RNAIII-Inhibiting Peptide Affects Biofilm Formation in a Rat Model of Staphylococcal Ureteral Stent Infection

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Ureteral stents coated with the quorum-sensing inhibitor RNAIII-inhibiting peptide (RIP) were implanted in rat bladders and shown to suppress Staphylococcus aureus formation on the stent and in urine and was especially effective when combined with teicoplanin. Coating ureteral stents with RIP thus increases the efficacy of teicoplanin in preventing ureteral stent-associated staphylococcal infections.

Bacterial biofilms that form on ureteral stents within hours of stent placement can result in infection (14, 16). Biofilms are up to 1,000-fold more tolerant of antibiotics (10). Resistance is multifactorial and includes cell-to-cell communication (quorum-sensing) systems (11, 12). Quorum sensing in staphylococci regulates biofilm architecture and toxin production, both of which are important for disease progression (3, 4).

RNAIII inhibiting peptide (RIP; YSPWTNF-NH2) inhibits staphylococcal biofilm formation and toxin production (5, 13). In the rat model, RIP has been shown to prevent biofilm formation and staphylococcal infections, whether it was applied to antibiotic-sensitive or antibiotic-resistant strains (6, 8). Its mechanism of action is different from that of common antibiotics, since instead of killing the bacteria, it inhibits cell-to-cell communication, leading to the prevention of cell adhesion and virulence in vivo.

Staphylococcus aureus is one of the common colonizers of urinary stents (14, 15), especially in patients with an indwelling urinary catheter or those who are immunocompromised. The aim of the present study was to assess the efficacy of RIP in the prevention of staphylococcal ureteral stent infections.

The S. aureus strain Smith diffuse (SD), a slime-producing strain with exopolysaccharides which are antigenically identical to many clinical S. aureus strains, was tested.

The MIC was determined according to the procedures outlined by the CLSI (9).

Adult female Wistar rats (weight range, 180 to 250 g) (n = 5) were used. The study included a control group (C0) without a bacterial challenge to evaluate the sterility of the surgical procedure; a challenged control group (C1) without antibiotic prophylaxis; and three challenged groups: (i) a group that received 10 mg/kg of body weight teicoplanin intraperitoneally immediately after stent implantation; (ii) a group that received RIP-coated stents, in which 0.2-cm² sterile stents (BiosoftDuo, Taborgues-Mentor, France) were incubated in 1 μg/ml RIP (Neo-system) solution for 30 min immediately before implantation; and (iii) a group that received stents coated with intraperitoneal teicoplanin (Aventis) that were incubated with RIP at the above-cited concentration. Experiments were performed in duplicate. The dose of teicoplanin was equivalent to that usually used in the human clinical setting, while the RIP dose was chosen on the basis of previous reports (1, 2). For statistical analysis, the data were pooled and refer to all 10 animals from each pair of groups.

The rats were anesthetized by an intramuscular injection of ketamine and xylazine (30 mg/kg and 8 mg/kg, respectively), the hair was shaved, and the skin was cleansed with a 10% povidone-iodine solution. The bladder was exposed through a suprapubic incision and opened at the dome (7). After cystotomy, stents were inserted into the bladder. Before implantation was performed, some of the ureteral stent segments were impregnated with RIP as described above. The bladder was sutured with 000 surgical silk. After the surgical intervention, a saline solution (1 ml) containing 2 × 10⁷ CFU/ml S. aureus (SD) (4, 17) was inoculated into the bladder, using a tuberculin syringe. Some of the animals received teicoplanin intraperitoneally immediately after stent implantation. The animals were returned to individual cages and were thoroughly examined daily. Twenty-four hours after stent placement, urine cultures were performed with a transvesicular sample taken by an insulin syringe to verify sterility or infection. Stents were explanted at day 5 following implantation. Enumeration 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. The organisms were quantitated by counting the number of CFU per plate (1). The limit of detection was approximately 10 CFU/ml. Culture results were presented as means ± standard deviations, and

