In Vivo Efficacies and Pharmacokinetics of DX-619, a Novel Des-Fluoro(6) Quinolone, against *Streptococcus pneumoniae* in a Mouse Lung Infection Model

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DX-619 is a novel des-fluoro(6) quinolone with potent activity against gram-positive pathogens. The in vivo activity of DX-619 against *Streptococcus pneumoniae* was compared with those of fluoro(6) quinolones, sitafloxacin, and ciprofloxacin in a mouse model. Two strains of *S. pneumoniae* were used: a penicillin-sensitive *S. pneumoniae* (PSSP) strain and a penicillin-resistant *S. pneumoniae* (PRSP) strain. Furthermore, these strains showed intermediate susceptibilities to ciprofloxacin. In murine lung infections caused by PSSP, the 50% effective doses (ED₅₀s) of DX-619, sitafloxacin, and ciprofloxacin were 9.15, 11.1, and 127.6 mg/kg of body weight, respectively. Against PRSP-mediated pneumonia in mice, the ED₅₀s of DX-619, sitafloxacin, and ciprofloxacin were 0.69, 4.84, and 38.75 mg/kg, respectively. The mean $\pm$ standard error of the mean viable bacterial counts in murine lungs infected with PSSP and treated with DX-619, sitafloxacin, ciprofloxacin (10 mg/kg twice daily), and saline (twice daily) were 1.75 ± 0.06 , 1.92 ± 0.23 , 6.48 ± 0.28 , and 7.57 ± 0.13 log₁₀ CFU/ml, respectively. Furthermore, the numbers of viable bacteria in lungs infected with PRSP and treated with the three agents and not treated (control) were 1.73 ± 0.04 , 2.28 ± 0.17 , 4.61 ± 0.59 , and 5.54 ± 0.72 log₁₀ CFU/ml, respectively. DX-619 and sitafloxacin significantly decreased the numbers of viable bacteria in the lungs compared to the numbers in the lungs of ciprofloxacin-treated and untreated mice. The pharmacokinetic parameter of the area under the concentration-time curve (AUC)/MIC ratio in the lungs for DX-619, sitafloxacin, and ciprofloxacin were 171.0, 21.92, and 1.22, respectively. The AUC/MIC ratio in the lungs was significantly higher for DX-619 than for sitafloxacin and ciprofloxacin. Our results suggest that DX-619 and sitafloxacin are potent against both PSSP and PRSP in our mouse pneumonia model.

Streptococcus pneumoniae is an important pathogen in many community-acquired respiratory infections, including community-acquired pneumonia, and in more invasive infections, such as meningitis and bacteremia. β -Lactam antibiotics were initially very effective against *S. pneumoniae*; however, resistance to this class of antibiotics has become an increasing problem. In Japan, the proportions of penicillin-resistant *S. pneumoniae* (PRSP) are reported to be 38.4 to 59.7% (24, 25). Resistance rates are as high as 50.3% in Hong Kong and 70.1% in South Korea (12). Unfortunately, *S. pneumoniae* is becoming increasingly resistant to a variety of antibiotics. Infections caused by PRSP may lead to clinical treatment failures. Concern over the emergence of penicillin-resistant and multidrug-resistant strains has led to the development of antipneumococcal fluoroquinolones, such as sparfloxacin, gatifloxacin, and moxifloxacin. These agents have high levels of activity against *S. pneumoniae* and are now approved for first-line therapy for community-acquired pneumonia (2, 17). Moreover, sitafloxacin (DU-6859a), which has more potent in vitro activity against *S. pneumoniae*

than sparfloxacin, gatifloxacin, and moxifloxacin, was synthesized (18, 27).

However, the use of many fluoroquinolones is limited because of the potential for adverse side effects (9, 15). Some fluoroquinolones have been removed from the market because of more serious adverse events (15). Furthermore, there is growing concern about the development of quinolone-resistant *S. pneumoniae*, with a recent survey revealing resistance to both the early fluoroquinolones and the newer quinolones, such as levofloxacin and moxifloxacin (4). Therefore, safer and more potent compounds must be developed to treat infections caused by multidrug-resistant *S. pneumoniae*.

It was initially thought that the fluoro(6) group was responsible for the enhanced bacterial penetration and killing via gyrase inhibition. Recently, however, compounds without this component were found to exhibit broad antimicrobial activities (28). Furthermore, the favorable safety profiles of the des-fluoro(6) quinolones have been elucidated by some groups (13, 14). In particular, it was reported that the single-dose toxicity and genotoxicity of des-fluoro(6) quinolones were less than those of fluoro(6) quinolones (13).

