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Comparative In Vitro Antimicrobial Activities of the Newly Synthesized Quinolone HSR-903, Sitafloxacin (DU-6859a), Gatifloxacin (AM-1155), and Levofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium* Complex

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We compared the in vitro antimycobacterial activity of a new fluoroquinolone, HSR-903, with strong activity against gram-positive cocci with those of levofloxacin (LVFX), sitafloxacin (STFX), and gatifloxacin (GFLX). The MICs of the quinolones for *Mycobacterium tuberculosis* and *Mycobacterium avium* complex were in the order STFX ≈ GFLX < LVFX ≦ HSR-903 and STFX ≦ GFLX ≦ HSR-903 ≦ LVFX, respectively. HSR-903 effectively eliminated intramacrophagial *M. tuberculosis*, as did LVFX, and exhibited bacteriostatic effects against *M. tuberculosis* replicating in type II alveolar cells.

The recent increase in AIDS-associated intractable mycobacterial infections, including multidrug-resistant tuberculosis (MDR-TB) and disseminated *Mycobacterium avium* complex (MAC) infections, has caused serious problems around the world (4, 7, 28). New quinolones are recommended for use as second-choice drugs in treatment of MDR-TB, since they have potent anti-*Mycobacterium tuberculosis* activity and good pharmacokinetics, in terms of tissue and cellular distribution, and have few adverse effects (1, 5, 6). Ciprofloxacin (CPFX), ofloxacin (OFLX), sparfloxacin (SPFX), and levofloxacin (LVFX) have good therapeutic efficacies against experimental *M. tuberculosis* infection in mice (9, 11) and are efficacious in clinical control of tuberculosis, including MDR-TB, when given in combination with other antituberculous drugs (1, 26).

HSR-903, a new fluoroquinolone [(*S*)-(−)-5-amino-7-(7-amino-5-azaspiro [2,4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid methanesulfonate], has a broad spectrum of action against both gram-positive and gram-negative bacteria. HSR-903 has more potent activity against *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Helicobacter pylori* than do other fluoroquinolones, including CPFX, SPFX, and LVFX (20, 24, 27). In pharmacological studies with mice, the levels of HSR-903 in the lungs were much higher than those in the plasma after oral administration, and concentrations of HSR-903 in lung were higher than those of CPFX and LVFX (13). In humans, the maximum concentration of drug in serum (C_{max}) of HSR-903 at 200 mg/kg of body weight was 0.86 μg/ml at 1.3 to 2.4 h (time to C_{max} [T_{max}]), and the half-life ($T_{1/2}\beta$) and area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) of HSR-903 were 18.0 h and 12.8 μg · h/ml, respectively (13a, 23). HSR-903 also exhibited potent therapeutic efficacy against experimental murine infections caused by penicillin-resistant *S. pneumoniae* and *H. influenzae* (27). In the present study, the in vitro antimicrobial activity of HSR-903 against *M. tuberculosis* and MAC was compared with those of several other fluoroquinolones, including LVFX, sitafloxacin (STFX; DU-6859a), and gatifloxacin (GFLX; AM-1155), which possess potent in vitro and in vivo antimycobacterial activities (9, 11, 14–18, 21).

M. tuberculosis (45 strains), *M. avium* (20 strains), and *Mycobacterium intracellulare* (20 strains) were isolated from sputum specimens of non-human immunodeficiency virus-infected patients with sporadic tuberculosis or MAC infection in several hospitals in Japan and grown in 7H9 medium. Each strain was isolated from a different patient. The *M. tuberculosis* isolates were divided into MDR *M. tuberculosis* with resistance to both rifampin (RMP) and isoniazid (INH) (MIC_{RMP} of ≧1.56 μg/ml and MIC_{INH} of ≧0.4 μg/ml) and non-MDR *M. tuberculosis* (MIC_{RMP} of ≦0.78 μg/ml and MIC_{INH} of ≦0.2 μg/ml) strains, according to the criteria of the Centers for Disease Control and Prevention (10). Alternatively, the *M. tuberculosis* isolates were divided into LVFX-susceptible (MIC_{LVFX} of ≦0.78 μg/ml) and LVFX-resistant (MIC_{LVFX} of ≧1.56 μg/ml) strains (17).

