Rapid bactericidal activity of sitafloxacin against *Streptococcus pneumoniae*

Hiroko Kanda[1)], Kazue Inoue[2)], Ryo Okumura[1)] and Kazuki Hoshino[1)]

[1] Biological Research Laboratories, R&D Division, Daiichi Sankyo Co., Ltd., Tokyo
[2] Vaccine Business Strategy Department, Vaccine Business Intelligence Division, Daiichi Sankyo Co., Ltd., Tokyo

(Received for publication November 7, 2012)

The initial bactericidal activity of quinolones against *Streptococcus pneumoniae* at the concentration equivalent to their respective peak serum concentration (C_{max}) and free drug fraction of C_{max} (fC_{max}) were investigated. The bactericidal activity of sitafloxacin (STFX), levofloxacin (LVFX), moxifloxacin (MFLX), and garenoxacin (GRNX) were compared by determining the actual killing of bacteria at C_{max} and fC_{max} for 1 and 2 hours based on the Japanese maximum dose per administration (100, 500, 400, and 400 mg, respectively). Against 4 quinolone-susceptible clinical isolates (wild-type), STFX with C_{max} and fC_{max} exhibited the most rapid bactericidal activity resulting in an average reduction of $\geq 3.0 \log_{10}$ colony forming units (CFU)/mL in 1 hour. STFX with C_{max} and fC_{max} also showed the most rapid and potent bactericidal activity against 9 clinical isolates with single *par* (*C*/*E*) mutation, resulting in $\geq 3.0 \log_{10}$ CFU/mL average reduction in viable cells in 1 hour. STFX showed a statistically significant advantage in initial bactericidal activity over other quinolones for single mutants ($P < 0.001$). The propensity that the difference in the initial bactericidal activity between STFX and other quinolones was higher in single mutants than wild-type strains, was confirmed using *S. pneumoniae* ATCC49619 (wild-type) and its laboratory single *parC* mutant. As a result, STFX showed a similar rapid and potent initial bactericidal activity against both strains, while initial bactericidal activity for other quinolones was significantly reduced in the single mutant ($P < 0.05$). In conclusion, STFX has the most rapid and potent initial bactericidal activity against wild-type and single mutants of *S. pneumoniae* and its bactericidal activity is not affected by the presence of a single *par* mutation compared to LVFX, MFLX, and GRNX.

Introduction

Streptococcus pneumoniae is one of the most significant pathogens of community-acquired respiratory tract infections and accounts for two-thirds of mortality of those patients requiring hospital treatment[1]. Due to increasing levels of resistance in *S. pneumoniae* to commonly used antimicrobials, such as penicillins and macrolides, fluoroquinolone with improved antibacterial activity against *S. pneumoniae* are being recommended and used for the treatment of patients with community acquired pneumonia（CAP）caused by multidrug resistant strains[2]. Emerging quinolone-resistant *S. pneumoniae* is also a growing concern because high rates of levofloxacin (LVFX) used by geographic region were reported to be associated with increased quinolone-resistant pneumococci[3], however, current global resistance rates remain low[4~6].

In pneumococci, resistance to quinolones is primarily mediated via mutations in the quinolone resistance-determining region (QRDR) of target enzymes[7]. Even in the LVFX-susceptible clinical isolates, 6–25% of strains with LVFX MIC at 1 μg/mL and 30–71% of strains with LVFX MIC at 2 μg/mL were reported to possess a single mutation in QRDR[8~10]. The frequency of selection of resistant mutants were higher in single QRDR mutants than wild-type strains[11~13]. It is necessary to use an appropriate quinolone and regimen to eradicate bacteria and to minimise the development of resistance.

Sitafloxacin (STFX) is a fluoroquinolone with enhanced broad-spectrum antibacterial activity against Gram-positive and Gram-negative aerobes and anaerobes[14]. STFX was confirmed to possess a potent antimicrobial activity against *S. pneumoniae*, including quinolone-resistant isolates, compared with other quinolones[15].

For the evaluation of the bactericidal activities of quinolones, many studies were conducted using the concentration based on the minimum inhibitory concentration (MIC). In the case of STFX, the MIC based study showed potent bactericidal activity of STFX against *S. pneumoniae*[16], however, the concentration should be examined considering the clinical setting.

Since the pharmacodynamics of quinolones are concentration-dependent[17,18], some investigators reported the antibactericidal activity using pharmacokinetics/pharmacodynamics parameters of the therapeutic efficacy of quinolones, AUC/MIC, peak serum concentration (C_{max})/MIC and C_{max}[19~22]. In this study, we investigated the initial bactericidal activities of STFX and other quinolones against wild-type and single QRDR mutants of *S. pneumoniae* based on their C_{max} and free drug fraction of C_{max} (fC_{max}), which might have an effect on the initial bactericidal activities of quinolones in the clinical setting.

Table 1. Amino acid substitutions in the quinolone resistance-determining regions of ParC and ParE and susceptibility to quinolones of used *Streptococcus pneumoniae* strains.