DX-619 is a novel des-fluoro(6) quinolone with excellent activity against clinical isolates of resistant gram-positive bacteria, including ciprofloxacin-resistant methicillin-resistant *Staphylococ-*

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cus aureus, methicillin-resistant coagulase-negative staphylococci, PRSP, and vancomycin-resistant enterococci in vitro (10).

In the present study, we examined the in vivo antimicrobial activity of DX-619 against penicillin-susceptible *S. pneumoniae* (PSSP) and PRSP in a noncompromised mouse model of pneumonia. Furthermore, we compared the activity of DX-619 with those of the newer fluoro(6) quinolone, sitafloxacin, and the early one, ciprofloxacin.

MATERIALS AND METHODS

Antimicrobial agents. DX-619 and sitafloxacin were provided by the Daiichi Pharmaceutical Co. Ciprofloxacin was extracted from commercial preparations purchased from Bayer Pharmaceutical Co. DX-619 and sitafloxacin were dissolved in 0.1 N NaOH and were then reconstituted with normal saline, in accordance with the manufacturer's instruction. Preliminary examinations showed that the vehicle had no bactericidal activity. Ciprofloxacin was dissolved in normal saline.

Microorganisms. Two strains of *S. pneumoniae* clinically isolated at the Nagasaki University School of Medicine were used in the present study. One strain was PSSP strain NU83127 (MIC of penicillin G, 0.03 µg/ml; serotype 4). The other was PRSP strain NU187 (MIC of penicillin G, 2 µg/ml; serotype 19). The bacteria were stored at -80°C until use.

Laboratory animals. Five-week-old female CBA/J specific-pathogen-free mice (body weight, 16 to 20 g) were purchased from Charles River Japan. The CBA/J mouse model of PRSP pneumonia has been described elsewhere (26). All mouse experiments were performed according to the guidelines of the Laboratory Animal Center for Biomedical Research, Nagasaki University School of Medicine.

Antibiotic susceptibility testing. The MICs of the antibiotics were determined by the broth dilution method with Muller-Hinton broth (Difco Laboratories, Detroit, MI) supplemented with 5% lysed horse blood. Microtiter plates containing 5.0×10^5 CFU/well were incubated with antibiotic at 35°C for 24 h, and the lowest concentration of drug that prevented visible growth was considered the MIC (19).

Experimental murine model of pneumococcal pneumonia. The *S. pneumoniae* strains were cultured on horse blood agar for 20 h at 37°C and then scraped and suspended in brain heart infusion broth (Becton Dickinson and Company, Sparks, MD) mixed with horse serum and cultured with shaking for 6 h at 37°C at 250 rpm. The bacteria were then harvested by centrifugation (800 × g, 3 min). The organisms were resuspended in normal saline; and the final numbers of bacteria prepared were approximately 10^5 CFU/ml for PSSP and 10^8 CFU/ml for PRSP, as determined by the optical density method. Infection was induced by intranasal inoculation of 0.05 ml of a bacterial suspension containing about 1×10^5 CFU of PSSP/ml (5×10^3 CFU/mouse) and 1×10^8 CFU of PRSP/ml (5×10^6 CFU/mouse) into mice under anesthesia (pentobarbital sodium, 40 mg/kg of body weight intraperitoneally) (20).

ED₅₀s. The experimental murine model of pneumococcal pneumonia was induced with PSSP and PRSP, as described above. Then, 0.2 ml of various doses of the test drugs were injected intraperitoneally into the mice ($n = 6$) twice daily for 14 days beginning 24 h after inoculation. Control mice also received 0.2 ml of normal saline intraperitoneally to confirm lung infection. Mortality was also recorded for 14 days, and the 50% effective dose (ED₅₀) of each drug was calculated by the probit method.

Bacteriological examinations. The drugs were injected intraperitoneally into the mice 24, 36, and 48 h after inoculation. Each single dose was 10 mg/kg. Mice ($n = 7$) from each group were killed by cervical dislocation 12 h after the third administration. For bacteriological examination, the lungs were dissected out under aseptic conditions and suspended in saline (1 ml). The organs were homogenized with a Polytron homogenizer (PT3000; Kinematica, Littau-Lucerne, Switzerland), quantitatively inoculated onto blood agar plates by serial dilutions, and incubated at 37°C for 20 h. The lowest detectable level was 50 CFU/ml. For statistical comparison, culture-negative samples were considered to contain just below the lower limit of detection (49 CFU/ml, or $1.69 \log_{10}$ CFU/ml).

