RNAIII inhibiting peptide affects biofilm formation in a rat model of staphylococcal ureteral stent infection

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ABSTRACT

Ureteral stents coated with quorum sensing inhibitor RIP were implanted in rats bladder and shown to suppress *Staphylococcus aureus* 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.

NOTE

Bacterial biofilms that form on ureteral stents within hours of placement can result in infection (15,18). Biofilms are up to 1000 fold more tolerant to antibiotics (10,12). Resistance is multifactorial also including cell-to-cell communication systems (quorum sensing, QS) (11,13). QS in staphylococci regulate biofilm architecture and toxin production, both important for disease progression (3,4).

RNAIII inhibiting peptide (RIP, YSPWTNF-NH2) inhibits staphylococcal biofilm formation and toxin production (5,14). RIP has been shown in rat models to prevent biofilm formation and staphylococcal infections, whether antibiotic sensitive or resistant strains (6,8). Its mechanism of action is different from common antibiotics, since instead of killing the bacteria, it inhibits cell-cell communication, leading to prevention of their adhesion and virulence in vivo.

*S. aureus* is one of the common colonizers of urinary stents (15,16), especially for patients with an indwelling urinary catheter or those who are immunocompromised (17). The aim of the present study was to assess the efficacy of RIP in the prevention of staphylococcal ureteral stent infections.

*S. aureus* strain Smith diffuse (SD), a slime producing strain with exopolysaccharides which are antigenically identical to many clinical *S. aureus* strains, was tested. Minimal inhibitory concentration (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. Study included a control group (C₀) without bacterial challenge to evaluate the sterility of the surgical procedure, a challenged control group (C₁) without antibiotic prophylaxis, and three challenged groups that received a) 10 mg/kg teicoplanin intraperitoneally, immediately after stent implantation, b) RIP-coated stents where 0.2-cm² sterile stents (Biosoft®Duo, Porges-Mentor, France) were incubated in 1 mg/L RIP (Neosystem) solution for 30 min immediately before implantation; and c) intraperitoneal teicoplanin (Aventis) plus RIP-coated stent at the above concentrations. Experiments were performed in duplicate. The dosage of teicoplanin resembled that usually used in the human clinical setting while RIP dosage was chosen on the basis of previous reports (1,2). For statistical analysis, the data were pooled and referred 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 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, 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) (4,19) 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 thoroughly examined daily. Twenty-four hours after stent placement, urine cultures were performed through a transvesical sample taken by an insulin syringe, to verify the 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 mean ± S.D. of the mean and statistical comparisons between groups were made using analysis of variance (ANOVA) on the log-
transformed data with Tukey-Kramer Honestly Significant Difference Test. Significance was accepted when the $P$ value was $\leq 0.05$.

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

In vitro teicoplanin exhibited MIC of 1.00 mg/l 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. Differently, all rats included in the challenged but untreated control group demonstrated evidence of infection, with quantitative culture results showing $6.6 \times 10^6 \pm 1.9 \times 10^6$ CFU/ml. Rats that received intraperitoneal teicoplanin showed bacterial counts of $3.8 \times 10^3 \pm 0.8 \times 10^3$ CFU/ml. Animals that had RIP-coated stents showed bacterial counts of $6.7 \times 10^4 \pm 1.4 \times 10^3$ (P<0.05). Animals that had RIP-coated stents and treated with teicoplanin had no bacterial counts (P<0.001), indicating that RIP combined with teicoplanin showed efficacies higher than that of each single compound alone. Urine cultures confirmed these microbiological data and were negative both for the uncontaminated group and for the combined treatment group. For the singly treated groups they were positive with a bacterial count of $10^4$ CFU/ml both for RIP- and for 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 shows that the use of RIP-impregnated stent with or without combination with conventional antibiotics caused significantly lowered bacterial loads. Similarly to teicoplanin, RIP caused a significant reduction in bacterial load on the ureteral stent tissue when compared with 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 not: in the clinical setting the positivity of an urine
culture is demonstrated by the presence of bacterial counts $>10^6$ CFU/ml. In our models these values have been detected only in the control group $C_1$. In the treated groups 1 and 2 values about $10^4$ CFU/ml demonstrated the efficacy of the agents but also the persistence of organisms.

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

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\textbf{REFERENCES}


Table 1. Activity of RIP and teicoplanin against *S. aureus* strain Smith diffuse in a rat model of ureteral stent infection.

<table>
<thead>
<tr>
<th>Group( ^a )</th>
<th>Stent-bonded drug( ^b )</th>
<th>Intraperitoneal drug( ^c )</th>
<th>Quantitative stent culture (CFU/ml)( ^d )</th>
<th>Urine culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (( C_0 ))</td>
<td>-</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Control (( C_1 ))</td>
<td>-</td>
<td>-</td>
<td>( 6.6 \times 10^5 \pm 1.9 \times 10^6 )</td>
<td>( 3.0 \times 10^6 \pm 0.3 \times 10^6 )</td>
</tr>
<tr>
<td>Group 1( ^e )</td>
<td>-</td>
<td>Teicoplanin</td>
<td>( 3.8 \times 10^3 \pm 0.8 \times 10^3 )</td>
<td>( 2.9 \times 10^4 \pm 0.6 \times 10^4 )</td>
</tr>
<tr>
<td>Group 2( ^f )</td>
<td>RIP</td>
<td>-</td>
<td>( 6.7 \times 10^3 \pm 1.4 \times 10^3 )</td>
<td>( 4.0 \times 10^4 \pm 1.1 \times 10^4 )</td>
</tr>
<tr>
<td>Group 3( ^f )</td>
<td>RIP</td>
<td>Teicoplanin</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

\( ^a \) *Staphylococcus aureus* Smith diffuse

\( ^b \) The ureteral stent segments were impregnated with 1 mg/l of RIP

\( ^c \) Each rat received intraperitoneally 10 mg/kg of teicoplanin

\( ^d \) The limit of detection for the method was \( \leq 10 \) CFU/ml.

\( ^e \) \( P < 0.05 \) when compared with the untreated control group

\( ^f \) \( P < 0.001 \) when compared with the untreated control group; \( P < 0.05 \) when compared with the singly treated groups