Subinhibitory concentrations of telithromycin, clarithromycin and azithromycin reduce methicillin-resistant *Staphylococcus aureus* coagulase in vitro and in vivo

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**Background:** Subinhibitory levels of clarithromycin and azithromycin have been shown to reduce the activity of bacterial virulence factors, but few studies have examined the effects of subinhibitory levels of telithromycin. Here, we examined the effects of telithromycin, clarithromycin and azithromycin on methicillin-resistant *Staphylococcus aureus* (MRSA) coagulase in vitro. We also examined the effects of these antibiotics on bacterial survival in a murine model of pulmonary infection, in which the number of bacteria in the lung correlates with the coagulase titre.

**Methods:** The coagulase titre in MRSA strain NUMR101, a clinical isolate, was measured after a 16 h treatment with telithromycin, clarithromycin or azithromycin at the MIC (512 mg/L) and 1/2, 1/4, 1/8 and 1/16 of the MIC. In addition, we examined the effect of these drugs in a murine model of pulmonary infection induced by the intravenous injection of *S. aureus* enmeshed in agar beads. Treatment was started 1 day before infection and mice were treated once a day for 7 days by oral administration of 10 or 100 mg/kg telithromycin, clarithromycin or azithromycin, and the number of viable bacteria in the lungs was counted 24 h after the injection of the bacteria.

**Results:** The coagulase titres in mice treated with 1/8 of the MIC of telithromycin, clarithromycin and azithromycin and in the control were 8, 4, 8 and 32, respectively. In the mouse model of infection, the log cfu/lung (mean ± SEM; n = 5 or 6) were 6.62 ± 0.81, 4.79 ± 0.41, 6.15 ± 0.38 and 8.41 ± 0.30 for mice treated with 100 mg/kg/day of telithromycin, clarithromycin and azithromycin and for controls, respectively (P < 0.05 for all groups versus control).

**Conclusions:** Subinhibitory concentrations of telithromycin inhibit MRSA coagulase in vitro. In addition, the in vivo results indicate that pre-treatment with telithromycin, clarithromycin or azithromycin can reduce the bacterial load in a murine model of pulmonary infection.

**Keywords:** pathogenesis, resistant bacteria, MRSA

**Introduction**

Telithromycin is the first ketolide antibacterial to be approved for clinical use. The ketolides represent a novel class of antibacterial agents structurally related to the macrolides, and they were developed to treat a wide spectrum of upper and lower respiratory tract infections caused by common and atypical pathogens, including strains that are resistant to currently used antibiotics. The ketolides are semi-synthetic erythromycin A derivatives that...
have a 3-keto group in place of the L-cladinose moiety at the C-3 position of the lactone ring.¹

Some reports have shown that subinhibitory levels of macrolides inhibit the activity of bacterial virulence factors. For example, subinhibitory levels of azithromycin reduce exotoxin A, total protease, elastase and phospholipase C production by Pseudomonas aeruginosa without affecting growth or total protein production.² Also, subinhibitory concentrations of erythromycin reduce the haemolytic activity of pneumolysin.³

Furthermore, we previously demonstrated that subinhibitory concentrations of clarithromycin and azithromycin reduce pneumolysin of high-level macrolide-resistant Streptococcus pneumoniae both in vitro and in vivo.⁴ However, to the best of our knowledge, the effects of subinhibitory levels of telithromycin on bacterial virulence factors have not been examined.

Staphylococcus aureus produces many extracellular products that may act as virulence factors, and, of these, staphylocoagulase has been considered one of the most important. We previously found that coagulase plays a role in the development of blood-borne staphylococcal pneumonia.⁵,⁶ In the current study, we examined the effect of telithromycin on staphylocoagulase in methicillin-resistant S. aureus (MRSA) in vitro and in vivo and we compared the effects of telithromycin with those of clarithromycin and azithromycin.

### Materials and methods

#### Bacterial strain

MRSA strain NUMR101 was a clinical isolate obtained from blood samples of a patient at Nagasaki University Hospital. The bacteria were stored at −70°C in brain heart infusion (BHI) broth (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 10% (v/v) glycerol and 5% (w/v) skimmed milk (Yukijirushi Co., Tokyo, Japan) until use. MRSA NUMR101 was cultured on a tryptone soy agar (BBL Microbiology Systems)-based sheep blood agar plate for 24 h at 37°C. The MIC of each agent was determined by the microplate dilution technique using Muller–Hinton medium, with an inoculum size of 5 × 10⁵ cfu/mL. The MIC was defined as the lowest concentration of the test agent that inhibited visible growth of bacteria after 18 h at 37°C. The MICs of telithromycin, clarithromycin and azithromycin for NUMR101 were 512 mg/L.

#### Effect of antibiotics on coagulase production in vitro

The S. aureus NUMR101 strain was cultured in the presence of antibiotics at the MIC and 1/2, 1/4, 1/8 and 1/16 of the MIC. Coagulase levels were determined using a modification of the method reported by Jordens et al.⁷ Overnight cultures in BHI broth were diluted 2-fold in fresh sterile BHI to a total volume of 100 mL. Next, 0.5 mL of 1:20 fresh-frozen dry rabbit plasma (Eiken Chemical Co., Tokyo, Japan) in BHI broth was added, and clot formation was assessed after 2 h at 37°C. The highest dilution giving a definite clot was considered the coagulase titre.