Strain No.	Amino acid substitution		MIC (μg/mL)			
	ParC	ParE	LVFX	MFLX	GRNX	STFX
GE00175	None[a]	None	1	0.25	0.06	0.06
GE01045	None	None	1	0.25	0.06	0.06
GE01085	None	None	1	0.25	0.06	0.06
05712	None	None	1	0.25	0.06	0.06
GE00215	D83Y	None	2	0.25	0.06	0.06
GE01064	D83A	None	1	0.12	0.03	0.03
05101	S79F	None	2	0.25	0.06	0.06
05667	S79F	None	2	0.25	0.12	0.06
05696	S79Y	None	1	0.12	0.06	0.03
05298	None	D435N	1	0.12	0.03	0.03
05674	None	D435E	2	0.25	0.03	0.06
05706	None	E494D	2	0.25	0.12	0.06
1026523	S79F	None	2	0.5	0.12	0.06
ATCC49619	None	None	1	0.12	0.03	0.03
60	S79Y	None	2	0.25	0.06	0.06

a: no amino acid substitution

Materials and Methods

Antimicrobial agents

STFX and LVFX were synthesized at Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan. Moxifloxacin (MFLX) was purchased from Sequoia Research Products Ltd., Pangbourne, UK and LKT Laboratories, Inc., Minnesota, USA. Garenoxacin mesilate (GRNX) was synthesized at Daiichi Sankyo Co., Ltd., Tokyo, Japan. The determination of MICs was performed by the broth dilution method according to the Clinical and Laboratory Standards Institute guidelines[23].

Microorganisms

The bacterial strains used in this study are shown in Table 1. Four quinolone-susceptible clinical isolates of *S. pneumoniae* (GE00175, GE01045, GE01085, and 05712), which did not harbour mutations in the QRDR of the *gyrA*, *gyrB*, *parC* and *parE* genes, and 9 clinical isolates (GE00215, GE01064, 05101, 05667, 05696, 05298, 05674, 05706, and 1026523) harbouring a single mutation relating to quinolone-resistance in the QRDR of *parC* or *parE* of topoisomelase

IV[24,25] isolated from Japan or UK were used in this study. Strains ATCC49619 and strain 60, which is a mutant of ATCC49619 harbouring a single QRDR mutation in *parC* (Ser79Tyr)[13], were also used in this study. The bacterium were stored at −80℃ before experiments.

Time-kill study

The bacterial strains were inoculated on heart infusion agar (HIA; Eiken Chemical Co., Ltd., Tokyo, Japan) containing 5% sheep-defibrinated blood and incubated overnight at 35℃. Several fresh colonies on the plate were suspended in brain heart infusion broth (BHI; Becton, Dickinson and Company (BD), NJ, USA) supplemented with 0.5% yeast extract (BD) (BHIY) and incubated at 37℃ with shaking, to obtain the culture containing approximately 10^6 colony forming units (CFU)/mL of bacteria. The initial bactericidal activities of STFX, LVFX, MFLX, and GRNX were examined with exposure of the culture to the concentration equivalent to their respective C_{max} at the Japanese maximum dose per administration (100, 500, 400, and 400 mg, respectively) and their fC_{max} at 37℃ for 2 hours. C_{max} of the quinolones was as follows: STFX 1.0 μg/mL[26], LVFX 8.0 μg/mL[27], MFLX 4.1 μg/mL[28], and GRNX 7.2 μg/mL[29]. fC_{max} of the quinolones which was calculated using their respective protein binding ratio by the ultrafiltration method[26~29] was as follows: STFX 0.5 μg/mL, LVFX 5.6 μg/mL, MFLX 2.05 μg/mL, and GRNX 1.44 μg/mL. Samples were taken for viable count determinations at 0, 1, and 2 hours after addition of the drug. Each sample was then diluted appropriately and spread on HIA plates. Antibacterial agents carryover was minimized by serial saline dilution. Saline dilution reduced antibacterial agents concentrations around the MIC for study organisms. The viable cell counts ($\log_{10}$CFU/mL) were determined based on the numbers of colonies grown on the plates after incubation at 35℃ overnight combined with dilution factors. The detection limit was 1×10^2 CFU/mL.

Analysis of time-kill data

Initial bactericidal activities were represented as the change in viable cell counts before and after treatment with quinolones. This was shown as the mean±SD of the results of quinolone-susceptible isolates or mutant strains, calculated by Microsoft Excel 2003.

Statistical analysis

The statistical significance between the STFX-treated group and other quinolone-treated groups was evaluated using Tukey's comparison test. The statistical significance between *S. pneumoniae* ATCC49619 and 60 treated with the same quinolone was evaluated using Student's *t*-test. These analyses were performed by SAS System Release 8.2 (SAS Institute Inc., NC, USA).

Results

Antimicrobial activity

The antimicrobial activity of STFX and other quinolones to *S. pneumoniae* strains used are shown in Table 1. The MICs of STFX, LVFX, MFLX, and GRNX against wild-type strains of *S. pneumoniae* were 0.03–0.06, 1, 0.12–0.25, and 0.03–0.06 μg/mL, respectively. The MICs of STFX, LVFX, MFLX, and GRNX against single mutants of *S. pneumoniae* were 0.03–0.06, 1–2, 0.12–0.5, and 0.03–0.12 μg/mL, respectively.

Bactericidal activity against wild-type and single QRDR mutant of *S. pneumoniae*

The changes in mean viable cell counts of 4 wild-type strains of *S. pneumoniae* after treatment with quinolones at C_{max} and fC_{max} for 1 and 2 hours are shown in Fig. 1 and Fig. 2, respectively. STFX at C_{max} and fC_{max} exhibited the most potent initial bactericidal activity resulting in 3.75 and 3.77 $\log_{10}$ CFU/mL reduction in viable counts at 1 hour and 4.46 and 4.13 $\log_{10}$ CFU/mL reduction in viable counts at 2 hours, respectively. Especially, STFX at fC_{max} exhibited significantly rapid bactericidal activity compared to other quinolones tested (1 hour: $P<0.001$ vs. LVFX and GRNX, $P<0.01$ vs. MFLX, 2 hours: $P<0.05$ vs. MFLX and GRNX).

