Comparative mutant prevention concentration and mutant selection window of sitafloxacin versus other quinolones using strains of *H. influenzae* with decreasing susceptibility to levofloxacin

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Introduction

The fluoroquinolones are widely used in the treatment of respiratory infections, and the potential for bacteria developing resistance to the fluoroquinolones is becoming a concern[1]. Although the low prevalence rate of fluoroquinolone resistant strains are reported (0.15%)[2], the cases of quinolone-resistant *Haemophilus influenzae* (*H. influenzae*) have been increasing recently[3].

The mutant prevention concentration (MPC) concept has been suggested as a useful tool for guiding the appropriate use of antimicrobial drugs in order to decrease the selection of resistant bacteria[4]. Additionally, the mutant selection window (MSW) is an antimicrobial concentration range extending from the minimal concentration required to block the growth of wild-type bacteria up to that required to inhibit the growth of the single-step mutant, and the upper boundary is also called the MPC[1,4]. Sitafloxacin is a new fluoroquinolone that has been reported to have a broader spectrum, and the greater activity against quinolone-resistant strains than that of other quinolones[5]. The purpose of this study was to evaluate the *in vitro* activity of sitafloxacin and compare its MPC and MSW to those of levofloxacin, moxifloxacin, garenoxacin, and ciprofloxacin.

Materials and Methods

Three *H. influenzae* strains, Rd, 27995, and 11438, were used to measure the MIC and the MPC; the strains 27995 and 11438 with decreasing susceptibility to levofloxacin were kindly provided by Meiji Seika Kaisha Ltd. (Tokyo, Japan). Stock solutions of sitafloxacin (Daiichi-Sankyo, Tokyo, Japan), levofloxacin (Daiichi-Sankyo, Tokyo, Japan), moxifloxacin (Shionogi & Co., Tokyo, Japan), ciprofloxacin (Bayer Corporation, Tokyo, Japan), and garenoxacin (Toyama Chemical & Co., Tokyo, Japan) were prepared according to the manufacturers' instructions and frozen at –80°C until needed. Susceptibilities were tested in duplicate for each isolate using inocula of 10^5 CFU / mL and determined by a broth dilution method using *Haemophilus* Test Medium (HTM) according to the Clinical and Laboratory Standards Institute (CLSI) recommendation[6].

To identify mutations in the QRDRs of *gyrA* and *parC* in these strains, PCR and direct DNA sequencing were used according to the method of Vila *et al*[7]. The QRDR DNA sequencing results were compared with the sequence of strain Rd (GenBank accession no. NC_000907), which was used as the wild-type standard strain in this study. The MPC is defined as the fluoroquinolone concentration at which no colony was recovered when more

than 10^{10} cells were applied to agar plates, and generally, it is also defined the lower limit of the MSW equal to the MIC[5]. The MPC methodology was adapted from the method outlined by Blondeau *et al*[1]. First, the bacterial isolates were grown up on chocolate II agar plates (10 plates per isolate) (Nissui Pharmaceutical Co., Tokyo, Japan) and incubated overnight at 37°C in 5% CO_2. Then, colonies from each strain were transferred from the plates to 200 ml of Mueller Hinton II broth (Beckton Dickinson, Oxford, UK) containing 2% lysed horse blood and 15 μg / ml NAD and then incubated with vigorous shaking for 2 h at 37°C. Each 200 ml culture was then centrifuged at 5000 rpm for 30 min. The pellets of the same strain were combined to make the final suspension of $\geq 3 \times 10^{10}$ CFU / mL. For the MPC determination, 100 μl of this concentrated bacterial suspension was used to inoculate each fluoroquinolone plates. These plates were incubated at 37°C in 5% CO_2 and were screened for colonies every 24 h over 96 to 120 h to ensure that the colony number had stabilized.

Results and Discussion

Table 1 appears to the MPC and MIC ratios which will give the idea of the MSW size. The MPC of sitafloxacin was the lowest of the five fluoroquinolones tested, and its MSW

was also narrow against the Rd strain (levofloxacin MIC is 0.003 μg/mL) (MPC/MIC ratio; 2.5). Similarly, the MPCs of sitafloxacin against the strain 27995, with levofloxacin MIC at the susceptibility breakpoint, and the strain 11438, with high levofloxacin MIC, were lower than the MPCs of the other quinolones tested. Moreover, the MPCs of sitafloxacin were lower than the MICs of other fluoroquinolones, and MSW of strain 27995 and 11438 were equivalent to or narrower than the MSWs of the other fluoroquinolones (MPC/MIC ratio; 8.3 and 2, respectively).

Because the MPC determination assay uses a higher inoculum size (typically 10^{10} to 10^{11} CFU / mL) than MIC, this technique should be further validated prior to adoption of clinical therapeutic standards that prevent the selection of antibiotic-resistant mutants. Fluoroquinolone resistance may arise largely from sporadic conditions in which abnormally low doses or patient characteristics, such as chronic lung disease, cause the drug concentration to fall into the MSW and allow the mutation of QRDR. In the present study, we demonstrated that sitafloxacin may have a low potential for selecting quinolone resistance in *H. influenzae* mutants. But further studies are warranted to evaluate the usefulness of sitafloxacin against infections caused by quinolone-resistant *H. influenzae*.

