Effects of DQ-113, a New Quinolone, against Methicillin- and Vancomycin-Resistant Staphylococcus aureus-Caused Hematogenous Pulmonary Infections in Mice

Yukihiro Kaneko, Katsunori Yanagihara, Yoshitsugu Miyazaki, Kazuhiro Tsukamoto, Yoichi Hiranaka, Kazunori Tomono, Jun-ichi Kadota, Takayoshi Tashiro, Ikuo Murata, and Shigeru Kohno

Second Department of Internal Medicine, Department of Pharmacotherapeutics, and Division of Molecular & Clinical Microbiology, Department of Molecular Microbiology & Immunology, Graduate School of Medical Sciences, Nagasaki University, Graduate School of Medical Science, Japan

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We compared the effects of DQ-113, a new quinolone, to those of vancomycin (VCM) and teicoplanin (TEIC) in murine models of hematogenous pulmonary infections caused by methicillin-resistant Staphylococcus aureus (MRSA) and VCM-insensitive S. aureus (VISA). The MICs of DQ-113, VCM, and TEIC for MRSA were 0.125, 1.0, and 0.5 μg/ml, respectively; and those for VISA were 0.25, 8.0, and 8.0 μg/ml, respectively. Treatment with DQ-113 resulted in a significant decrease in the number of viable bacteria in the lungs of the mice in the MRSA infection model (counts in mice treated with DQ-113, VCM, and TEIC and control mice, 6.33 ± 0.22, 7.99 ± 0.14, 7.36 ± 0.20, and 8.47 ± 0.22 log₁₀ CFU/lung [mean ± standard error of the mean], respectively [P < 0.01 for the group treated with DQ-113 compared with the group treated with VCM or TEIC or the untreated group]). Mice infected with VISA were pretreated with cyclophosphamide, and the survival rate was recorded daily for 10 days. At the end of this period, 90% of the DQ-113-treated mice were still alive, whereas only 45 to 55% of the mice in the other three groups were still alive (P < 0.05 for the group treated with DQ-113 compared with the group treated with VCM or TEIC or the untreated group). DQ-113 also significantly (P < 0.05) reduced the number of viable bacteria in the lungs compared with those in the lungs of the other three groups (counts in mice treated with DQ-113, VCM, and TEIC and control mice, 5.76 ± 0.39, 7.33 ± 0.07, 6.90 ± 0.21, and 7.44 ± 0.17 log₁₀ CFU/lung, respectively). Histopathological examination revealed milder inflammatory changes in DQ-113-treated mice than in the mice in the other groups. Of the antibiotics analyzed, the parameters of area under the concentration-time from 0 to 6 h (AUCₐ₀₆) and the time that the AUC ₐ₀₆ exceeded the MIC were the highest for DQ-113. Our results suggest that DQ-113 is potent and effective for the treatment of hematogenous pulmonary infections caused by MRSA and VISA strains.

Methicillin-resistant Staphylococcus aureus (MRSA) was first identified in the 1960s and was reported to colonize the upper respiratory tract and to cause severe infections, such as pneumonia, pulmonary abscesses, and septicemia. MRSA infection develops mainly in inpatients with risk factors related to health care (5), although it has also recently been described in the general population (1). Glycopeptides, such as vancomycin (VCM) and teicoplanin (TEIC), are the most reliable antibiotics and the pathogenesis of blood-borne staphylococcal pneumonia. In the present study, we used the model to evaluate the antibacterial and histopathological effects of DQ-113 against MRSA and VISA by comparing these effects with those of VCM and TEIC.

MATERIALS AND METHODS

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

Bacterial strain. Two strains of S. aureus were examined. Strain NUMR101 was isolated clinically at Nagasaki University Hospital from blood samples of infected patients. Mu50, a VCM-insensitive strain, was kindly provided by K.
Hiramatsu (Juntendo University, Tokyo, Japan) (3). The bacteria were stored at
−70°C in brain heart infusion broth (BBL Microbiology System, Cockeysville,
Md.) supplemented with 10% (vol/vol) glycerol and 5% (wt/vol) skim milk
(Yukijirushi Co., Tokyo, Japan) until use.

MIC determinations. DQ-113 (Daichi Pharmaceutical Co., Tokyo, Japan)
dissolved in 0.01 N NaOH to a concentration of 106 CFU/ml in sterilized water
immediately before use. The MIC of each agent was determined by the microplate dilution
technique using Mueller-Hinton medium and an inoculum of 5 × 104 CFU/ml. The MIC
was defined as the lowest concentration of the test drug that inhibited visible growth
of the bacteria after 18 h of incubation at 37°C.

Inoculum. The method of inoculation was described previously (8). S. aureus
was cultured on a Trypticase soy agar (BBL Microbiology System) blood agar plate for 24 h at 37°C. The bacteria were suspended in endotoxin-free
sterile saline and harvested by centrifugation (3,000 × g, 4°C, 10 min). The microorganisms were resuspended in cold sterile saline and diluted to 2 × 10^6 to 4 × 10^7 CFU/ml as estimated by turbidimetry. The suspension was warmed to 45°C, and then 10 ml of the suspension was mixed with 10 ml of 4% (wt/vol) molten Noble agar (Difco Laboratories, Detroid, Mich.) at 45°C. The agar-
bacterium suspension (1.0 ml) was placed in a 1.0-ml syringe, and the suspension
and femur were separated after the blood had clotted. Four animals were used for each group.