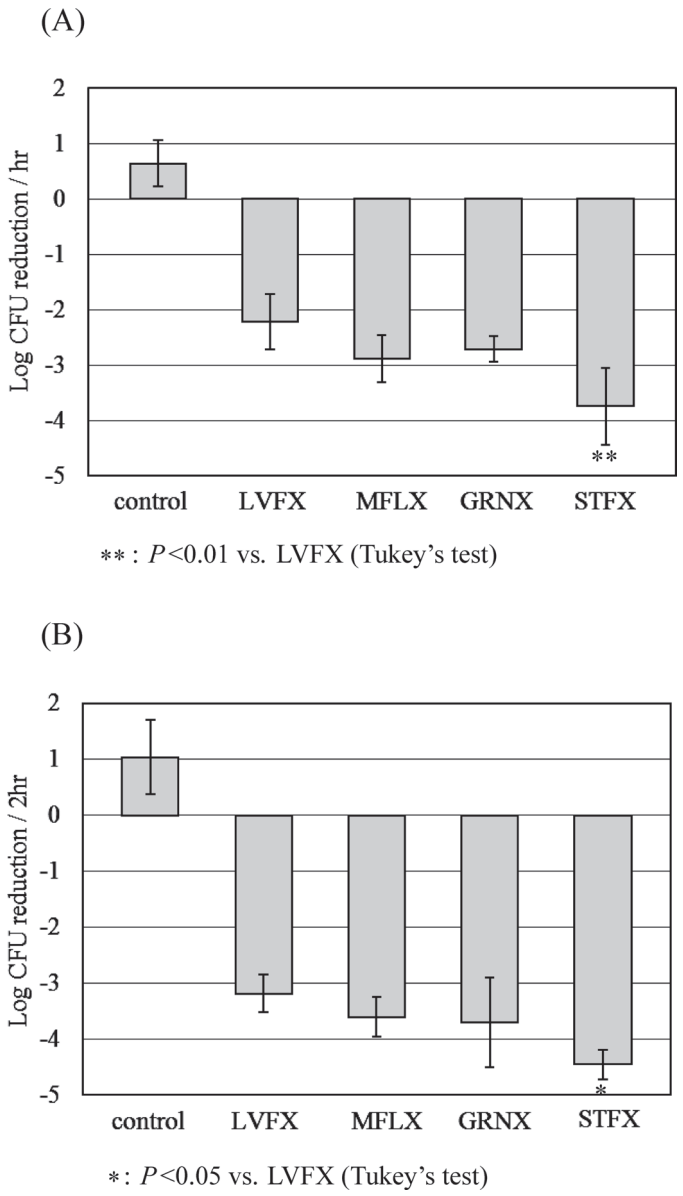
The changes in mean viable cell counts of 9 single mutants of *S. pneumoniae* after treatment with quinolones at C_{max} or fC_{max} for 1 and 2 hours are shown in Fig. 3 or Fig. 4, respectively. STFX at C_{max} and fC_{max} exhibited the most potent initial bactericidal activity resulting in 3.75 and 3.26 $\log_{10}$ CFU/mL reduction in viable counts at 1 hour and 3.96 and 3.85 $\log_{10}$ CFU/mL reduction in viable counts at 2 hours, respectively. STFX showed a statistically significant advantage in initial bactericidal activity over other quinolones tested for single mutants at any condition ($P<0.001$).

The changes in the mean viable cell counts after treatment with STFX at C_{max} for 1 and 2 hours were 0.87–1.53 and 0.75–1.27 $\log_{10}$ CFU/mL, respectively in the wild-type strains and 2.14–3.05 and 1.27–2.37 $\log_{10}$ CFU/mL, respectively in the single mutants. The changes in the mean viable cell counts after treatment with STFX at fC_{max} for 1 and 2 hours were 1.35–2.22 and 0.97–1.25 $\log_{10}$ CFU/mL, respectively in the wild-type strains and 1.98–2.56 and 1.73–2.39 $\log_{10}$ CFU/mL, respectively in the single mutants. This means that the STFX showed a more rapid bactericidal activity in the single mutants than wild type strains.

Bactericidal activity against wild-type and its laboratory single *parC* mutant of *S. pneumoniae*

The initial bactericidal activity of wild-type *S. pneumoniae* ATCC49619 (parent strain) and strain 60, which is a single *parC* mutant of ATCC49619, was investigated. The mean changes in viable cell counts with 3 times of individual experiments after treatment of two strains with quinolones at C_{max} or fC_{max} for 1 and 2 hours are shown in Fig. 5 or Fig. 6, respectively. STFX at

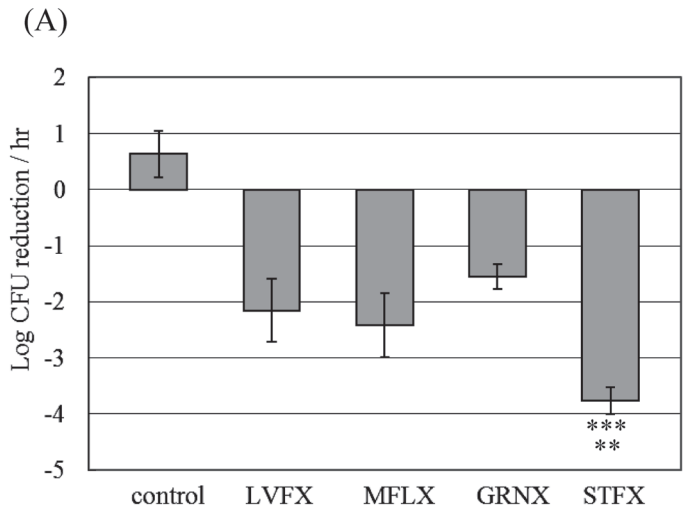
Fig. 1. Change in viable cell count of wild-type *Streptococcus pneumoniae* after (A) 1 hour and (B) 2 hour treatment with quinolones at C_{max} in humans.