In this study, the activities of the following drugs were measured: HSR-903 (Hokuriku Pharmaceutical Co., Fukui, Japan), LVFX (Daiichi Pharmaceutical Co., Tokyo, Japan), STFX (Daiichi Pharmaceutical Co.), GFLX (Kyorin Pharmaceutical Co., Tokyo), RMP (Daiichi Pharmaceutical Co.), clarithromycin (CAM) (Taisho Pharmaceutical Co., Tokyo), and INH (Daiichi Pharmaceutical Co.).

MICs of test drugs were determined as previously reported (18) by either the agar dilution method with Middlebrook 7H11 medium (Difco Laboratories, Detroit, Mich.) or the broth dilution method in microculture wells with 7HSF medium as described by Yajuko et al. (25).

The activities of test drugs against intracellular *M. tuberculosis* were measured as follows. The Mono Mac 6 human macrophage (Mϕ)-like cell line (MM6-Mϕs; German Collection of Microorganisms and Cell Cultures, Mascheroder, Germany)

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TABLE 1. MICs of HSR-903, STFX, GFLX, LVFX, RMP, INH, and CAM for *M. tuberculosis* and MAC strains[a]

Strains	No. of strains	MIC_{50} (μg/ml)							MIC_{90} (μg/ml)						
		HSR-903	STFX	GFLX	LVFX	RMP	INH	CAM	HSR-903	STFX	GFLX	LVFX	RMP	INH	CAM
M. tuberculosis															
Non-MDR[b]	23	0.78	0.1	0.1	0.39	≦0.05	≦0.05	25	0.78	0.1	0.2	0.39	0.39	1.56	50
MDR[c]	22	3.13	0.39	0.39	3.13	50	12.5	6.25	25	1.56	1.56	6.25	100	>100	25
LVFX-S[d]	27	0.78	0.1	0.1	0.39	≦0.05	0.1	25	1.56	0.1	0.2	0.78	100	6.25	50
LVFX-R[e]	18	6.25	0.78	0.78	3.13	12.5	25	6.25	50	1.56	3.13	12.5	100	>100	25
M. avium	20	6.25	1.56	3.13	6.25	50	6.25	12.5	25	6.25	6.25	25	>100	12.5	25
M. intracellulare	20	12.5	6.25	6.25	25	3.13	6.25	6.25	12.5	6.25	12.5	25	6.25	100	12.5

[a] MICs were determined by the agar dilution method with 7H11 medium.
[b] Either MIC_{RMP} of ≦0.78 μg/ml or MIC_{INH} of ≦0.2 μg/ml.
[c] MIC_{RMP} of ≧1.56 μg/ml and MIC_{INH} of ≧0.4 μg/ml.
[d] LVFX-S, LVFX susceptible (MIC_{LVFX} of ≦0.78 μg/ml).
[e] LVFX-R, LVFX resistant (MIC_{LVFX} of ≧1.56 μg/ml).

and A-549 human type II lung epithelial cell line (A-549 cells; American Type Culture Collection, Rockville, Md.) were used as host cells for *M. tuberculosis* infection. Cultured MM6-Mϕs and A-549 cells (4×10^4 cells) suspended in RPMI 1640 medium and Ham's F-12K medium containing 5% fetal bovine serum (FBS) (BioWhittaker Co., Walkersville, Md.), respectively, were seeded on round-bottom microculture wells. The resulting cells were then infected with *M. tuberculosis* "Kurono" [$MIC_{RMP(7H11)}$ of ≦0.05 μg/ml and $MIC_{INH(7H11)}$ of ≦0.05 μg/ml] at a multiplicity of infection (MOI) of 30 for 3 h and at an MOI of 10 for 2 h, respectively. (These conditions yielded comparable loads of mycobacterial infection for MM6-Mϕs and A-549 cells.) After being washed with 2% FBS–Hanks' balanced salt solution, *M. tuberculosis*-infected cells were cultured in corresponding medium (0.2 ml) containing 1% FBS in the presence or absence of test drugs for up to 7 days. At intervals, the cells were lysed with 0.07% sodium dodecyl sulfate and washed with distilled water by centrifugation, and the number of recovered CFU was counted on 7H11 agar plates.

