, Division of Infectious Diseases, School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts; 3Department of General Surgery, National Institute for Research and Therapy in the Elderly, and 4Institute of Infectious Diseases and Public Health, Polytechnic University of Marche, Ancona, Italy; 5Department of Human Microbiology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

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) (YSPWTNF-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 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.

Infection by Staphylococcus aureus and S. epidermidis is often associated with the use of implantable medical devices [1]. Such infections are common causes of morbidity and mortality and, because of biofilm resistance to antibiotics, are difficult to treat [2]. Pathogenic mechanisms involved are the ability to adhere to either polymer surfaces or host cells and the production of a biofilm by toxin-producing staphylococci [3]. In staphylococci, pathogenesis is achieved by a complex regulatory process termed “quorum sensing,” involving cell-cell communication through the secretion of chemical signals [4, 5].

Biofilm formation and toxin production can be inhibited by the quorum-sensing inhibitor RNAIII-inhibiting peptide (RIP) [5–7]. RIP is a heptapeptide (YSPWTNF-NH2) that inhibits the adhesion of S. aureus and S. epidermidis to both epithelial cells and plastic polymers and thus prevents biofilm from forming [8]. RIP does not kill bacteria; rather, it disrupts bacterial cell-cell communication by inhibiting the phosphorylation of RIP’s target protein, called “target of RNAIII-activating protein” (TRAP) [9].

Materials and methods. To test whether the disruption of bacterial cell-cell communication by RIP is sufficient to eliminate medical device–associated infections by staphylococci, a vascular-graft rat model was used. For the vascular-graft rat model, a subcutaneous pocket was created by making a 1.5-cm incision on each side of the median line on anesthetized male Wistar rats (n = 15; 250–300 g) (University of Ancona, Animal Facility, National Institute for Research and Therapy in the Elderly); then 1-cm² sterile collagen-sealed Dacron grafts (Albograft) were aseptically implanted into the pockets. Immediately before implantation, the grafts were soaked for 20 min in either sterile saline only, sterile saline with RIP (YSPWTNF-NH2; Neosystem) at a concentration of 20 μg/mL, or sterile saline with an inactive RIP analogue (YKPETNF-NH2; Neosystem) at a concentration of 20 μg/mL. The pockets were closed by use of skin clips, and then 1 mL of saline, either with or without 2 × 10⁵ exponentially growing S. aureus or S. epidermidis, was inoculated onto the graft surface. Selected strains were as follows: methicillin-susceptible S. epidermidis (MSSE) and methicillin-susceptible S. aureus (MSSA), methicillin-resistant S. epidermidis (MRSE) and methicillin-resistant S. aureus (MRSA), and glycopeptide–intermediately sensitive S. epidermidis (GISE) and glycopeptide–intermediately sensitive S. aureus (GISA). As a model for parenteral surgical prophylaxis, some of the rats were also injected intraperitoneally with a single dose (10 mg/kg) of RIP, administered 30 min before the implantation of the graft. Grafts were explanted 7 days later and were placed in sterile tubes, were washed in sterile saline, were placed in tubes containing 10 mL of phosphate-buffered saline, and were sonicated for 2 min, to remove the adherent bacteria from the grafts. Quantitation of viable bacteria was performed by culturing serial 10-fold dilutions of the bacterial suspension on blood agar plates. All plates were
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.

Table 1. Prevention of infection, in rats, by strains of 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>Bacterial concentration, mean ± SD, 10$^4$ cfu/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>710 ± 150 to 680 ± 110</td>
</tr>
<tr>
<td>Inactive RIP analogue</td>
<td>540 ± 80 to 708 ± 210</td>
</tr>
<tr>
<td>RIP</td>
<td>0.08 ± 0.024 to 0.087 ± 0.023</td>
</tr>
<tr>
<td>Parentera onlya</td>
<td>0.067 ± 0.019 to 0.052 ± 0.017</td>
</tr>
<tr>
<td>Local plus parenterala</td>
<td>0 to 0</td>
</tr>
</tbody>
</table>

* $P<.05$ compared with both the untreated control group and the inactive-RIP-analogue control group; threshold of detection, 10 cfu/mL.

References