Pharmacokinetic studies. Studies were undertaken to determine the pharmacokinetic profiles of DX-619, sitafloxacin, and ciprofloxacin in mice with pneumonia caused by PRSP. At 24 h postinfection, groups of four mice each were administered DX-619, sitafloxacin, and ciprofloxacin at a dose of 10 mg/kg each (20). The animals were killed by cervical dislocation; and serum samples and lungs were collected from the mice at 0.25, 0.5, 1, 2, 4, and 6 h after treatment. The sera and lungs were immediately frozen and stored at -80°C until they were

TABLE 1. Protective effects of DX-619, sitafloxacin, and ciprofloxacin in mice infected with *S. pneumoniae*

Infection	Drug	MIC (µg/ml)	ED ₅₀ (mg/kg/day) (95% confidence limits)
PSSP ^a	DX-619	0.03	9.152 (-2.101 to 24.37)
	Sitafloxacin	0.125	11.05 (3.161 to 23.66)
	Ciprofloxacin	2.0	127.6 (70.32 to 176.9)
PRSP ^b	DX-619	0.03	0.694 (0.239 to 1.646)
	Sitafloxacin	0.125	4.835 (2.769 to 24.08)
	Ciprofloxacin	1.0	38.75 (19.08 to 192.0)

^a Groups of six mice each were infected intranasally with 0.05 ml of a PSSP suspension (5×10^3 CFU/mouse).

^b Groups of six mice each were infected intranasally with 0.05 ml of PRSP suspension (5×10^6 CFU/mouse).

assayed. To determine the concentrations of each drug in the lungs, we measured the weight of the lungs and then added three times that weight of 0.1 M phosphate buffer (pH 7.0). Then, the lungs were homogenized in a Physcotron homogenizer (NS-310E; Niti-on, Tokyo, Japan). After centrifugation, the concentrations of DX-619 and sitafloxacin in the supernatant and serum were determined by a microbiological agar diffusion assay with *Bacillus subtilis* ATCC 6633, and the concentration of ciprofloxacin was determined by a microbiological agar diffusion assay with *Escherichia coli* NIHJ. Prior to the assay, a check was performed to determine that the antibiotic-free samples had no inhibitory effect on the test organisms. The lower limits of sensitivity of the assays were 0.02 µg/ml for DX-619, 0.039 µg/ml for sitafloxacin, and 0.02 µg/ml for ciprofloxacin. The assay precision, as determined by the coefficient of variation for control samples with antibiotic concentrations ranging from 0.039 to 2.5 µg/ml, were as follows: DX-619-buffer, 0.0 to 12.2%; sitafloxacin-buffer, 0.0 to 6.3%; and ciprofloxacin-buffer, 2.1 to 15.2%. Pharmacokinetics parameters were calculated from the arithmetic means of serum and lung tissue concentrations (23).

Statistical analysis. Data are expressed as the mean ± standard error of the mean (SEM). Differences between numbers of viable bacteria in lungs were evaluated by analysis of variance. *P* values of less than 0.05 were considered statistically significant.

RESULTS

In vitro susceptibility. For the PSSP and PRSP strains used, the MICs of DX-619, sitafloxacin, and ciprofloxacin were 0.03 and 0.03 µg/ml, 0.125 and 0.125 µg/ml, and 2.0 and 1.0 µg/ml, respectively (Table 1). Both strains showed intermediate susceptibilities to ciprofloxacin, according to European Committee on Antimicrobial Susceptibility Testing standards (6).

In vivo efficacy against PSSP. As shown in Table 1, in murine lung infections caused by PSSP, the ED₅₀s of DX-619, sitafloxacin, and ciprofloxacin were 9.15, 11.1, and 127.6 mg/kg, respectively. All control mice died within 2 days. In the bacteriological study, the mean viable bacterial counts in the lungs of the DX-619-, sitafloxacin-, and ciprofloxacin-treated mice and the untreated control mice were 1.75 ± 0.06 , 1.92 ± 0.23 , 6.48 ± 0.28 , and $7.57 \pm 0.13 \log_{10}$ CFU/ml, respectively. DX-619 and sitafloxacin eradicated viable bacteria from four and three of seven murine lungs, respectively. The numbers of viable bacteria in the lungs of DX-619- and sitafloxacin-treated mice were significantly less than those in the lungs of ciprofloxacin-treated and untreated mice ($P < 0.0001$ for each comparison) (Fig. 1). There was no statistically significant difference between the number of viable bacteria in the lungs of DX-619-treated mice and sitafloxacin-treated mice ($P = 0.56$).