Experimental model. We injected 0.20 to 0.25 ml of the agar beads that
contained the bacteria and that were suspended in saline into the tail vein of each
mouse (10 mg/kg of body weight). Before the bacteria were emmeshed in the agar beads, we verified their numbers by inoculating duplicates of serial dilutions onto
blood agar plates and counting the number of CFU after 48 h of incubation at
37°C. The method used to induce infection has previously been described in
detail (8). Treatment commenced a day after inoculation by intraperitoneal administration of antibiotics. For the study with MRSA, 45 animals received one of the following eight treatments: DQ-113 (40 mg/kg of body weight/day; n = 6), VCM (40, 80, or 160 mg/kg/day; n = 6, 6, and 6, respectively), TEIC (40, 80, or 160 mg/kg-day; n = 6, 6, and 5, respectively), or no treatment (controls; n = 6). We investigated the number of viable bacteria in the
lungs after 7 days of treatment.

Bacteriological, survival, and histopathological examinations. Each group of
animals was killed by cervical dislocation at specific time intervals. After exan-
gamation, the lungs were dissected and removed under aseptic conditions. The
organisms used for bacteriological analyses were homogenized and cultured quan-
titatively by serial dilution on blood agar plates. Lung tissue for histological
examination was fixed in 10% buffered formalin and stained with hematoxylin-
eosin.

Lung and serum drug concentrations in mice. The strains were killed by
cervical dislocation at 0.25, 0.5, 1, 2, 4, and 6 h after treatment. Serum
was separated after the blood had clotted. Four animals were used for each group. The
lungs were removed, washed briefly, and cryohomogenized with saline. These samples were immediately frozen and stored at −80°C for a few days until
the assay was performed. The concentration of DQ-113 was measured by the
paper disk (bioassay) method (4). The test organism was Bacillus subtilis ATCC
6633. The concentrations of VCM and TEIC were measured by fluorescence
polarization immunoassay (7). Pharmacokinetic parameters were calculated from
the arithmetic mean concentrations in serum and lung tissue.

Statistical analysis. Bacteriological data were expressed as means ± standard
eerrors of the means (SEMs). Survival data were compared by plotting Kaplan-
Meier curves. Differences between groups were examined for statistical signif-
cance by the unpaired t test for MRSA and the log-rank test for VISA. A P value
less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Therapeutic effects of antibiotics. Treatment with VCM (40 or 80 mg/kg/day) did not reduce the number of viable bacteria in the lungs relative to the number in the lungs of the controls (VCM at 40 mg/kg/day, 7.6 ± 0.14 log10 CFU/lung; VCM at 80 mg/kg/day, 8.1 ± 0.18 log10 CFU/lung; controls, 8.4 ± 0.22 log10 CFU/lung [n = 6, 6, and 5, respectively]). Treatment
with VCM (160 mg/kg/day) or TEIC (40, 80, or 160 mg/kg/day) reduced the number of viable bacteria in the lungs relative to the number in the lungs of the controls (VCM at 160 mg/kg/day, 7.1 ± 0.15 log10 CFU/lung; TEIC at 40 mg/kg/day, 7.3 ± 0.20 log10 CFU/lung; TEIC at 80 mg/kg/day, 7.6 ± 0.18 log10 CFU/lung; TEIC at 160 mg/kg/day, 6.9 ± 0.27 log10 CFU/lung [n = 6, 6, 5, and 5, respectively]). In contrast, administration of
DQ-113 at 40 mg/kg/day resulted in a significant decrease in the number of viable bacteria compared with the number in the other groups (6.3 ± 0.22 log10 CFU/lung [n = 6] [P < 0.05 versus the counts for the other groups]). The data are repre-
sentative of those from three independent experiments. In the VISA study, 90% of mice treated with DQ-113 were still alive at the end of the study, while the survival rates were only 45 to
55% for the other three groups (Fig. 1). The differences in survival rates between the group treated with DQ-113 and the other three groups were significant (P < 0.05 for each com-
parison). The data are representative of those from two independ-
ent experiments. DQ-113 also significantly (P < 0.05) reduced the number of viable bacteria in the lungs compared with the number in the lungs of the other three groups (for DQ-113, VCM, TEIC, and the controls, 5.7 ± 0.39, 7.3 ± 0.07, 6.9 ± 0.21, and 7.4 ± 0.17 log10 CFU/lung, respectively [n = 6 for each group]).

Histopathological examination. At 7 days after treatment, microscopic examination of lung tissue specimens of mice infected with VISA Mu50 showed lung abscesses consisting of a central zone comprising a bacterial colony with infiltration of acute inflammatory cells (Fig. 2). Findings for the mice treated with VCM (Fig. 2c) and TEIC (Fig. 2b) were similar to those for the control mice (Fig. 2a). DQ-113-treated mice (Fig. 2d)
exhibited fewer abscesses and milder inflammatory processes relative to those for the other groups.

