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In Vitro Activities and Spectrum of the Novel Fluoroquinolone Lascufloxacin (KRP-AM1977)

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ABSTRACT Lascufloxacin exhibited a broad spectrum of activity against various clinical isolates. Furthermore, lascufloxacin showed the most potent activity against Gram-positive bacteria among the quinolones tested and incomplete cross-resistance against existing quinolone-resistant strains. Enzymatic analysis indicated that lascufloxacin had potent inhibitory activity against both wild-type and mutated target enzymes. These results suggest that lascufloxacin may be useful in treating infections caused by various pathogens, including quinolone-resistant strains.

KEYWORDS antimicrobial activity, antimicrobial agents, quinolones

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Recently, the emergence of strains resistant to commercially available antibacterial drugs, including β-lactams, macrolides, tetracyclines, and aminoglycosides, has become a clinical problem around the world. Quinolones are also widely used for treatment of various infectious diseases, and the development of quinolone resistance in various bacteria has become a clinical concern. For example, a large proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates showed resistance to quinolones (1, 2). For *Streptococcus pneumoniae*, a major causative organism of respiratory tract infections, the acquisition of resistance to quinolones has been reported in various areas of the world, including Southeast Asia (3). Therefore, the development of more-potent agents against resistant Gram-positive bacteria is required in preparation for the emergence of resistant strains.

Lascufloxacin is a novel fluoroquinolone antibacterial agent newly developed in Japan (Fig. 1). This study was performed to determine the antibacterial activities of lascufloxacin against various clinical isolates. In addition, the activities of lascufloxacin against sequentially selected quinolone-resistant *S. aureus*, *S. pneumoniae*, and *Escherichia coli* were evaluated, and the inhibitory activities of lascufloxacin against target enzymes of wild-type and quinolone-resistant *S. aureus* were determined. (This work was presented in part at the Interscience Conference on Antimicrobial Agents and Chemotherapy/International Congress of Chemotherapy and Infection, San Diego, CA, 2015 [4].)

Lascufloxacin and garenoxacin were synthesized by Kyorin Pharmaceutical Co., Ltd. (Tokyo, Japan). Other drugs were purchased from Eiken Chemical Co., Ltd. (Tokyo, Japan), Sigma-Aldrich (St. Louis, MO), LKT Laboratories, Inc. (St. Paul, MN), or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Various clinical strains were isolated from patients in Japanese hospitals from 2013 to 2015. Most of the clinical strains, except for *Enterococcus faecalis* and methicillin-susceptible *S. aureus* (MSSA), were limited to one isolate per patient; in some cases, two different bacterial strains, i.e., *E. faecalis* and MSSA, were isolated from one patient. Clinical strains of *S. aureus* MS5935, MS5952, MR5867, and MR6009 and their stepwise quinolone-resistant mutant strains were described previously (5). The quinolone-resistant *S. pneumoniae* isolates selected from strain IID553 were described previously (6). The quinolone-resistant *E. coli* GF4-3, CP4-1, and SP4-1 isolates were selected from K-12 strain KL-16 with gatifloxacin, ciprofloxacin,

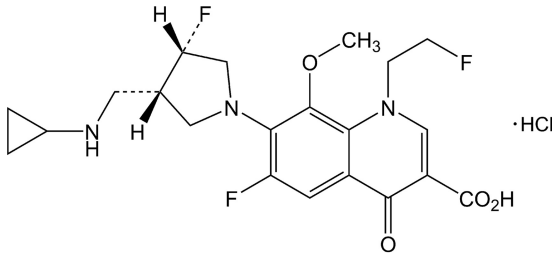

FIG 1 Structure of lascufloxacin.

and sparfloxacin, respectively. The MICs against various strains other than *Mycoplasma pneumoniae* were determined by agar or broth dilution methods as described in the Clinical and Laboratory Standards Institute guidelines (7). The MICs against *M. pneumoniae* were determined by the broth microdilution method according to Yamaguchi et al. (8), with some modifications.