In vivo efficacies against PRSP. As shown in Table 1, in murine lung infections caused by PRSP, the ED₅₀s of DX-619, sitafloxacin, and ciprofloxacin were 0.69, 4.84, and 38.75 mg/kg,

FIG. 1. Efficacies of DX-619, sitafloxacin (STFX), and ciprofloxacin (CIP) against PSSP. The numbers of viable bacteria in lungs after treatment with three drugs (10 mg/kg twice daily) and a control treatment (twice daily) are indicated. Data are the means $\pm$ SEMs for seven mice. DX-619 and STFX significantly reduced the numbers of viable bacteria compared to those achieved by treatment with ciprofloxacin and saline (control). *, $P < 0.0001$.

FIG. 2. Efficacies of DX-619, sitafloxacin (STFX), and ciprofloxacin (CIP) against PRSP. The numbers of viable bacteria in lungs after treatment with three drugs (10 mg/kg twice daily) and a control treatment (twice daily) are indicated. Data are the means $\pm$ SEMs for seven mice. DX-619 and STFX significantly reduced the numbers of viable bacteria compared to those achieved by treatment with ciprofloxacin and saline (control). *, $P < 0.0001$.

respectively. All control mice died within 9 days. In the bacteriological study, the mean $\pm$ SEM of viable bacteria in the lungs of DX-619-, sitafloxacin-, and ciprofloxacin-treated mice and untreated control mice were 1.73 ± 0.04 , 2.28 ± 0.17 , 4.61 ± 0.59 , and 5.54 ± 0.72 log₁₀ CFU/ml, respectively. DX-619 and sitafloxacin eradicated viable bacteria from four and one of seven murine lungs, respectively. The numbers of viable bacteria in the lungs of DX-619- and sitafloxacin-treated mice were significantly less than those in the lungs of ciprofloxacin-treated and untreated mice ($P < 0.0001$ for each comparison) (Fig. 2). There was no statistically significant difference between the numbers of viable bacteria in the lungs of DX-619-treated mice and sitafloxacin-treated mice ($P = 0.43$).

Lung and serum concentrations of DX-619, sitafloxacin, and ciprofloxacin in mice. Table 2 shows the pharmacokinetic-pharmacodynamic parameters of the test drugs in the sera and lungs after intraperitoneal injection into mice infected with PRSP. The ratios of the area under the concentration-time curve (AUC) in the lungs to that in serum for DX-619, sitafloxacin, and ciprofloxacin (dose, 10 mg/kg) were 5.34, 4.72 and 1.61, respectively. The AUC/MIC ratio in the lungs for DX-619 was 171.0 at a dose of 10 mg/kg, which was significantly higher than those of sitafloxacin and ciprofloxacin.

DISCUSSION

In the present study, the in vivo activity of DX-619, a new des-fluoro(6) quinolone, against *S. pneumoniae* strains with intermediate susceptibilities to ciprofloxacin was compared with those of two fluoro(6) quinolones, sitafloxacin and ciprofloxacin, through the evaluation of bacteriological and pharmacological effects.

Data from the MICs and ED₅₀s showed that DX-619 had potent activity against *S. pneumoniae*. Furthermore, the ED₅₀ of DX-619 against PSSP was as potent as that of sitafloxacin, and against PRSP, DX-619 was more potent than sitafloxacin. Moreover, DX-619 and sitafloxacin treatment significantly decreased the numbers of viable bacteria compared with the numbers obtained with ciprofloxacin treatment. Even though the dose of ciprofloxacin was lower than an equivalent clinical dose, we administered ciprofloxacin at the same dose (10 mg/kg) at which the other two drugs were administered. Our results showed that DX-619 and sitafloxacin are efficacious against two ciprofloxacin-nonsusceptible *S. pneumoniae* isolates.

The AUC/MIC ratio is an important pharmacodynamic parameter that influences the outcome of fluoroquinolone treatment (7, 21). In this study, the AUC/MIC ratio in the lungs for DX-619 was higher than those for sitafloxacin and ciprofloxacin.