Serum and lung DQ-113, VCM, and TEIC concentrations in mice. Figure 3a and b shows the mean concentrations of DQ-113, VCM, and TEIC in the sera and lungs of the mice 0.25, 0.5, 1, 2, 4, and 6 h after administration. These data are for MRSA-infected mice, and each drug was administered at 20 mg/kg once a day after inoculation. The peak concentrations of DQ-113, VCM, and TEIC in serum were $1.30 \pm 0.23$, $20.46 \pm 2.98$, and $60.40 \pm 1.47 \mu g/ml$, respectively (mean $\pm$ SEM; $n = 4$). The peak concentrations of DQ-113, VCM and TEIC in lung tissue were $3.38 \pm 0.19$, $15.51 \pm 4.04$, and $3.18 \pm 1.34 \mu g/ml$, respectively (mean $\pm$ SEM; $n = 4$). Table 1 shows the pharmacodynamic and pharmacokinetic parameters in the

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<th>Antibiotic</th>
<th>Strain</th>
<th>MIC (µg/ml)</th>
<th>AUC$_{0,6}$ (µg·h/ml)</th>
<th>$C_{max}$ (µg/ml)</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC$_{0,6}$/MIC (h)</th>
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* The pharmacokinetic data are for MRSA-infected mice. Each drug was administered at 20 mg/kg once a day after inoculation. Pharmacokinetic parameters were calculated from the arithmetic means of the concentrations in lung tissue (mean values for four animals). $C_{max}$, maximum concentration; $t_{1/2}$, half-life.
Efficacy of DQ-113 Against *S. aureus* Lung Infection

**DISCUSSION**

In the present study, we were successful in inducing severe pneumonia and lung abscesses in VISA Mu50-infected immunocompromised mice, resulting in the death of 60% of the mice at 10 days after infection. While VCM and TEIC had no effect on the survival rate, DQ-113 protected the mice against fatal pneumonia and resulted in a significant reduction in the number of bacteria in MRSA and VISA hematogenous infection models and significantly improved the rates of survival of immunocompromised mice infected with VISA compared with the rates achieved with VCM and TEIC.

**ACKNOWLEDGMENT**

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**REFERENCES**


**FIG. 3.** Pharmacokinetics of DQ-113 (20 mg/kg), VCM (20 mg/kg), and TEIC (20 mg/kg) in the sera (a) and lungs (b) of MRSA-infected mice. Each drug was administered intraperitoneally after infection. The results are presented as means ± SEMs. ●, DQ-113; •, VCM; ▲, TEIC.

Lung tissues of mice with MRSA and VISA infections. The MICs of DQ-113, VCM, and TEIC for NUMR101 were 0.125, 1.0, and 0.5 µg/ml, respectively; and those for Mu50 were 0.25, 8.0, and 8.0 µg/ml, respectively. Of the antibiotics analyzed, the parameters of the area under the concentration-time curve from 0 to 6 h (AUC<sub>0-6</sub>/MIC and the time that the AUC<sub>0-6</sub> exceeded the MIC (AUC<sub>0-6</sub> > MIC)/MIC (AUIC<sub>0-6</sub>) were the highest for DQ-113.

In our model of hematogenous pulmonary infection, the new oxazolidinone antimicrobial linezolid significantly reduced the number of MRSA organisms and improved the survival rates of mice infected with VISA compared to the effects of VCM and TEIC (12). Our present data suggest that DQ-113 is a potent antimicrobial agent against VISA infection as well as MRSA, similar to linezolid.

VCM-resistant *S. aureus* was recently isolated in the United States. In a study published by the Centers for Disease Control and Prevention (2), the MICs of VCM, TEIC, and oxacillin for VISA were >128, 32, and 16 mg/ml, respectively. The isolate contained the vanA VCM resistance gene from enterococci, which is consistent with the glycopeptide MIC profiles (2).

The new oxazolidinone antimicrobial linezolid has been approved for use for the treatment of infections caused by various gram-positive bacteria, including MRSA and VCM-resistant enterococci. However, one MRSA strain resistant to linezolid has already been isolated from a patient treated with this agent for dialysis-associated peritonitis (11). These reports emphasize the need to develop antimicrobial agents potent against VISA. The available in vitro data (10) and the present results suggest that DQ-113 is a promising and potent candidate. In the pharmacokinetic study, the AUC/MIC and AUIC values for DQ-113 were the highest of those for the antibiotics analyzed. A recent brief report showed that DQ-113 accumulates at higher concentrations than other quinolones, suggesting that both its high intracellular concentrations and its inhibitory activities against target enzymes contribute to its potent antibacterial activity (M. Tanaka, T. Akasaka, Y. Onodera, M. Yoshihara, T. Takemura, and K. Sato, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 552, 2001).

In conclusion, we have demonstrated in the present study that DQ-113, a novel antibacterial quinolone, effectively reduced the number of bacteria in MRSA and VISA hematogenous infection models and significantly improved the rates of survival of immunocompromised mice infected with VISA compared with the rates achieved with VCM and TEIC.


