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In Vitro Activities of DC-159a, a Novel Fluoroquinolone, against *Mycobacterium* Species[▽]

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The *in vitro* activities of DC-159a against seven species of *Mycobacterium* were compared with moxifloxacin, gatifloxacin, levofloxacin, and rifampin. DC-159a was the most active compound against quinolone-resistant multidrug-resistant *M. tuberculosis* (MIC_{90}, 0.5 μg/ml) as well as drug-susceptible isolates (MIC_{90}, 0.06 μg/ml). The anti-tubercle bacilli activity of DC-159a was 4- to 32-fold more potent than those of currently available quinolones. DC-159a also demonstrated the highest activities against clinically important nontuberculous mycobacteria.

To be effective, tuberculosis (TB) treatment with a multidrug regimen must be continued for at least 6 months. This complicated regimen makes TB control difficult because of the long treatment duration, resulting in nonadherence of treatment, the development of multidrug-resistant TB (MDR-TB), and an additional problem of TB and human immunodeficiency virus (HIV) coinfection. Consequently, new anti-TB agents are urgently needed to overcome these problems (14).

Quinolones such as ofloxacin and levofloxacin are classified and used as second-line drugs for MDR-TB cases because they inhibit the supercoiling action of DNA gyrase, which is different from the target enzymes of first-line drugs; however, their bactericidal activities against *Mycobacterium tuberculosis* are weak in clinical use (15). Currently, moxifloxacin and gatifloxacin, which are 8-methoxy fluoroquinolones, have proven to have more potent activities and may enable the duration of treatment to be shortened. Therefore, combination regimens consisting of new quinolones are expected to improve the treatment of TB (3, 11, 13). However, there is a problem of cross-resistance among quinolones caused by the previous usage of old quinolones in the treatment of MDR-TB and other respiratory infections. Thus, the emergence of quinolone-resistant *M. tuberculosis* is a concern (4).

DC-159a is a newly synthesized broad-spectrum 8-methoxy fluoroquinolone. This compound has been shown previously to have potent activities against various respiratory pathogens, including quinolone-resistant strains (2, 7). The present study was performed to compare the *in vitro* antimycobacterial activities of DC-159a with three currently available quinolones and also rifampin against *M. tuberculosis*, including quinolone-resistant MDR (QR-MDR) strains and six species of clinically important nontuberculous mycobacteria (NTM).

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Bacterial strains were isolated from Japanese patients between 1997 and 2003. The 32 *M. tuberculosis* isolates included drug-susceptible isolates ($n = 21$) and QR-MDR isolates ($n = 11$; levofloxacin MICs, ≥2 μg/ml; rifampin MICs, ≥6 μg/ml; isoniazid MICs, ≥4 μg/ml). The NTM isolates comprised slowly growing mycobacteria, including *M. kansasii* ($n = 22$), *M. avium* (serovar 4 [$n = 10$] and serovar 8 [$n = 23$]), and *M. intracellulare* serovar 16 ($n = 17$), and rapidly growing mycobacteria, including *M. fortuitum* ($n = 10$), *M. abscessus* ($n = 12$), and *M. chelonae* ($n = 10$). The bacterial strains were isolated from clinical specimens and cultured in Middlebrook 7H9 broth medium (Difco, MD) supplemented with 10% albumin–dextrose–catalase enrichment and 0.2% glycerol to provide 10^7-CFU/ml stock cultures and stored at −80°C until use.

Antimicrobial agents were obtained as pure substances from their manufacturers: DC-159a and levofloxacin (LVX; both from Daiichi-Sankyo Pharmaceuticals Co., Ltd., Tokyo, Japan), moxifloxacin (MXF; Bayer Yakuhin, Ltd., Osaka, Japan), gatifloxacin (GAT; Kyorin Co., Ltd., Tokyo, Japan), rifampin (RIF; Sigma-Aldrich Co., Tokyo, Japan).

MICs were determined according to the standard agar dilution method recommended by NCCLS (10). A multipoint inoculator (Microplanter; Sakuma Seisakusho, Tokyo, Japan) was used to inoculate approximately 10^4 CFU per 2-μl spot onto Middlebrook 7H10 agar (Difco, MD) supplemented with 10% oleic acid–albumin–dextrose–catalase and 0.5% glycerol. The tested agar plates contained antimicrobial agents ranging from 0.015 to 128 μg/ml in 2-fold dilutions. The inoculated agar plates were incubated at 37°C for 21 days for *M. tuberculosis*, 14 days for *M. avium* and *M. intracellulare*, 7 days for *M. kansasii*, and 3 days for *M. fortuitum* and *M. abscessus*. *M. chelonae* was incubated at 30°C for 3 days. The MIC was defined as the lowest drug concentration yielding no visible colony formation on the agar surface.