TABLE 2. Pharmacokinetic-pharmacodynamic parameters of DX-619, sitafloxacin, and ciprofloxacin in serum and lungs of mice infected with PRSP^a

Drug (MIC)	Compartment	C _{max} (μg/ml)	T _{max} (h)	t _{1/2} (h)	AUC (mg · h/ml)	Lung AUC/serum AUC ratio	AUC/MIC ratio
DX-619 (0.03)	Serum	1.26	0.25	0.56	0.96	5.34	32.0
	Lung	5.99	0.25	0.63	5.13		171.0
STFX (0.125)	Serum	0.33	1.00	1.49	0.58	4.72	4.64
	Lung	1.99	1.00	1.14	2.74		21.92
CIP (1.0)	Serum	1.00	0.25	0.59	0.76	1.61	0.76
	Lung	1.96	0.25	0.59	1.22		1.22

^a Each drug was administered at a dose of 10 mg/kg to four mice in each group. Abbreviations: STFX, sitafloxacin; CIP, ciprofloxacin; C_{max}, maximum concentration of drugs in serum or lungs; T_{max}, time to maximum concentration of drugs in serum or lungs; t_{1/2}, half-life.

cin. The differences in the AUC/MIC ratios are largely driven by differences in MICs because the lung AUCs of the three drugs varied only slightly. The ED₅₀ against PSSP and the number of viable bacteria in the lungs of DX-619-treated mice infected with PSSP were higher than expected. We think that this contradiction between the AUC/MIC ratio and the efficacy of DX-619 against PSSP may be associated with the high pathogenesis of PSSP. Thus, DX-619 exhibited good in vivo efficacy against both PSSP and PRSP.

Inagaki et al. (10) reported that DX-619 had excellent in vitro activity against clinical isolates of resistant gram-positive bacteria. The MICs at which 90% of isolates are inhibited for ciprofloxacin-resistant methicillin-resistant *S. aureus*, methicillin-resistant coagulase-negative staphylococci, PRSP, and vancomycin-resistant enterococci were 0.015, 0.12, 0.03, and 2.0 µg/ml, respectively. Indeed, the activity was comparable to or greater than those of DQ-113, linezolid, quinupristin-dalfopristin, vancomycin, and teicoplanin. This is the first study of the in vivo efficacy of DX-619 against *S. pneumoniae*.

The guidelines of the Infectious Diseases Society of America recommend the use of cefotaxime, ceftriaxone, a respiratory quinolone, or vancomycin for the treatment of pneumonia caused by PRSP for immunocompetent adults (17). Visalli et al. (27) reported on an MIC and time-kill study of sitafloxacin, ciprofloxacin, levofloxacin, cefotaxime, imipenem, and vancomycin against nine penicillin-sensitive and -resistant pneumococci; of all quinolone and nonquinolone compounds tested, sitafloxacin showed the lowest MICs and the fastest killing. In our study, DX-619 yielded good results, similar to those for sitafloxacin, in murine pneumococcal pneumonia models.

Pediatric respiratory tract infections are one of the most commonly encountered illnesses in primary care, and they are becoming more difficult to treat because of drug resistance. Therefore, the use of effective antimicrobials should be considered for the treatment of respiratory tract infections in children. Higher doses of amoxicillin or the amoxicillin component of high-dose amoxicillin-clavulanate fulfill these criteria, but these compounds cannot be used in cases of severe penicillin allergy (11). Fluoroquinolones also meet this description; however, these agents are not recommended for use by children and growing adolescents, because fluoroquinolone-treated juvenile animals developed articular cartilage lesions (15). Although the mechanism of this chondrotoxicity is largely unknown (3), the absence of the fluorine atom at position 6 of the quinolone nucleus in these molecules may reduce the frequency of toxic side effects (1). Thus, DX-619 might be a good candidate for use for the treatment of pediatric respiratory infections.

Recently, the emergence of *S. pneumoniae* strains with reduced susceptibilities to the fluoroquinolones has been described in Canada, Spain, and Hong Kong (5, 8, 16). Roychoudhury et al. (22) developed 8-methoxy, nonfluorinated quinolones and reported that the nonfluorinated quinolones were less affected in vitro by preexisting and characterized target mutations that reduce the potencies of quinolones against *S. pneumoniae*. DX-619 may also have potent efficacy against ciprofloxacin-resistant *S. pneumoniae*, but further evaluation is needed.

In conclusion, a new des-fluoro(6) quinolone, DX-619, had high degrees of efficacy against PSSP and PRSP in a mouse

model of pulmonary infection that were similar to those of sitafloxacin. Thus, DX-619 may be a promising candidate for the treatment of serious human infections due to PRSP.

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