Table 1 summarizes the MICs at which 50 and 90% of the test strains were inhibited (MIC_{50} and MIC_{90}, respectively) of HSR-903, STFX, GFLX, LVFX, RMP, INH, and CAM for *M. tuberculosis* and MAC. The MIC_{50} and MIC_{90} of test quinolones were distributed over a range from 0.1 to 0.78 μg/ml and 0.39 to 25 μg/ml for non-MDR *M. tuberculosis* and MDR *M. tuberculosis* strains, respectively. Their MICs were in the order STFX ≈ GFLX < LVFX ≦ HSR-903. The MICs of RMP and INH were lowest among test drugs for non-MDR *M. tuberculosis* strains, but markedly increased in the case of MDR-*M. tuberculosis* strains. The MICs of CAM were high for both non-MDR-*M. tuberculosis* and MDR-*M. tuberculosis* strains.

Notably, the MICs of test quinolones for MDR-*M. tuberculosis* isolates were 4 to 32 times higher than their MICs for non-MDR-*M. tuberculosis* strains. This finding is not surprising, since in the present study, most MDR-*M. tuberculosis* strains with increased quinolone resistance were isolated from patients who had been treated with antituberculous regimens containing fluoroquinolones, such as OFLX and CPFX. Moreover, certain MDR-*M. tuberculosis* isolates with susceptibility to quinolones as high as those of non-MDR-*M. tuberculosis* strains were isolated from patients who had never undergone quinolone treatment. Indeed, it was previously reported that MICs of these quinolones were not increased in MDR-*M. tuberculosis* isolates (8). Similarly, the MICs of HSR-903, STFX, and GFLX for LVFX-resistant *M. tuberculosis* strains (MIC_{LVFX} of ≧1.56 μg/ml) were 8 to 32 times higher than those for LVFX-susceptible *M. tuberculosis* strains (MIC_{LVFX} of ≦0.78 μg/ml).

The MIC_{50} and MIC_{90} of test quinolones for *M. avium* were distributed over a range from 1.56 to 25 μg/ml and in the order STFX ≦ GFLX < HSR-903 = LVFX. Their MICs for *M. intracellulare* were distributed from 6.25 to 25 μg/ml and in the order STFX ≦ GFLX ≦ HSR-903 < LVFX. Thus, the activities of these quinolones against MAC can be ranked as STFX ≧ GFLX ≧ HSR-903 ≧ LVFX. The activities of both HSR-903 and LVFX are poor against MAC. Notably, the MIC_{50}s of the quinolones for *M. avium* tended to be lower than those for *M. intracellulare*, as previously reported (22). In contrast, the MICs of RMP and CAM for *M. avium* were higher than those for *M. intracellulare*.

Next, we examined the antimicrobial activity of HSR-903 against intracellular *M. tuberculosis*. Figure 1 shows the effects of HSR-903 and LVFX on the mode of intracellular growth of *M. tuberculosis* "Kurono" residing in MM6-Mϕs and A-549 cells, when these drugs were added to the culture medium at the C_{max} in blood (0.86 and 2.0 μg/ml, respectively) (3, 23). The *M. tuberculosis* bacteria in MM6-Mϕs were progressively killed in similar fashions by HSR-903 and LVFX during a 7-day cultivation. While gradual but progressive killing by LVFX was noted for *M. tuberculosis* bacteria residing in A-549 cells, HSR-903-mediated bacterial elimination was somewhat incomplete for the organisms in A-549 cells. In separate experiments, when these drugs were added at the MIC (7HSF medium) (0.25 μg/ml each for HSR-903 and LVFX), HSR-903 displayed bacteriostatic activity against the organisms residing in MM6-Mϕs, while LVFX did not (data not shown). In the case of A-549 cells, only weak bacteriostatic effects were observed for the two quinolones against intracellular *M. tuberculosis* (data not shown).