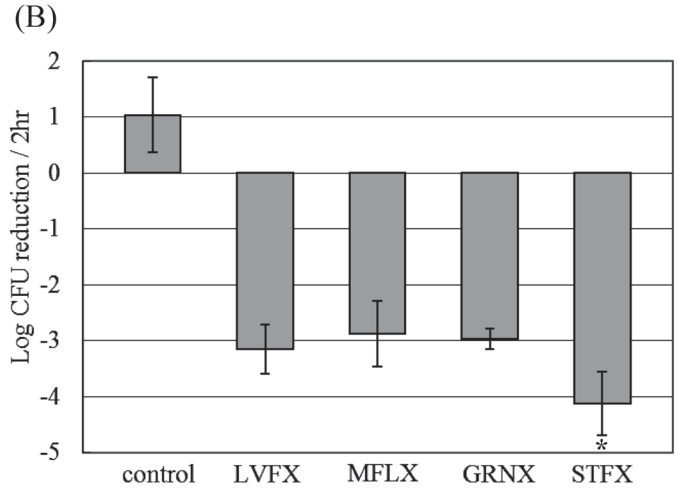
Each bar represents the mean±S.D. of the change in viable cell count of 4 strains of wild-type *S. pneumoniae*. The statistical significance between the STFX-treated group and other quinolone-treated groups was evaluated using Tukey's comparison test.

C_{max} exhibited the most potent early bactericidal activity against wild-type and a single *parC* mutant, resulting in 2.92 and 3.00 $\log_{10}$ CFU/mL reduction in viable counts at 1 hour, respectively, and 3.70 and 3.71 $\log_{10}$ CFU/mL reduction in viable counts at 2 hours, respectively. No significant effect on the initial bactericidal activity of STFX by a single *parC* mutation was confirmed.

Fig. 2. Change in viable cell count of wild-type *Streptococcus pneumoniae* after (A) 1 hour and (B) 2 hour treatment with quinolones at fC_{max} in humans.



*** : $P < 0.001$ vs. LVFX and GRNX, ** : $P < 0.01$ vs. MFLX (Tukey's test)

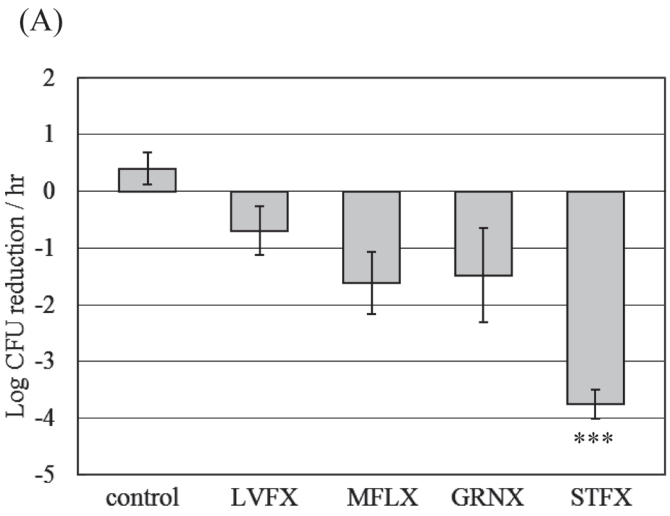


*: $P<0.05$ vs. MFLX and GRNX (Tukey's test)

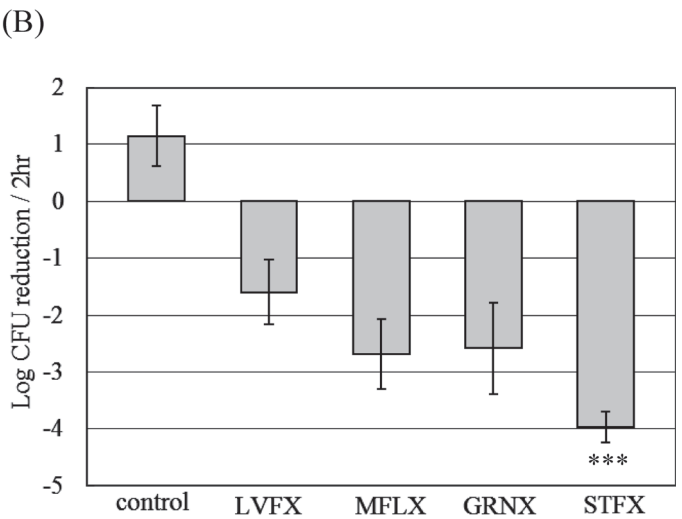
Each bar represents the mean±S.D. of the change in viable cell count of 4 strains of wild-type *S. pneumoniae*. The statistical significance between the STFX-treated group and other quinolone-treated groups was evaluated using Tukey's comparison test.

On the contrary, in the case of other quinolones tested, significant reduction of bactericidal activity was confirmed in a single *parC* mutant compared to wild-type ($P<0.01$ vs. LVFX at 1 and 2 hours, $P<0.05$ vs. MFLX and GRNX at 1 and 2 hours). STFX at fC_{max} exhibited the most potent early bactericidal activity against wild-type and a single *parC* mutant resulting in 2.80 and 2.41 $\log_{10}$ CFU/mL reduction in viable counts at 1 hour, respectively and 3.55 and 3.24 $\log_{10}$ CFU/mL reduction in viable counts at 2 hours, respectively. No significant effect on the initial

Fig. 3. Change in viable cell count of *Streptococcus pneumoniae* with a single mutation after (A) 1 hour and (B) 2 hour treatment with quinolones at C_{max} in humans.