References

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Figure 1

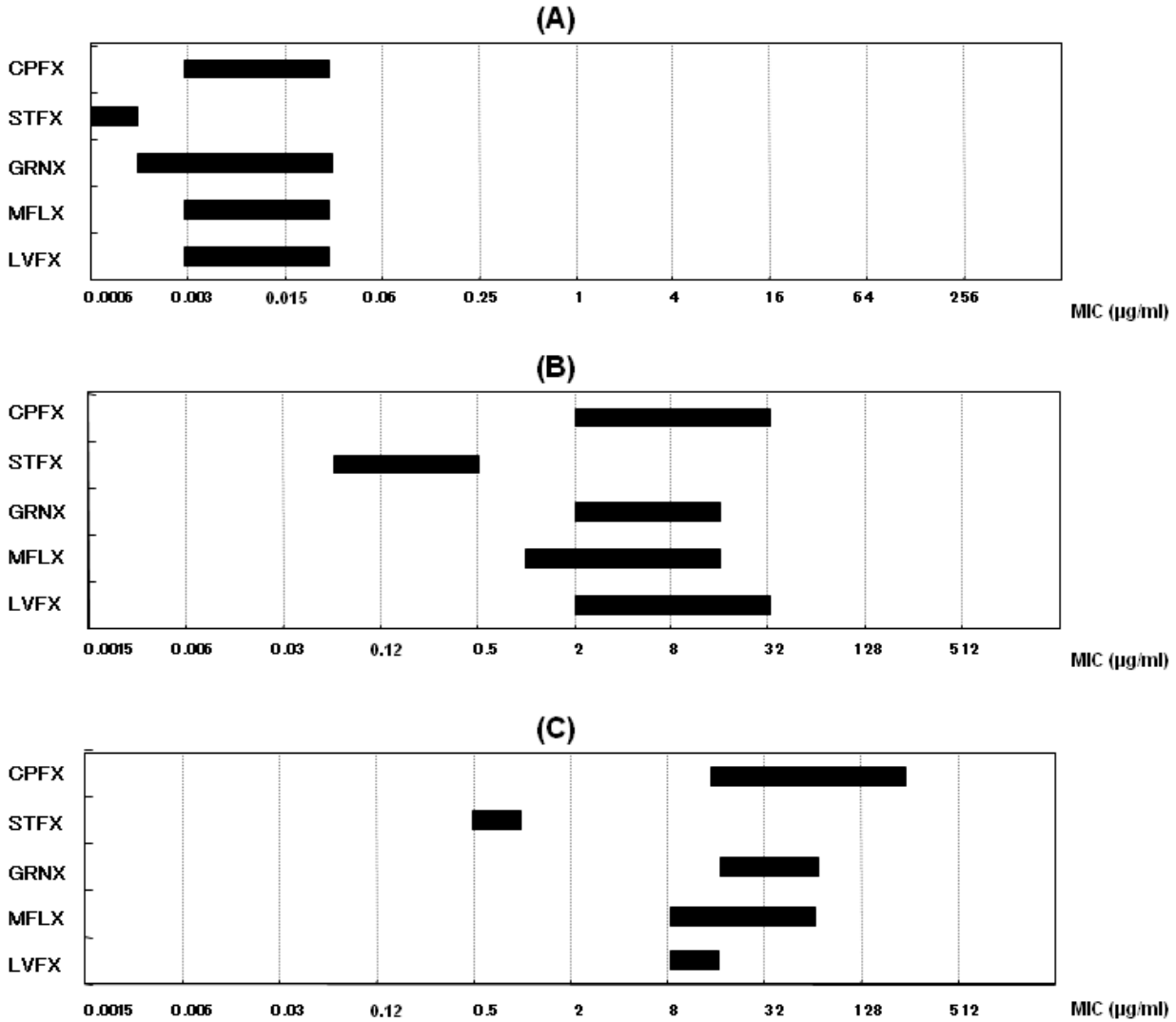
(A)
CPFX
STFX
GRNX
MFLX
LVFX
0.0005
0.003
0.015
0.06
0.25
1
4
16
64
256
MIC (μg/ml)
(B)
CPFX
STFX
GRNX
MFLX
LVFX
0.0015
0.006
0.03
0.12
0.5
2
8
32
128
512
MIC (μg/ml)
(C)
CPFX
STFX
GRNX
MFLX
LVFX
0.0015
0.006
0.03
0.12
0.5
2
8
32
128
512
MIC (μg/ml)

Table MICs of quinolones and QRDR mutation in the gyrA and parC of *H. influenzae*

strain	amino acid change		MIC of quinolones(mg/L)				
	gyrA	parC	LVFX	MFLX	CPFX	GRNX	STFX
Rd	(-)	(-)	0.003	0.003	0.003	0.0015	0.0006
27995	Ser84→Leu	Ser84→Ile	2	1	2	2	0.06
11428	Ser84→Leu Asp88→Tyr	Ser84→Arg Glu88→Ala	8	8	16	16	0.5

LVFX; levofloxacin MFLX; moxifloxacin CPFX; ciprofloxacin GRNX; garenoxacin STFX; sitafloxacin