The antibacterial activities of lascufloxacin and reference compounds against clinical isolates (600 strains of 14 bacterial species in total) of Gram-positive and Gram-negative bacteria are shown in Table 1. Lascufloxacin exhibited the most potent activities against Gram-positive pathogens, such as MSSA, MRSA, *Staphylococcus epidermidis*, *E. faecalis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and penicillin-susceptible and penicillin-resistant *S. pneumoniae*, among the quinolones tested. Lascufloxacin showed more-potent activities against *S. epidermidis* and *E. faecalis* than any of the other agents tested. The MIC_{90} against MRSA was 2 μg/ml. The activity of lascufloxacin against MRSA was almost the same as those of linezolid and vancomycin, and it was 32- to >64-fold superior to those of levofloxacin, garenoxacin, and ciprofloxacin.

The activities of lascufloxacin against Gram-negative bacteria and *M. pneumoniae* were similar to those of existing quinolones. In the Gram-negative bacteria, lascufloxacin showed antibacterial activities against *Moraxella catarrhalis* and β-lactamase-negative ampicillin-susceptible and ampicillin-resistant strains of *Haemophilus influenzae*, with an MIC_{90} value of 0.06 μg/ml in all cases. The MIC_{90} values against *Enterobacter* spp., *Klebsiella pneumoniae*, and *Acinetobacter* spp. were 0.25, 0.25, and 0.5 μg/ml, respectively. The activities of lascufloxacin against *E. coli* and *P. aeruginosa* were almost the same as or lower than those of the other quinolones tested. The MIC_{50} and MIC_{90} values of lascufloxacin against *M. pneumoniae* were 0.12 and 0.25 μg/ml, respectively, which were similar to or slightly superior to those of levofloxacin. The emergence of macrolide resistance in *M. pneumoniae* has been reported (9), and macrolide-resistant isolates were detected in the present study. The MIC_{90}s of clarithromycin and azithromycin were >16 and 16 μg/ml, respectively; on the other hand, lascufloxacin showed potent activity against macrolide-resistant *M. pneumoniae* clinical isolates.

Table 2 shows the antibacterial activities of lascufloxacin against clinical strains of *S. aureus* and their sequentially selected quinolone-resistant mutant strains. As the number of mutations of the target enzyme increased, the susceptibility of bacteria against quinolones decreased. However, compared with the other quinolones tested, lascufloxacin showed more potent activities even against third- and fourth-step mutants of *S. aureus*. The MICs of lascufloxacin against parent strains ranged from 0.008 to 0.015 μg/ml, and those against fourth-step *parC*, *gyrA*, *parC*, and *gyrA* mutant strains were all 2 μg/ml. A similar increase in MICs through the level of resistance to quinolones was found in other quinolones tested. The MICs of levofloxacin increased from 0.125 to 128 μg/ml, and those of garenoxacin increased from 0.015 or 0.03 μg/ml to 32 or 64 μg/ml. The MIC ratios of lascufloxacin between parent strains and their fourth-step mutant strains were 128 to 256, whereas those of other quinolones tested were 128 to 4,096. The increases in the MICs of lascufloxacin with the acquisition of resistance were smaller than those of levofloxacin and garenoxacin. These results suggest that lascufloxacin

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TABLE 1 *In vitro* antibacterial activities of lascufloxacin against various clinical isolates