Summaries of the *in vitro* activities of DC-159a against *M. tuberculosis* and NTM in comparison with those of the other three quinolones and RIF are shown in Tables 1 and 2, respectively. MICs of currently available quinolones were generally similar to those of previous reports (1, 5, 8). DC-159a was the most active compound against *M. tuberculosis*. The MIC_{90} of

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TABLE 1. Comparison of MICs of DC-159a with other quinolones and rifampin against *M. tuberculosis*

M. tuberculosis isolate type (*n*)	Drug	MIC (μg/ml)		
		Range	50%	90%
Drug susceptible (21)	DC-159a	0.03–0.125	0.06	0.06
	MXF	0.125–0.5	0.25	0.25
	GAT	0.06–0.25	0.125	0.25
	LVX	0.25–1	0.5	0.5
	RIF	≤0.015–0.25	0.06	0.125
QR-MDR (11)	DC-159a	0.125–0.5	0.125	0.5
	MXF	1–4	2	4
	GAT	0.5–2	1	2
	LVX	2–16	8	16
	RIF	16 to >128	128	>128

DC-159a against drug-susceptible *M. tuberculosis* was 0.06 μg/ml, which was 2-fold lower than that of RIF and 4- to 8-fold lower than those of other tested quinolones. The MIC_{90} of DC-159a against QR-MDR *M. tuberculosis* was 0.5 μg/ml, which was 4- to 32-fold lower than those of other tested quinolones and equal to that of LVX against drug-susceptible *M. tuberculosis*.

Furthermore, DC-159a inhibited the growth of slowly growing NTM and yielded the lowest MIC_{90} among the tested quinolones with *M. kansasii* (MIC_{90}, 0.125 μg/ml), *M. avium* (MIC_{90}, 2 μg/ml), and *M. intracellulare* (MIC_{90}, 4 μg/ml). The MIC_{90} of DC-159a against the *M. avium-M. intracellulare* complex was 2- to 16-fold lower than those of other tested quinolones.

In the rapidly growing mycobacteria, it is well known that *M. fortuitum* is susceptible to quinolones, but *M. chelonae* and *M. abscessus* are naturally resistant to quinolones (6). DC-159a also showed the same pattern of activity against rapidly growing NTM as the other quinolones. The MIC_{90} of DC-159a against *M. fortuitum* (0.25 μg/ml) was comparable to those of MXF and GAT; however, the MIC_{90}s of DC-159a against *M. chelonae* (8 μg/ml) and *M. abscessus* (16 μg/ml) were 4- to 8-fold lower than those of other tested quinolones.

In summary, the order of susceptibility of *Mycobacterium* species to DC-159a was *M. tuberculosis* > *M. kansasii* > *M. fortuitum* > *M. avium* > *M. intracellulare* > *M. chelonae* > *M. abscessus*.

TABLE 2. Comparison of MICs of DC-159a with other quinolones and rifampin against nontuberculous mycobacteria

Mycobacterium species (no. of isolates)	Drug	MIC (μg/ml)		
		Range	50%	90%
Slowly growing mycobacteria				
M. kansasii (22)	DC-159a	0.03–0.25	0.06	0.125
	MXF	0.06–1	0.125	0.25
	GAT	0.06–1	0.125	0.5
	LVX	0.25–2	0.25	1
	RIF	0.06–128	0.25	0.25
M. avium (33): serovar 4 (10) and serovar 8 (23)	DC-159a	0.25–8	1	2
	MXF	1–16	2	4
	GAT	1–32	2	8
	LVX	2–64	8	32
	RIF	0.25 to >128	32	128
M. intracellulare serovar 16 (17)	DC-159a	0.25–8	1	4
	MXF	0.5–32	2	8
	GAT	1–32	4	16
	LVX	2–32	8	32
	RIF	0.25–128	2	32
Rapidly growing mycobacteria				
M. fortuitum (10)	DC-159a	0.03–0.25	0.125	0.25
	MXF	0.06–0.5	0.125	0.25
	GAT	0.03–0.25	0.125	0.25
	LVX	0.06–2	0.25	0.5
	RIF	1–128	32	64
M. chelonae (10)	DC-159a	4–16	4	8
	MXF	8–64	32	32
	GAT	4–64	16	32
	LVX	4–128	32	64
	RIF	>128	>128	>128
M. abscessus (12)	DC-159a	4–32	8	16
	MXF	16–128	64	128
	GAT	8–64	32	64
	LVX	16 to >128	64	128
	RIF	>128	>128	>128

In this study, we compared the *in vitro* activities of DC-159a with those of LVX, MXF, GAT, and RIF as reference drugs. DC-159a showed the lowest MICs and the narrowest MIC distribution against *M. tuberculosis* and NTM isolates for the tested drugs. A good activity of DC-159a against quinolone-resistant strains in other bacteria has been reported previously (2, 7). The results of this study also confirmed the characteristic advantage of DC-159a against QR-MDR *M. tuberculosis*. This finding may be attributed to the high inhibitory activities of DC-159a against the mutant GyrA enzymes in quinolone-resistant *M. tuberculosis* (12). The exact mechanism of DC-159a resistance in *M. tuberculosis* is under investigation.

According to the pharmacokinetic study in a monkey model in which an oral dose of 5 mg/kg of body weight was administered, DC-159a achieved a higher peak concentration (C_{max}; 2.20 μg/ml) and area under the concentration-time curve from 0 to 24 h (AUC_{0-24}; 16.9 μg · h/ml) than the MIC against *M. tuberculosis*, showed better pharmacokinetic properties than LVX (C_{max}, 1.68 μg/ml; AUC_{0-24}, 15.3 μg · h/ml), and lacked interaction with cytochrome P450 3A4 (9).

For substantiation of DC-159a as a novel agent for TB, MDR-TB, extensively drug-resistant TB, and TB/HIV coinfection cases, further intracellular, pharmacokinetic, drug-drug interaction, and *in vivo* evaluation studies are essential. DC-159a deserves further study as a promising candidate for a better TB cure, with a focus on the establishment of a 3- to 4-month new standard regimen in the near future.

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