*** : $P<0.001$ vs. LVFX, MFLX, and GRNX (Tukey's test)



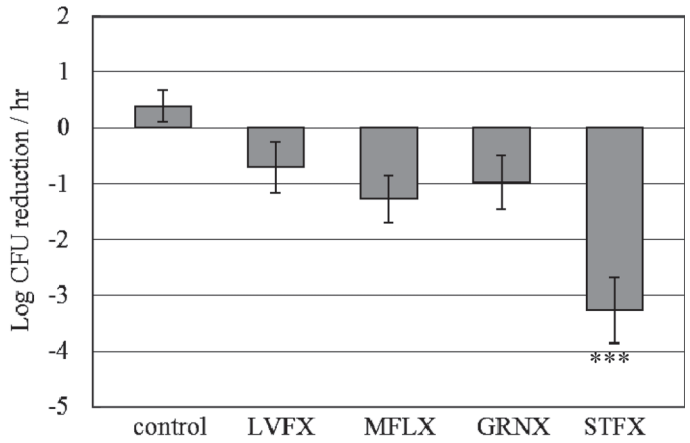
*** : $P<0.001$ vs. LVFX, MFLX, and GRNX (Tukey's test)

Each bar represents the mean±S.D. of the change in viable cell count of 9 strains of *S. pneumoniae* with a single mutation. The statistical significance between the STFX-treated group and other quinolone-treated groups was evaluated using Tukey's comparison test.

bactericidal activity of STFX by the substitution of single amino acid was confirmed. In contrast, in the case of other quinolones tested, significant reductions of bactericidal activities were confirmed in the single mutant compared to wild-type strain ($P<0.01$ vs. LVFX at 1 and 2 hours, MFLX at 2 hours, GRNX at 1 hour, $P<0.05$ vs. MFLX at 1 hour and GRNX at 2 hours).

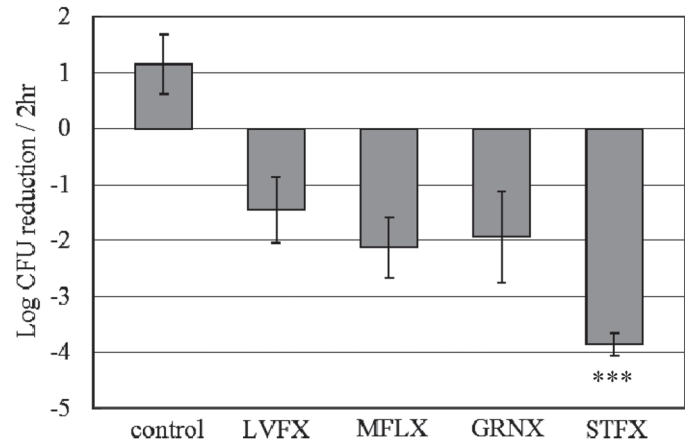
Fig. 4. Change in viable cell count of *Streptococcus pneumoniae* with a single mutation after (A) 1 hour and (B) 2 hour treatment with quinolones at fC_{max} in humans.

(A)



*** : P<0.001 vs. LVFX, MFLX, and GRNX (Tukey's test)

(B)



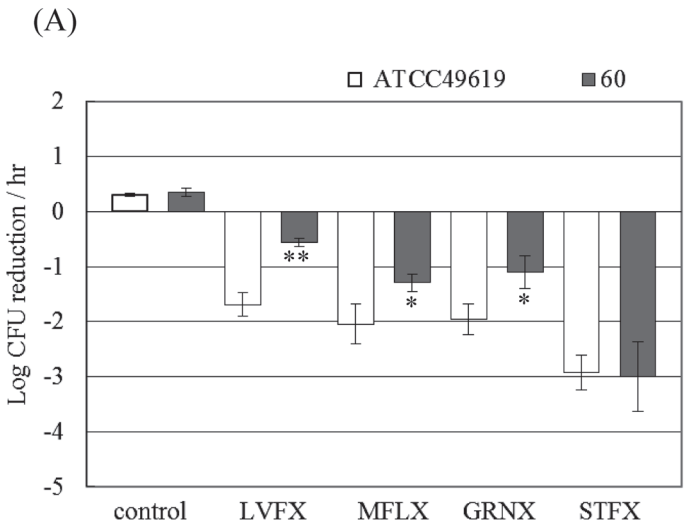
*** : P<0.001 vs. LVFX, MFLX, and GRNX (Tukey's test)

Each bar represents the mean±S.D. of the change in viable cell count of 9 strains of *S. pneumoniae* with a single mutation. The statistical significance between the STFX-treated group and other quinolone-treated groups was evaluated using Tukey's comparison test.