Organism[a] (no. of isolates)	Drug	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
MSSA (30)	Lascufloxacin	≤0.008 to 0.015	0.015	0.015
	Levofloxacin	0.12 to 0.25	0.25	0.25
	Garenoxacin	≤0.008 to 0.03	0.015	0.03
	Azithromycin	1 to >64	2	>64
	Ceftriaxone	2 to 4	2	4
	Cefcapene	1 to 2	1	2
	Meropenem	≤0.06 to 0.12	≤0.06	≤0.06
	Oxacillin	0.12 to 1	0.25	0.5
MRSA (100)	Lascufloxacin	0.03 to 2	0.5	2
	Levofloxacin	0.25 to >128	64	>128
	Garenoxacin	0.06 to 64	8	64
	Ciprofloxacin	0.5 to >128	128	>128
	Ceftazidime	16 to >128	>128	>128
	Imipenem	0.06 to >128	32	128
	Vancomycin	0.5 to 2	1	1
	Teicoplanin	0.5 to 8	1	4
	Oxacillin	16 to >128	>128	>128
	Arbekacin	0.25 to 8	1	2
	Daptomycin	0.25 to 1	0.5	0.5
	Linezolid	1 to 4	2	2
S. epidermidis (30)	Lascufloxacin	0.015 to 0.12	0.03	0.12
	Levofloxacin	0.12 to 8	0.25	4
	Garenoxacin	0.03 to 1	0.06	1
	Azithromycin	0.25 to >64	0.5	>64
	Ceftriaxone	1 to >128	4	32
	Cefcapene	0.12 to >128	0.5	8
	Meropenem	≤0.06 to 32	0.5	4
	Oxacillin	≤0.06 to >128	1	32
E. faecalis (30)	Lascufloxacin	0.06 to 0.5	0.06	0.12
	Levofloxacin	1 to >16	1	2
	Garenoxacin	0.12 to 2	0.12	0.25
	Azithromycin	4 to >64	>64	>64
	Ceftriaxone	64 to >128	>128	>128
	Cefcapene	64 to >128	>128	>128
	Meropenem	2 to 8	4	8
S. pyogenes (30)	Lascufloxacin	0.03 to 0.12	0.03	0.06
	Levofloxacin	0.5 to 2	0.5	1
	Garenoxacin	0.03 to 0.12	0.06	0.12
	Azithromycin	0.12 to >16	0.12	16
	Ceftriaxone	0.015	0.015	0.015
	Cefcapene	≤0.008	≤0.008	≤0.008
	Meropenem	≤0.008	≤0.008	≤0.008
S. agalactiae (30)	Lascufloxacin	0.06 to 1	0.06	0.5
	Levofloxacin	0.5 to >16	1	>16
	Garenoxacin	0.06 to 4	0.06	4
	Azithromycin	0.06 to >16	0.06	>16
	Ceftriaxone	0.06 to 0.25	0.06	0.12
	Cefcapene	0.03 to 0.12	0.03	0.06
	Meropenem	0.03 to 0.06	0.03	0.06
PSSP (30)	Lascufloxacin	0.03 to 0.06	0.06	0.06
	Levofloxacin	0.5 to 1	1	1
	Garenoxacin	0.03 to 0.06	0.06	0.06
	Azithromycin	0.03 to >16	>16	>16
	Ceftriaxone	0.015 to 0.25	0.12	0.25
	Cefcapene	≤0.008 to 0.5	0.25	0.5
	Meropenem	≤0.008 to 0.015	≤0.008	0.015
	Benzylpenicillin	0.015 to 0.06	0.03	0.06

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TABLE 1 (Continued)

