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Improved Antimicrobial Activity of DU-6859a, a New Fluoroquinolone, against Quinolone-Resistant *Klebsiella pneumoniae* and *Enterobacter cloacae* Isolates with Alterations in GyrA and ParC Proteins

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MICs of DU-6859a, a novel fluoroquinolone, for 18 *Klebsiella pneumoniae* isolates and 21 *Enterobacter cloacae* isolates with altered GyrA or altered GyrA and ParC ranged from ≤0.025 to 6.25 μg/ml and from 0.1 to 3.13 μg/ml, respectively. Based on the MICs at which 90% of the isolates were inhibited for these strains of *K. pneumoniae* and *E. cloacae*, DU-6859a exhibited 16- to 256-fold-greater activity than currently available fluoroquinolones.

Selected fluoroquinolones have often been administered to patients with urinary tract infections and have been effective in treating such infections. However, we have noted the isolation of quinolone-resistant bacterial strains from the urinary tract and fluoroquinolone treatment failures associated with the development of quinolone resistance in bacterial pathogens. Among the mechanisms of quinolone resistance, alterations in the GyrA subunit of DNA gyrase have been demonstrated to play a central role in conferring high-level quinolone resistance in several gram-negative bacterial species (1, 4, 8, 16, 17). In addition, alterations in the ParC subunit of topoisomerase IV seem to play a complementary role in increasing resistance to fluoroquinolones (1, 6, 7, 9, 11). Therefore, to maintain the efficacy of fluoroquinolones for the treatment of urinary tract infections, the agents must exhibit potent activity against quinolone-resistant strains harboring alterations in GyrA and ParC.

DU-6859a is a novel fluorinated quinolone currently undergoing clinical trials. Initial susceptibility data indicated that this compound had a broad antimicrobial spectrum and potent activity against gram-positive and gram-negative bacteria (14). In addition, Kitamura et al. reported that DU-6859a caused significantly greater inhibition of altered DNA gyrase purified from quinolone-resistant strains of *Pseudomonas aeruginosa* and was much more active against quinolone-resistant clinical isolates of *P. aeruginosa* than currently used fluoroquinolones (10). However, there is little information available on the activity of this agent against strains of other bacterial species harboring altered DNA gyrase and topoisomerase IV (3). *Klebsiella pneumoniae* and *Enterobacter cloacae* strains as well as *P. aeruginosa* are common pathogens causing urinary tract infections. In our previous studies, we determined the correlation of alterations in GyrA and ParC with high levels of ciprofloxacin resistance in urinary tract-derived strains of *K. pneumoniae* and *E. cloacae* (2, 5). In the present study, we attempted to assess whether DU-6859a was active against *K. pneumoniae* and *E. cloacae* clinical isolates with altered GyrA and ParC.

The type strain of *K. pneumoniae*, ATCC 13883, and that of *E. cloacae*, ATCC 13047, were purchased from the American Type Culture Collection. The 26 *K. pneumoniae* and 26 *E. cloacae* clinical isolates used in the present study were isolated from 1991 through 1993 from Japanese patients with complicated urinary tract infections. No patients were receiving antimicrobial agents at the time of isolation of *K. pneumoniae* or *E. cloacae*, and no information about prior use of antimicrobials was available. In our previous studies, the type strains and all of the clinical strains were examined for mutations in the region corresponding to the quinolone resistance-determining region of the *Escherichia coli gyrA* gene (16) and the analogous region of the *parC* gene, and they were tested for susceptibility to ciprofloxacin (2, 5). In this study, their antimicrobial susceptibilities to DU-6859a, norfloxacin, ofloxacin, and sparfloxacin were determined by the agar dilution method.

Statistical analysis was conducted with the Wilcoxon rank-sum test to compare the MIC distributions for DU-6859a and each of the other fluoroquinolones tested. Differences between groups were considered to be statistically significant at P of <0.05.

Tables 1 and 2 summarize the antimicrobial activities of DU-6859a and other fluoroquinolones against *K. pneumoniae* and *E. cloacae* strains. A single amino acid change in GyrA was sufficient to decrease susceptibility to DU-6859a and other fluoroquinolones, and the accumulation of alterations in GyrA and the simultaneous presence of alterations in ParC were associated with the development of higher-level resistance to fluoroquinolones, including DU-6859a. However, some isolates exhibited inconsistencies between the status of alterations in GyrA and ParC and the fluoroquinolone MICs. Other mechanisms, including alterations in GyrB of DNA gyrase and ParE of topoisomerase IV and the prevention of drug access to bacterial target enzymes by permeability barriers and active efflux, were able to influence the level of quinolone resistance.