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TABLE 1. Activity of RIP and teicoplanin against the S. aureus strain SD in a rat model of ureteral stent infection

<table>
<thead>
<tr>
<th>Test group</th>
<th>Stent-bonded drug</th>
<th>Intraperitoneal drug</th>
<th>Quantitative bacterial count (CFU/ml) ± SDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C0)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control (C1)</td>
<td></td>
<td></td>
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<tr>
<td>Group 1f</td>
<td></td>
<td>Teicoplanin</td>
<td></td>
</tr>
<tr>
<td>Group 2f</td>
<td>RIP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3f</td>
<td>RIP</td>
<td>Teicoplanin</td>
<td></td>
</tr>
</tbody>
</table>

a Infected with S. aureus SD.

b The ureteral stent segments were impregnated with 1 μg/ml of RIP.

c Each rat received 10 mg/kg teicoplanin intraperitoneally.

d The values shown are means ± standard deviations (SD). The limit of detection for the method was ≤10 CFU/ml.

e P < 0.05 compared with the untreated control group.

f P < 0.001 compared with the untreated control group; P < 0.05 compared with the single-agent-treated groups.

statistical comparisons between groups were made using analysis of variance with the log-transformed data with the Tukey-Kramer honestly significant difference test. Significance was accepted when the P value was ≤0.05.

Toxicity was evaluated on the basis of the presence of any drug-related adverse effects, i.e., signs of local inflammation, weight loss, diarrehea, fever, and behavioral alterations.

In vitro teicoplanin exhibited a MIC of 1.00 μg/ml, while as expected, RIP did not inhibit growth of the staphylococcal strain.

None of the animals included in the uncontaminated control group had microbiological evidence of stent infection. By contrast, all rats included in the challenged but untreated control group demonstrated evidence of infection, with quantitative culture results showing 6.6 × 10⁶ ± 1.9 × 10⁶ CFU/ml. Rats that received intraperitoneal teicoplanin showed bacterial counts of 3.8 × 10⁶ ± 0.8 × 10⁶ CFU/ml. Animals that had RIP-coated stents showed bacterial counts of 6.7 × 10⁴ ± 1.4 × 10⁴ (P < 0.05). Animals that had RIP-coated stents and were treated with teicoplanin had no bacterial counts (P < 0.001), indicating that RIP combined with teicoplanin showed efficacies that were higher than that of each single compound alone.

Urine cultures confirmed these microbiological data and were negative both for the uncontaminated group and the combined treatment group. The single-agent-treated groups were positive, with a bacterial count of 10⁷ CFU/ml both for the RIP- and for the teicoplanin-treated group (Table 1). None of the animals included in any group died or had any clinical evidence of drug-related adverse effects.

Data presented here show that the use of a RIP-impregnated stent with or without a combination of conventionally used antibiotics caused significantly lower bacterial loads. Similar to teicoplanin, RIP caused a significant reduction in bacterial load on the ureteral stent tissue compared with that from control untreated animals, and when teicoplanin and RIP were combined, no evidence of bacteria was detected on the stent or in the urine. In summary, not only did RIP by itself reduce bacterial load, it also enhanced the effect of teicoplanin. Our data showed the presence of large errors for bacteriological counts in each of the treated animal group. We cannot affirm that some bladders have been cured and others have not: in the clinical setting, the positivity of a urine culture is demonstrated by the presence of bacterial counts of >10⁵ CFU/ml. In our models, these values have been detected only in the control group, C₁. In treated groups 1 and 2, values of about 10⁴ CFU/ml demonstrated the efficacy of the agents but also the persistence of organisms.

RIP has been shown to be effective against every staphylococcal strain tested so far, including methicillin- and glycopeptide-intermediate-resistant S. aureus and Staphylococcus epidermidis (2, 6), suggesting that RIP-impregnated ureteral stents may benefit from lower rates of infections.

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The study was approved by the animal research ethics committee of the I.N.R.C.A.–I.R.R.C.S.

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