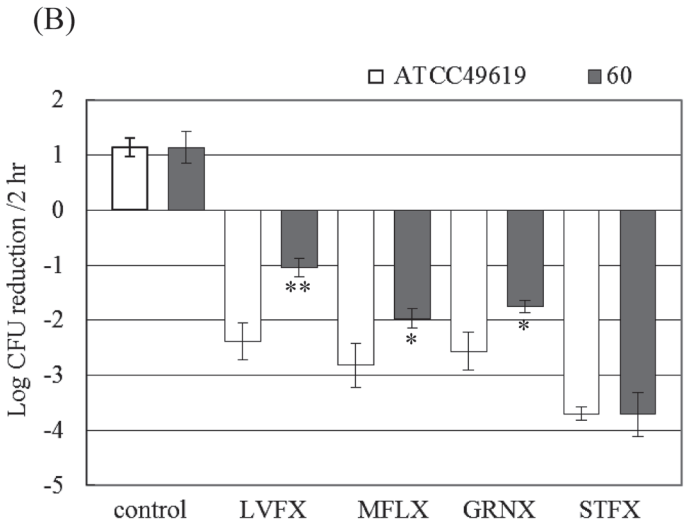
Discussion

The *in vitro* study described here was performed with the aim of determining the initial bactericidal activities of STFX, LVFX, MFLX, and GRNX against wild-type strains and single

Fig. 5. Change in viable cell count of *Streptococcus pneumoniae* ATCC49619 and 60 after (A) 1 hour and (B) 2 hour treatment with quinolones at C_{max} in humans.



**: $P<0.01$, *: $P<0.05$ compared to ATCC49619 (Student's *t*-test)

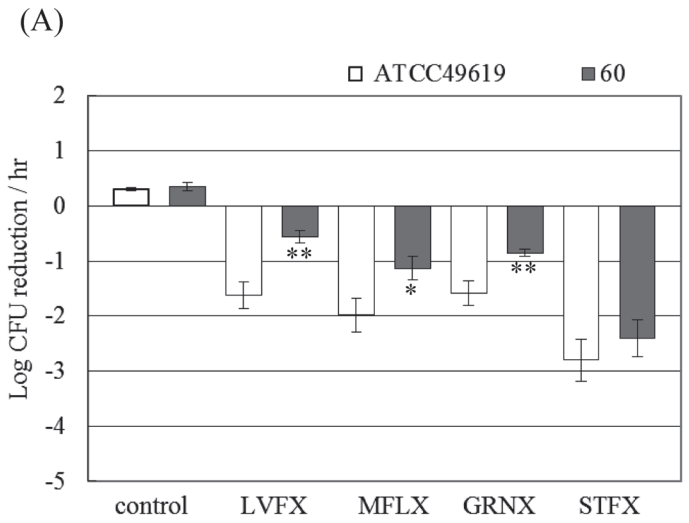


**: $P<0.01$, *: $P<0.05$ compared to ATCC49619 (Student's *t*-test)

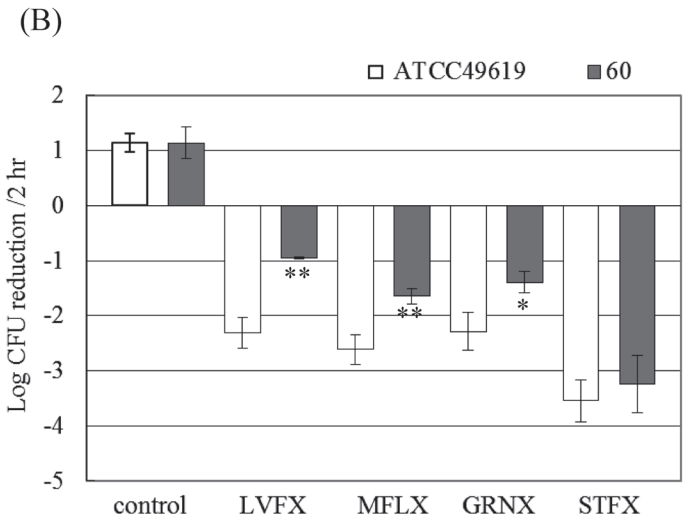
Each bar represents the mean±S.D. of the change in viable cell count of 3 times of individual experiments. The statistical significance between ATCC49619 and 60 treated with the same quinolone was evaluated using Student's *t*-test.

QRDR mutants of *S. pneumoniae* at the concentration equivalent to their C_{max} and fC_{max} in 2 hours to predict the initial bactericidal activities of quinolones in the clinical setting. We revealed that STFX not only at C_{max} but also fC_{max} exhibited distinguished potent and rapid bactericidal activity against *S. pneumoniae*, compared to LVFX, MFLX, and GRNX. The advantage of the

Fig. 6. Change in viable cell count of *Streptococcus pneumoniae* ATCC49619 and 60 after (A) 1 hour and (B) 2 hour treatment with quinolones at fC_{max} in humans.



**：$P<0.01$，*：$P<0.05$ compared to ATCC49619 (Student's *t*-test)



**：$P<0.01$，*：$P<0.05$ compared to ATCC49619 (Student's *t*-test)

Each bar represents the mean±S.D. of the change in viable cell count of 3 times of individual experiments. The statistical significance between ATCC49619 and 60 treated with the same quinolone was evaluated using Student's *t*-test.

bactericidal activity of STFX over other quinolones tested stood out in single QRDR mutants. Among the other quinolones tested, GRNX was confirmed to possess an almost equivalent antibacterial activity to STFX against *S. pneumoniae* and the C_{max} and fC_{max} of GRNX were about 7 times and 3 times higher than those of STFX, respectively. However, the initial antibactericidal

activity of GRNX at C_{max} and fC_{max} were lower than those of STFX at C_{max} and fC_{max}. YOKOTA *et al.* demonstrated a similar experiment except for the concentration of STFX. They used C_{max} and fC_{max} for the dosage of STFX 50 mg, which was half of our study and reported a different result that the antibactericidal activity of STFX 50 mg at C_{max} and fC_{max} was lower than GRNX[21]. Since the protein binding ratio of STFX is 50%, fC_{max} of STFX 100 mg is almost equal to C_{max} of STFX 50 mg. However, the initial bactericidal activity of fC_{max} of STFX 100 mg was comfirmed to be higher than that of C_{max} of GRNX 400 mg in our study. This difference is considered to be attributable to the difference of bacterial strains, medium, and bacterial counts at the beginning of drug treatment between the two studies. On the contrary, in terms of LVFX and MFLX, SCHAFER, J. *et al.* reported that MFLX initially displayed faster killing than LVFX against a *parC* mutant with a Ser-79Phe mutation[19]. It was confirmed in the report by ANDEREGG, T. R. *et al.* that GRNX and LVFX showed slow initial bactericidal activity against wild-type and QRDR mutant strains of *S. pneumoniae* at clinically achievable serum concentration[22], where these data support our study.