In this study, we compared the in vitro activity of a newly synthesized fluoroquinolone, HSR-903, with those of LVFX, STFX, and GFLX as reference drugs. First, the MICs of HSR-903 for *M. tuberculosis* isolates were the same as or twice as high as those of LVFX. HSR-903 exhibited a broader MIC distribution for MDR *M. tuberculosis* isolates than did LVFX, with a peak around 3.13 μg/ml, the same as that of LVFX (data not shown). It thus appears that HSR-903 has somewhat weak-

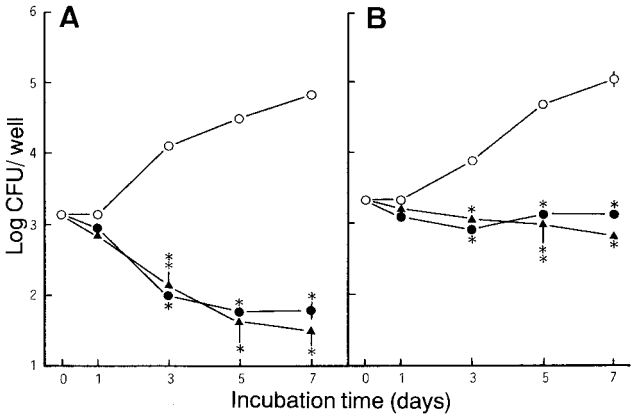

FIG. 1. Antimicrobial activity of HSR-903 (●) and LVFX (▲) against *M. tuberculosis* "Kurono" residing in MM6-Mϕs (A) and A-549 cells (B). These drugs were added to culture medium of *M. tuberculosis*-infected cells at the C_{max} in blood (0.86 and 2.0 μg/ml, respectively) after oral administration (HSR-903, 4 mg/kg; LVFX, 20 mg/kg). The numbers of cells recovered were increased by 2.0 and 2.9 times during a 7-day cultivation of MM6-Mϕs and A-549 cells, respectively. Each symbol indicates the mean ± standard error ($n = 3$ [error bars were omitted when values were <0.1]). ○, control culture without addition of drugs. Asterisks indicate significant differences between the number of bacterial CFU recovered from drug-treated cells versus that recovered from untreated cells (*, $P < 0.01$; **, $P < 0.05$ [Student's t test]).

er but comparable activity against *M. tuberculosis* compared to LVFX. In any case, the activities of both HSR-903 and LVFX are poor against MDR-*M. tuberculosis*. Second, both STFX and GFLX exhibited much more potent anti-*M. tuberculosis* activity than did LVFX and HSR-903. Notably, STFX and GFLX were eight times more active against MDR-*M. tuberculosis* strains than LVFX in terms of MIC_{50}, suggesting that these new quinolones might be useful in clinical control of MDR-TB. Third, HSR-903 at C_{max} displayed the same level of bactericidal activity as LVFX against *M. tuberculosis* organisms residing in MM6-Mϕs. Thus, HSR-903 may be as efficacious as LVFX in displaying in vivo antimicrobial activity against intramacrophagial *M. tuberculosis* at sites of infection. HSR-903 added at the MIC exhibited more marked bacteriostatic activity against *M. tuberculosis* within MM6-Mϕs than did LVFX added at the MIC. This finding suggests that the efficacy of HSR-903 delivery to phagosomes containing bacteria in *M. tuberculosis*-infected Mϕs may be greater than that for LVFX.

The antimicrobial activities of HSR-903 and LVFX against *M. tuberculosis* residing in A-549 alveolar cells, which are nonprofessional phagocytes, were significantly lower than those against *M. tuberculosis* within MM6-Mϕs, which are professional phagocytes (Fig. 1). *M. tuberculosis* replicates in A-549 cells more vigorously than in Mϕs (2, 12), presumably because of the inability of A-549 cells to produce nitric oxide, an important antimycobacterial effector molecule (19). Therefore, mobilization of nitric oxide-dependent antimicrobial mechanisms in host cells may be critical for quinolone-mediated elimination of intracellular *M. tuberculosis*. Alternatively, it is also possible that the delivery of quinolones to internalized *M. tuberculosis* is less efficient in A-549 cells than in MM6-Mϕs; this may lead to reduced anti-*M. tuberculosis* activity by drugs in the case of A-549 cells. Further studies to examine the profiles of quinolone delivery in MM6-Mϕs and A-549 cells are currently under way.

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