The MICs of DU-6859a for 18 isolates of *K. pneumoniae* consisting of 12 isolates with altered GyrA only and 6 isolates with both altered GyrA and altered ParC ranged from ≤0.025 to 6.25 μg/ml (Table 1). The MICs of DU-6859a inhibiting 50% (MIC_{50}) and 90% (MIC_{90}) of these 18 isolates were 0.39 and 1.56 μg/ml, respectively, whereas the MIC_{50}s and MIC_{90}s of norfloxacin, ofloxacin, ciprofloxacin, and sparfloxacin were 12.5 and 200 μg/ml, 6.25 and 50 μg/ml, 3.13 and 50 μg/ml, and

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TABLE 1. Alterations in GyrA and ParC and antimicrobial activities of DU-6859a, norfloxacin, ofloxacin, ciprofloxacin, and sparfloxacin against clinical isolates of *K. pneumoniae*

Strain	Amino acid[a] at the indicated position in:				MIC (μg/ml)[b]				
	GyrA		ParC		DU-6859a	NFLX	OFLX	CPFX	SPFX
	83	87	80	84					
Type strain	Ser	Asp	Ser	Glu	≤0.025	0.2	0.1	0.05	0.05
102	—	—	—	—	≤0.025	0.2	0.1	≤0.025	0.05
193	—	—	—	—	≤0.025	0.2	0.1	≤0.025	≤0.025
424	—	—	—	—	≤0.025	0.2	0.2	0.05	0.05
544	—	—	—	—	≤0.025	0.05	0.1	≤0.025	≤0.025
600	—	—	—	—	≤0.025	0.2	0.1	≤0.025	≤0.025
743	—	—	—	—	≤0.025	0.2	0.1	≤0.025	≤0.025
196	—	—	—	—	0.1	1.56	0.78	0.39	0.78
413	—	—	—	—	0.1	0.78	0.78	0.2	0.39
377	—	Gly	—	—	≤0.025	0.78	0.39	0.1	0.1
338	Phe	—	—	—	≤0.025	3.13	0.78	0.39	0.39
017	Tyr	—	—	—	0.05	6.25	1.56	0.78	0.78
166	Tyr	—	—	—	0.05	3.13	1.56	0.39	0.2
430	Phe	—	—	—	0.1	0.78	1.56	0.39	0.39
227	Tyr	—	—	—	0.1	1.56	1.56	0.78	0.78
555	Phe	—	—	—	0.2	12.5	3.13	3.13	3.13
356	—	Gly	—	—	0.39	25	6.25	3.13	1.56
397	Tyr	—	—	—	0.39	6.25	6.25	1.56	1.56
143	Phe	Gly	—	—	0.39	3.13	3.13	1.56	0.78
467	Phe	Asn	—	—	1.56	25	25	12.5	12.5
596	Phe	Asn	—	—	1.56	50	25	6.25	3.13
237	Phe	Asn	—	Gly	0.39	50	6.25	6.25	6.25
043	Phe	Ala	Ile	—	0.78	50	12.5	12.5	12.5
802	Phe	Asn	Ile	—	0.78	50	25	25	12.5
300	Phe	Gly	—	Lys	1.56	400	50	50	25
211	Phe	Asn	Arg	—	3.13	200	200	50	50
199	Phe	Gly	—	Gly	6.25	800	100	400	100

[a] Amino acids in GyrA and ParC were analyzed in our previous study (2). —, identical to the type strain.
[b] NFLX, norfloxacin; OFLX, ofloxacin; CPFX, ciprofloxacin; SPFX, sparfloxacin. Ciprofloxacin MICs were derived in our previous study (2).

TABLE 2. Alterations in GyrA and ParC and antimicrobial activities of DU-6859a, norfloxacin, ofloxacin, ciprofloxacin, and sparfloxacin against clinical isolates of *E. cloacae*