Organism[a] (no. of isolates)	Drug	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
PRSP (30)	Lascufloxacin	0.03 to 0.06	0.06	0.06
	Levofloxacin	0.5 to 1	1	1
	Garenoxacin	0.03 to 0.06	0.06	0.06
	Azithromycin	1 to >16	>16	>16
	Ceftriaxone	0.5 to 4	1	2
	Cefcapene	0.5 to 4	1	2
	Meropenem	0.25 to 1	0.5	1
	Benzylpenicillin	2 to 4	2	4
E. coli (30)	Lascufloxacin	0.06 to >16	0.25	>16
	Levofloxacin	0.015 to >16	0.06	16
	Garenoxacin	0.015 to >16	0.12	>16
	Azithromycin	4 to 64	8	64
	Ceftriaxone	≤0.06 to >128	≤0.06	>128
	Cefcapene	0.25 to >128	0.5	128
	Meropenem	≤0.06	≤0.06	≤0.06
Enterobacter spp. (30)	Lascufloxacin	0.12 to 0.5	0.25	0.25
	Levofloxacin	0.03 to 0.06	0.06	0.06
	Garenoxacin	0.06 to 0.25	0.12	0.12
	Azithromycin	8 to 32	16	16
	Ceftriaxone	≤0.06 to 32	0.12	16
	Cefcapene	0.5 to 32	0.5	32
	Meropenem	≤0.06 to 0.12	≤0.06	≤0.06
K. pneumoniae (30)	Lascufloxacin	0.12 to 0.5	0.25	0.25
	Levofloxacin	0.03 to 0.12	0.06	0.06
	Garenoxacin	0.06 to 0.25	0.12	0.12
	Azithromycin	8 to 16	16	16
	Ceftriaxone	≤0.06	≤0.06	≤0.06
	Cefcapene	0.25 to 1	0.5	1
	Meropenem	≤0.06	≤0.06	≤0.06
Acinetobacter spp. (30)	Lascufloxacin	0.06 to 16	0.25	0.5
	Levofloxacin	0.03 to 8	0.06	0.25
	Garenoxacin	0.015 to 8	0.03	0.12
	Azithromycin	0.5 to >64	1	64
	Ceftriaxone	2 to >128	8	16
	Cefcapene	4 to 128	16	16
	Meropenem	0.12 to 2	0.25	0.5
P. aeruginosa (30)	Lascufloxacin	1 to >16	4	>16
	Levofloxacin	0.25 to >16	0.5	>16
	Garenoxacin	0.5 to >16	1	>16
	Azithromycin	8 to >64	64	>64
	Ceftriaxone	8 to >128	32	>128
	Cefcapene	16 to >128	32	>128
	Meropenem	0.12 to 64	0.25	8
M. catarrhalis (30)	Lascufloxacin	0.06 to 0.12	0.06	0.06
	Levofloxacin	0.03	0.03	0.03
	Garenoxacin	≤0.008 to 0.015	≤0.008	0.015
	Azithromycin	≤0.06 to 0.12	≤0.06	≤0.06
	Ceftriaxone	≤0.06 to 1	0.5	1
	Cefcapene	≤0.06 to 1	0.5	1
	Meropenem	≤0.06	≤0.06	≤0.06
BLNAS (30)	Lascufloxacin	0.03 to 0.06	0.03	0.06
	Levofloxacin	0.015 to 0.03	0.015	0.03
	Garenoxacin	≤0.004 to 0.015	0.008	0.015
	Azithromycin	0.5 to 4	1	2
	Ceftriaxone	≤0.008 to 0.25	≤0.008	0.25
	Cefcapene	≤0.008 to 2	0.015	1
	Meropenem	0.015 to 0.06	0.03	0.06
	Ampicillin	0.12 to 1	0.5	1

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TABLE 1 (Continued)

Organism[a] (no. of isolates)	Drug	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
BLNAR (30)	Lascufloxacin	0.03 to 0.12	0.03	0.06
	Levofloxacin	0.015 to 0.06	0.015	0.03
	Garenoxacin	≤0.004 to 0.03	0.008	0.015
	Azithromycin	0.5 to 4	1	2
	Ceftriaxone	0.03 to 0.25	0.25	0.25
	Cefcapene	0.12 to 2	2	2
	Meropenem	0.03 to 0.5	0.25	0.25
	Ampicillin	2 to 8	4	4
M. pneumoniae (50)	Lascufloxacin	0.06 to 0.25	0.12	0.25
	Levofloxacin	0.25 to 0.5	0.5	0.5
	Garenoxacin	0.008 to 0.03	0.015	0.03
	Clarithromycin	≤0.001 to >16	>16	>16
	Azithromycin	≤0.001 to 16	8	16