#### Laboratory animals

Six-week-old, male, ddY, specific pathogen-free mice (25–30 g body weight) were purchased from Shizuoka Agricultural Cooperative Association Laboratory Animals (Shizuoka, Japan). All animals were housed in a pathogen-free environment in the Laboratory Animal Centre for Biomedical Science at Nagasaki University and received sterile food and water ad libitum. All experimental protocols described in this study were approved by the Ethics Review Committee for Animal Experimentation at our institution.

#### Bacteriological analysis

Treatment was started 1 day before infection and mice were treated once a day for 7 days by oral administration of 10 or 100 mg/kg telithromycin, clarithromycin or azithromycin. Each group of animals was sacrificed by cervical dislocation 6 days after infection. After exsanguination, the lungs were dissected and removed under aseptic conditions. Organs used for bacteriological analyses were homogenized, serially diluted and cultured on blood agar plates.

#### Statistical analysis

Bacteriological data were expressed as means ± SEM. Differences between groups were examined for statistical significance using an unpaired t-test. A P value less than 0.05 was considered to indicate a statistically significant difference.

#### Results

#### Effect of subinhibitory concentrations of antibiotics on coagulase activity in vitro

At concentrations of 1/2, 1/4 and 1/8 of the MIC, telithromycin, clarithromycin and azithromycin inhibited coagulase production by S. aureus (Table 1). These concentrations did not, however, affect the number of bacteria.

#### Therapeutic effects of antibiotics

We next examined the effect of telithromycin, clarithromycin and azithromycin on the number of viable bacteria in a murine model of haematogenous pulmonary infection. According to previous reports, the peak concentrations in the lung are
reduce the level of coagulase protein. Specifically, we showed that telithromycin and macrolides are a therapeutic option for preventing infection by resistant bacteria. We previously reported that the inhibition of staphylocoagulase by a short interfering RNA could be an effective means of controlling MRSA infection. We previously examined the role of coagulase in a murine model of haematogenous pulmonary infection with MRSA, and we found a significant correlation between the coagulase titre and the number of viable bacteria recovered from the lung. Here, we found that treatment with a low dose of telithromycin (10 mg/kg) did not cause a change in the number of viable bacteria in the lungs in comparison with the control [7.75 ± 0.45 and 8.23 ± 0.21 log_{10} cfu/lung (n = 6), respectively; Table 2]. In contrast, a high dose of telithromycin (100 mg/kg) significantly reduced the number of viable bacteria when compared with control [6.62 ± 0.81 log_{10} cfu/lung (n = 6); P = 0.0167 versus control; Table 2]. Similarly, treatment with a low dose of clarithromycin or azithromycin (10 mg/kg) did not change the number of viable bacteria in the lungs, whereas a high dose of these drugs (100 mg/kg) significantly reduced the number of viable bacteria (Table 2).

Table 1. Effect of subinhibitory concentrations of antibiotics on coagulase titre of S. aureus

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Coagulase titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no drug</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>32</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>32</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>32</td>
</tr>
</tbody>
</table>

Table 2. Effect of antibiotics on bacteria numbers in vivo

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Log_{10} cfu/lung of S. aureus (n = 5 or 6)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>7.75 ± 0.45</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>7.65 ± 0.59</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>7.55 ± 0.83</td>
</tr>
<tr>
<td>Control</td>
<td>8.23 ± 0.21</td>
</tr>
</tbody>
</table>

*Values are means ± SEM. **P = 0.00167 versus control. ***P = 0.0001 versus control.

Discussion

In this study, we demonstrated that subinhibitory concentrations of telithromycin, clarithromycin and azithromycin reduce the level of MRSA coagulase in vitro and in vivo. Our results suggest that telithromycin and macrolides can be used as a new therapeutic option for preventing infection by resistant bacteria. Specifically, we showed that telithromycin and macrolides reduce the level of coagulase protein in vitro and significantly lowered the number of viable MRSA in vivo. We previously reported that the inhibition of staphylocoagulase by a short interfering RNA could be an effective means of controlling MRSA infection. The current results further show that subinhibitory concentrations of telithromycin, clarithromycin and azithromycin are effective against infection by MRSA in vivo. According to previous reports, the peak concentrations in the lung are 7 (10 mg/kg) and 40–72.8 (100 mg/kg) mg/L. These data suggested that the high dose (100 mg/kg) of antibiotics should have a sub-MIC effect against MRSA. We already reported that the number of bacteria recovered from the lung tissue correlated with the titre of staphylocoagulase. Thus, we decided that the in vivo inhibition of coagulase induced the lower bacteria number.

Previous reports indicated that telithromycin has effects against Gram-positive cocci and Helicobacter pylori at sub-MIC concentrations. Telithromycin has also been reported to reduce the number of viable bacteria during P. aeruginosa infection by inhibiting biofilm formation. These reports suggest that telithromycin has effects at sub-MIC concentrations. Furthermore, many investigators have reported that sub-MIC concentrations of macrolides can reduce pathogenic factors in vitro and in vivo. Here, we showed that telithromycin had a similar effect to macrolides. Finally, the antibiotics did not have an effect in the murine model of infection when administered 24 h after injection of bacteria (data not shown), indicating that this treatment is only effective prior to infection.

In conclusion, we showed that, similar to clarithromycin and azithromycin, a subinhibitory concentration of telithromycin reduces the level of MRSA coagulase. The in vivo results further revealed that pre-treatment with telithromycin, clarithromycin or azithromycin can reduce the bacterial load in a murine model of pulmonary infection.

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Transparency declarations

None to declare.

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