STFX was confirmed to possess a more potent and balanced dual-target inhibition against wild-type and single mutated DNA gyrase and topoisomerase IV of *S. pneumoniae* than other quinolones[30,31]. Moreover, OKUMURA *et al.* reported that the rapid bactericidal activity of STFX was due to its ability to induce autolysis mediated by LytA[32], which is resposible for autolysis in *S. pneumoniae*[33]. We speculate that these features are part of the reasons why STFX showed rapid and potent bactericidal activity against wild-type and single QRDR mutant strains of *S. pneumoniae*.

In conclusion, STFX at around the C_{max} of the clinical dose (100 mg) showed the most potent and rapid antibactericidal activity against *S. pneumoniae* regardless of existence or non-existence of single QRDR mutation. These results suggest that STFX may show potent and rapid bactericidal activity in the treatment of infectious disease caused by *S. pneumoniae*.

Acknowledgment

We gratefully acknowledge the technical assistance of MIHO TANABE.

References

1) GARAU, J. & E. CALBO: Community-acquired pneumonia. Lancet 371: 455～458, 2008
2) FILE, JR. T. M.: Clinical implications and treatment of multiresistant *Streptococcus pneumoniae* pneumonia. Clin. Microbiol. Infect. 12 (Suppl. 3): 31～41, 2006
3) BHAVNANI, S. M.; J. P. HAMMEL, R. N. JONES, *et al.*: Relationship between increased levofloxacin use and decreased susceptibility of *Streptococcus pneumoniae* in the United States. Diagn. Microbiol. Infect. Dis. 51: 31～37, 2005
4) FARRELL, D. J.; M. CASTANHEIRA, R. E. MENDES, *et al.*: *In vitro* activity of ceftaroline against multidrug-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae*: a review of published stud-

ies and the AWARE surveillance program (2008–2010). Clin. Infect. Dis. 55 (Suppl. 3): S206～214, 2012
5) Watanabe, A.; K. Yanagihara, T. Matsumoto, *et al.*: Nationwide surveillance of bacterial respiratory pathogens conducted by the Surveillance Committee of Japanese Society of Chemotherapy, Japanese Association for Infectious Diseases, and Japanese Society for Clinical Microbiology in 2009: general view of the pathogens' antibacterial susceptibility. J. Infect. Chemother. 5: 609～620, 2012
6) Tao, L. L.; B. J. Hu, L. X. He, *et al.*: Etiology and antimicrobial resistance of community-acquired pneumonia in adult patients in China. Chin. Med. J. 125: 2967～2972, 2012
7) Munoz, R. & A. G. De La Campa: ParC subunit of DNA topoisomerase IV of *Streptococcus pneumoniae* is a primary target of fluoroquinolones and cooperates with DNA gyrase A subunit in forming resistance phenotype. Antimicrob. Agents Chemother. 40: 2252～2257, 1996
8) Canton, R.; M. Morosini, M. C. Enright, *et al.*: Worldwide incidence, molecular epidemiology and mutations implicated in fluoroquinolone-resistant *Streptococcus pneumoniae*: data from the global PROTEKT surveillance programme. J. Antimicrob. Chemother. 52: 944～952, 2003
9) Lim, S.; D. Bast, A. McGeer, *et al.*: Antimicrobial susceptibility breakpoints and first-step *parC* mutations in *Streptococcus pneumoniae*: redefining fluoroquinolone resistance. Emerg. Infect. Dis. 9: 833～837, 2003
10) Davies, T. A.; A. Evangelista, S. Pfleger, *et al.*: Prevalence of single mutations in topoisomerase type II genes among levofloxacin-susceptible clinical strains of *Streptococcus pneumoniae* isolated in the United States in 1992 to 1996 and 1999 to 2000. Antimicrob. Agents Chemother. 46: 119～124, 2002
11) Jones, M. E.; D. F. Sahm, N. Martin, *et al.*: Prevalence of *gyrA*, *gyrB*, *parC*, and *parE* mutations in clinical isolates of *Streptococcus pneumoniae* with decreased susceptibilities to different fluoroquinolones and originating from worldwide surveillance studies during the 1997–1998 respiratory season. Antimicrob. Agents Chemother. 44: 462～466, 2000
12) Weigel, L. M.; G. J. Anderson, R. R. Facklam, *et al.*: Genetic analyses of mutations contributing to fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. 45: 3517～3523, 2001
13) Okumura, R.; T. Hirata, Y. Onodera, *et al.*: Dual-targeting properties of 3-aminopyrrolidyl quinolones, DC-159a and sitafloxacin, against DNA gyrase and topoisomerase IV: Contribution in reducing *in vitro* emergence of quinolone-resistant *Streptococcus pneumoniae*. J. Antimicrob. Chemother. 62: 98～104, 2008
14) Sato, K.; K. Hoshino, M. Tanaka, *et al.*: Antimicrobial activity of DU-6859, a new potent fluoroquinolone, against clinical isolates. Antimicrob. Agents Chemother. 36: 1491～1498, 1992
15) Touyama, M.; F. Higa, C. Nakasone, *et al.*: *In vitro* activity of sitafloxacin against clinical strains of *Streptococcus pneumoniae* with defined amino acid substitutions in QRDRs of gyrase A and topoisomerase IV. J. Antimicrob. Chemother. 58: 1279～1282, 2006
16) Tanaka, M.; K. Hoshino, M. Hohmura, *et al.*: Effect of growth conditions on antimicrobial activity of DU-6859a and its bactericidal activity determined by the killing curve method. J. Antimicrob. Chemother. 37: 1091～1102, 1996
17) Andes, D. & W. A. Craig: Animal model pharmacokinetics and pharmacodynamics: a critical review. Int. J. Antimicrob. Agents. 19: 261～268, 2002
18) Craig, W. A.: Does dose matter? Clin. Infect. Dis. 33 Suppl. 3: S233～237, 2001
19) Schafer, J.; L. B. Hovde, D. Simonson, *et al.*: *In vitro* pharmacodynamics of moxifloxacin versus