[a]PSSP and PRSP, penicillin-susceptible and penicillin-resistant *S. pneumoniae*, respectively; BLNAS and BLNAR, β-lactamase-negative ampicillin-susceptible and ampicillin-resistant strains of *Haemophilus influenzae*, respectively.

showed incomplete cross-resistance against these mutant strains. Small MIC ratios of lascufloxacin between wild-type and quinolone-resistant strains were also observed in *S. pneumoniae* and *E. coli* isolates (Table 2). The activities of lascufloxacin against first- and second-step mutant strains of *S. pneumoniae* were more potent than the activities

TABLE 2 Antibacterial activity of lascufloxacin against quinolone-resistant strains

Strain[a]	Mutation		MIC (μg/ml) for:			
	GyrA	ParC	Lascufloxacin	Levofloxacin	Garenoxacin	Ciprofloxacin
S. aureus						
MS5935	None	None	0.015	0.125	0.015	0.25
MS5935 *gyrA* mutant	Ser84Leu	None	0.03	0.25	0.06	0.25
MS5935 1st	None	Ser80Phe	0.03	0.5	0.03	2
MS5935 2nd	Ser84Leu	Ser80Phe	0.125	8	2	16
MS5935 3rd	Ser84Leu	Ser80Phe, Glu84Lys	0.5	32	8	32
MS5935 4th	Ser84Leu, Glu88Val	Ser80Phe, Glu84Lys	2	128	32	32
MS5952	None	None	0.015	0.125	0.03	0.25
MS5952 1st	None	Ser80Tyr	0.03	0.5	0.06	2
MS5952 2nd	Ser84Leu	Ser80Tyr	0.06	2	1	8
MS5952 3rd	Ser84Leu	Ser80Tyr, Ala116Val	0.25	16	0.5	32
MR5867	None	None	0.015	0.125	0.015	0.25
MR5867 1st	None	Glu84Lys	0.03	0.5	0.03	2
MR5867 2nd	Ser84Leu	Glu84Lys	0.125	8	0.5	16
MR5867 3rd	Ser84Leu	Glu84Lys, Ser80Phe	0.5	16	4	32
MR5867 4th	Ser84Leu, Glu88Lys	Glu84Lys, Ser80Phe	2	128	32	32
MR6009	None	None	0.008	0.125	0.015	0.25
MR6009 1st	None	Ser80Tyr	0.03	0.5	0.03	2
MR6009 2nd	Glu88Lys	Ser80Tyr	0.125	4	0.25	8
MR6009 3rd	Glu88Lys	Ser80Tyr, Glu84Lys	0.25	8	0.25	32
MR6009 4th	Glu88Lys, Ser84Leu	Ser80Tyr, Glu84Lys	2	128	64	32
S. pneumoniae						
IID553	None	None	0.06	1	0.06	1
NF9884	None	Ser79Tyr	0.125	2	0.125	2
CF9842	None	Asp83Asn	0.06	2	0.125	2
SF9863	Ser81Phe	None	0.06	2	0.25	2
GF9821	Ser81Tyr	None	0.125	2	0.25	2
ST9941	Ser81Phe	Ser79Phe	0.5	16	2	64
SN9981	Ser81Phe	Asp83Tyr	0.25	16	1	32
E. coli						
KL-16	None	None	0.125	0.03	0.03	0.008
GF4-3	Asp87Tyr	None	0.5	0.125	0.125	0.125
CP4-1	Asp87Asn	None	0.5	0.25	0.125	0.125
SP4-1	Ser83Leu	None	0.5	0.25	0.25	0.125

[a]The mutant strains of *S. aureus*, *S. pneumoniae*, and *E. coli* were sequentially selected from quinolone-susceptible strains.