Strain	Amino acid[a] at the indicated position in:				MIC (μg/ml)[b]				
	GyrA		ParC		DU-6859a	NFLX	OFLX	CPFX	SPFX
	83	87	80	84					
Type strain	Ser	Asp	Ser	Glu	≤0.025	0.05	0.05	≤0.025	≤0.025
009	—	—	—	—	≤0.025	0.1	0.05	≤0.025	≤0.025
092	—	—	—	—	≤0.025	0.1	0.1	≤0.025	≤0.025
192	—	—	—	—	≤0.025	0.05	≤0.025	≤0.025	≤0.025
219	—	—	—	—	≤0.025	0.1	0.05	≤0.025	≤0.025
261	—	—	—	—	≤0.025	0.39	0.1	≤0.025	0.05
023	Tyr	—	—	—	0.1	3.13	1.56	0.39	0.78
167	Tyr	—	—	—	0.2	3.13	1.56	0.39	0.2
199	Phe	—	—	—	0.39	25	6.25	3.13	1.56
546	Tyr	—	—	Gln	0.1	25	6.25	3.13	1.56
126	Phe	His	—	—	3.13	200	50	25	12.5
316	Phe	Gly	—	—	3.13	200	50	25	12.5
033	Tyr	Ala	Ile	—	0.39	200	12.5	25	25
160	Tyr	Asn	Ile	—	0.39	200	12.5	25	12.5
170	Tyr	Asn	Ile	—	0.78	400	25	25	25
001	Tyr	Val	—	Lys	1.56	100	50	25	50
050	Tyr	Asn	Ile	—	1.56	200	50	50	50
051	Tyr	Asn	Ile	—	1.56	100	50	25	50
119	Tyr	Asn	Ile	—	1.56	200	50	25	50
284	Tyr	Ala	Ile	—	1.56	200	50	50	25
509	Tyr	Ala	Ile	—	1.56	800	50	50	50
008	Tyr	Asn	Ile	—	3.13	400	100	50	100
075	Tyr	Asn	Ile	—	3.13	200	100	50	100
177	Tyr	Ala	Ile	—	3.13	200	50	50	50
181	Tyr	Asn	Ile	—	3.13	>800	200	100	200
186	Tyr	Asn	Ile	—	3.13	800	100	100	100
605	Tyr	Asn	Ile	—	3.13	800	100	100	100

[a] Amino acids in GyrA and ParC were analyzed in our previous study (5). —, identical to the type strain.
[b] NFLX, norfloxacin; OFLX, ofloxacin; CPFX, ciprofloxacin; SPFX, sparfloxacin. Ciprofloxacin MICs were derived in our previous study (5).

1.56 and 25 μg/ml, respectively. There were significant differences in MIC distribution between DU-6859a and each of the other fluoroquinolones. Based on MIC_{90}s, DU-6859a exhibited 16- to 128-fold-greater activity against these *K. pneumoniae* isolates. For the six isolates with double amino acid changes in GyrA and single amino acid changes in ParC, which exhibited remarkably decreased susceptibilities to other fluoroquinolones (MICs, 50 to 800 μg/ml for norfloxacin, 6.25 to 100 μg/ml for ofloxacin, 6.25 to 400 μg/ml for ciprofloxacin, and 6.25 to 100 μg/ml for sparfloxacin), the MICs of DU-6859a ranged from 0.39 to 6.25 μg/ml.

The MICs of DU-6859a for 21 isolates of *E. cloacae*, which included 5 isolates with alterations only in GyrA and 16 with alterations in both GyrA and ParC, were 0.1 to 3.13 μg/ml, with a MIC_{50} of 1.56 μg/ml and a MIC_{90} of 3.13 μg/ml (Table 2). For these isolates, the MIC_{50}s and MIC_{90}s of norfloxacin, ofloxacin, ciprofloxacin, and sparfloxacin were 200 and 800 μg/ml, 50 and 100 μg/ml, 25 and 50 μg/ml, and 25 and 100 μg/ml, respectively. DU-6859a was significantly more active than the other fluoroquinolones, exhibiting 32- to 256-fold-greater activity against these *E. cloacae* strains based on the MIC_{90}s. For the 15 strains with double amino acid changes in GyrA and single amino acid changes in ParC, which showed resistance to the fluoroquinolones (MICs, 200 to >800 μg/ml for norfloxacin, 12.5 to 200 μg/ml for ofloxacin, 25 to 100 μg/ml for ciprofloxacin, and 12.5 to 200 μg/ml for sparfloxacin) (13), the MICs of DU-6859a were 0.39 to 3.13 μg/ml.

A previous study on the structure-activity relationship of DU-6859a demonstrated that the introduction of the C-8 chloride into DU-6859a contributed to its great inhibitory effect on altered DNA gyrase of *P. aeruginosa*, leading to its potent activity against quinolone-resistant *P. aeruginosa* strains (10). In the present study, DU-6859a was found to be significantly more active than other tested fluoroquinolones against the isolates of *K. pneumoniae* and *E. cloacae* with altered GyrA and ParC. This result suggests that the potent antimicrobial activity of DU-6859a may be attributable to its strong inhibitory effect not only on altered DNA gyrases but also on mutant quinolone-resistant topoisomerase IV.

DU-6859a is absorbed rapidly and completely from the gastrointestinal tract and is excreted principally into the urine without undergoing change (12). However, the antimicrobial activity of this compound is somewhat lower in the urine under acidic conditions and in the presence of magnesium and ferrous ions (15). Further studies are required to determine the clinical efficacy of DU-6859a. However, this novel fluoroquinolone appears to be a potentially useful agent for treating patients with urinary tract infections involving fluoroquinolone-resistant strains because of its improved potent antimicrobial activity against strains bearing altered DNA gyrase and topoisomerase IV.

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