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TABLE 3 Frequencies of appearance of mutant strains by selection with lascufloxacin

Strain	Drug	MIC (μg/ml)	Frequency at the following multiple of MIC[a]:		
			4 × MIC	8 × MIC	16 × MIC
S. aureus ATCC 29213	Lascufloxacin	0.008	$<2.1 \times 10^{-9}$	$<2.1 \times 10^{-9}$	$<2.1 \times 10^{-9}$
	Levofloxacin	0.125	$<2.1 \times 10^{-9}$	$<2.1 \times 10^{-9}$	$<2.1 \times 10^{-9}$
	Garenoxacin	0.015	8.2×10^{-9}	$<2.1 \times 10^{-9}$	$<2.1 \times 10^{-9}$
	Ciprofloxacin	0.25	6.2×10^{-9}	3.1×10^{-9}	$<2.1 \times 10^{-9}$
S. pneumoniae ATCC 49619	Lascufloxacin	0.06	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$
	Levofloxacin	1	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$
	Garenoxacin	0.06	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$
	Ciprofloxacin	1	1.1×10^{-8}	$<1.1 \times 10^{-8}$	$<1.1 \times 10^{-8}$
E. coli ATCC 25922	Lascufloxacin	0.06	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$
	Levofloxacin	0.015	3.9×10^{-9}	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$
	Garenoxacin	0.03	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$
	Ciprofloxacin	0.008	1.2×10^{-8}	$<1.9 \times 10^{-9}$	$<1.9 \times 10^{-9}$

[a]$n = 2$, average.

of other quinolones, and the MICs of lascufloxacin against *gyrA* and *parC* double mutants were 0.25 to 0.5 μg/ml.

To evaluate the selectivity of mutant strains, the frequency of resistance of lascufloxacin was determined against *S. aureus*, *S. pneumoniae*, and *E. coli* (Table 3). Bacteria were incubated for ~70 h on Mueller-Hinton agar (*S. aureus* and *E. coli*) or Mueller-Hinton agar containing 5% defibrinated sheep blood (*S. pneumoniae*) with quinolones at 4, 8, and 16 times the MICs. It was revealed that resistant strains were not selected at all concentrations of lascufloxacin against three bacterial species. The frequencies of lascufloxacin were the same as or lower than those of other quinolones tested. These results and the MIC data shown in Table 2 suggest that the potent activity of lascufloxacin against mutant strains might contribute to the low level of resistance selectivity.

To clarify the mechanism underlying the incomplete cross-resistance of lascufloxacin, the inhibitory activities of lascufloxacin against DNA gyrase and topoisomerase IV of wild-type and quinolone-resistant *S. aureus* were determined (Table 4). The DNA gyrase and topoisomerase IV of *S. aureus* isolates were cloned and expressed in *E. coli*, and each subunit was purified (10). Human topoisomerase II was purchased from TopoGen (Buena Vista, CO). The activities of DNA gyrase and topoisomerase IV were determined as described previously (10). The inhibitory activities of quinolones against topoisomerases were assayed by determining the concentrations required to inhibit 50% of the enzyme reaction (IC_{50}). Against human topoisomerase II, the IC_{50} of lascufloxacin was >2,400 μg/ml, and high selectivity to bacterial topoisomerases was observed. Lascufloxacin showed the most potent inhibitory activity against all target enzymes among the quinolones tested. The ratio of the IC_{50} against DNA gyrase of the quinolone-resistant strain to that of the wild-type strain was 10 for lascufloxacin, which was the lowest value among the quinolones tested. The smallest IC_{50} ratio of lascu-

TABLE 4 Inhibitory activities of lascufloxacin against DNA gyrase and topoisomerase IV of *S. aureus* and human topoisomerase II

Drug	IC_{50} (μg/ml)[a]					IC_{50} ratio of QR to wild-type strain	
	Wild-type DNA gyrase	QR[b] DNA gyrase	Wild-type topoisomerase IV	QR[b] topoisomerase IV	Human topoisomerase II	DNA gyrase	Topoisomerase IV
Lascufloxacin	1.7 ± 0.4	17 ± 3	0.73 ± 0.06	2.8 ± 0.3	>2400	10	3.8
Levofloxacin	16 ± 6	1300 ± 100	2.8 ± 0.9	86 ± 19	1400 ± 100	81	31
Garenoxacin	11 ± 1	420 ± 80	1.9 ± 0.6	27 ± 1	750 ± 280	38	14
Ciprofloxacin	25 ± 5	>1200	1.8 ± 0.7	69 ± 7	>1100	>48	38

[a]$n = 3$, average ± SD.
[b]QR, quinolone resistant. Mutations of DNA gyrase and topoisomerase IV were GyrA Ser84Leu and ParC Ser80Phe, respectively.

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floxacin was also observed in the topoisomerase IV assay. The ratio of the IC_{50} of lascufloxacin against mutated topoisomerase IV to that of the wild-type strain was 3.8. Lascufloxacin showed potent inhibitory activities against not only wild-type but also mutated enzymes; therefore, the ratios of the IC_{50}s of lascufloxacin of mutated enzymes to wild-type enzymes were lower than those of the other quinolones. These results support incomplete cross-resistance of the antibacterial activity of lascufloxacin against quinolone-resistant strains.

In summary, lascufloxacin exhibited potent antibacterial activity against various pathogens. In particular, lascufloxacin showed the most potent activity against Gram-positive bacteria, such as *S. aureus*, *S. epidermidis*, *E. faecalis*, *S. pyogenes*, *S. agalactiae*, and *S. pneumoniae*, among the quinolones tested. The MIC_{90}s of lascufloxacin against *H. influenzae*, *M. catarrhalis*, and *M. pneumoniae* were 0.06, 0.06, and 0.25, respectively, although the clinical isolates used in this study included various resistant strains. Moreover, lascufloxacin showed potent antibacterial activity against MRSA that was similar to those of linezolid and vancomycin, suggesting the high efficacy of lascufloxacin for severe infectious diseases caused by MRSA. The results presented here suggested that lascufloxacin showed incomplete cross-resistance against existing quinolone-resistant strains and potent antibacterial activities against sequentially selected quinolone-resistant mutant strains. The inhibitory activities of lascufloxacin resulted in only a small decrease against the quinolone-resistant enzymes induced by genetic mutation of the targets, compared with those from the wild-type strain. The greater inhibitory activities of lascufloxacin against mutated target enzymes were likely responsible for the more-potent antibacterial activities against quinolone-resistant bacteria compared to those of the other quinolones examined in this study.

The present study revealed that lascufloxacin showed high selectivity to bacterial topoisomerases, with little activity against the human topoisomerase II (Table 4). In the toxicity studies, lascufloxacin showed similar or decreased class effects of quinolones, such as genotoxicity, articular toxicity, and phototoxicity, than other quinolones in animals. In humans, lascufloxacin was well tolerated in oral single doses up to 800 mg and multiple doses up to 400 mg once daily for 7 days and exhibited appropriate pharmacokinetic (PK) profiles (11). The plasma concentration of lascufloxacin achieved a mean maximum concentration of drug in serum (C_{max}) of 0.732 μg/ml and an area under the serum concentration-time curve from time zero to infinity (AUC_{inf}) of 12.7 $\mu g \cdot h \cdot ml^{-1}$ and was eliminated with a mean half-life ($t_{1/2}$) of 16.1 h at the 100-mg oral single dose in healthy subjects. The C_{max} and AUC for lascufloxacin increased in an approximately dose-proportional manner up to 800 mg, and the plasma protein binding was 74.0%. Furthermore, a clinical pharmacological study indicated that lascufloxacin showed excellent distribution in the lung; the average ratio of epithelial lining fluid to plasma concentration ranged from 15.0 to 22.4 (12).

Based on its remarkable PK profile and potent antibacterial activity against respiratory pathogens, lascufloxacin may be a promising agent for the treatment of various infectious diseases, including lower respiratory tract infections. Further clinical studies are required to determine the applicability of lascufloxacin.

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