levofloxacin against 4 strains of *Streptococcus pneumoniae*: 1 wild type, 2 first-step *parC* mutants, and 1 pump mutant. Diagn. Microbiol. Infect. Dis. 60: 155～161, 2008

20) Blaser, J.; B. B. Stone, M. C. Groner, *et al.*: Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. Antimicrob. Agents Chemother. 31: 1054～1060, 1987
21) Yokota, S.; Y. Okoshi & N. Fujii: Evaluation of four quinolones against fluoroquinolone-nonsusceptible clones of *Streptococcus pneumoniae* and *Haemophilus influenzae*. Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi, Japanese 21: 17～24, 2011
22) Anderegg, T. R. & R. N. Jones: Bactericidal activity of garenoxacin tested by kill-curve methodology against wild type and QRDR mutant strains of *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. 50: 213～217, 2004
23) Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Eighth Edition, M7-A8. Clinical and Laboratory Standards Institute, Wayne, Pa, 2009
24) Pan, X. S.; J. Amber, S. Mehtar, *et al.*: Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. 40: 2321～2326, 1996
25) Jorgensen, J. H.; L. M. Weigel, M. J. Ferraro, *et al.*: Activities of newer fluoroquinolones against *Streptococcus pneumoniae* clinical isolates including those with mutations in the *gyrA*, *parC*, and *parE* loci. Antimicrob. Agents Chemother. 43: 329～334, 1999
26) Gracevit tablets, fine granules [package insert]. Tokyo, Japan: Daiichi Sankyo Co. Ltd., 2008
27) Cravit tablets, fine granules [package insert]. Tokyo, Japan: Daiichi Sankyo Co. Ltd., 2009
28) Averox tablets [package insert]. Osaka, Japan: Bayer Yakuhin Ltd., 2005
29) Geninax tablets 200 mg [package insert]. Tokyo, Japan: Astellas Pharma Inc., 2007
30) Okumura, R.; T. Hirata, Y. Onodera, *et al.*: Dual targeting properties of the 3-aminopyrrolidyl quinolones, DC-159a and sitafloxacin, against DNA gyrase and topoisomerase IV: contribution to reducing *in vitro* emergence of quinolone-resistant *Streptococcus pneumoniae*. J. Antimicrob. Chemother. 62: 98～104, 2008
31) Kanda, H.: Pharmacological profile and clinical efficacy of sitafloxacin, a novel quinolone antibacterial agent. Nihon Yakurigaku Zasshi 133: 43～51, 2009
32) Okumura, R.; K. Hoshino, T. Otani, *et al.*: Quinolones with enhanced bactericidal activity induced autolysis in *Streptococcus pneumoniae*. Chemotherapy 55: 262～269, 2009
33) Lewis, K.: Programmed death in bacteria. Microbiol. Mol. Biol. Rev. 64: 503～514, 2000

Sitafloxacinの*Streptococcus pneumoniae*に対する迅速な殺菌効果

神田裕子[1)]・井上和恵[2)]・奥村　亮[1)]・星野一樹[1)]
[1)] 第一三共株式会社研究開発本部生物医学研究所
[2)] 第一三共株式会社ワクチン事業本部ワクチン事業部

キノロン系抗菌薬の*Streptococcus pneumoniae*に対する初期殺菌効果を，最高血漿中濃度（C_{max}）及びフリー体C_{max}（fC_{max}）で検討した。すなわち，Sitafloxacin（STFX），Levofloxacin（LVFX），Moxifloxacin（MFLX），及びGarenoxacin（GRNX）を健康成人に国内における1回あたり最高用量（それぞれ100 mg, 500 mg, 400 mg, 及び400 mg）を投与した際のC_{max}及びfC_{max}を作用させ，薬剤作用1時間後及び2時間後における殺菌効果を比較検討した。その結果，STFXは野生型の臨床分離*S. pneumoniae*菌株，計4株に対し，C_{max}, fC_{max}いずれの濃度を作用した場合も，薬剤作用1時間以内に平均生菌数を3 log colony forming units（CFU）/mL以上減少させ，最も高い初期殺菌効果を示した。STFXは，*par*（*C*/*E*）に1ヵ所変異を有する*S. pneumoniae*（一変異型*S. pneumoniae*），計9株に対しても，最も高い初期殺菌効果を示し，薬剤作用1時間以内に平均生菌数を3logCFU/mL以上減少させた。STFXの一変異型*S. pneumoniae*に対する初期殺菌効果は，対照キノロン系抗菌薬と比較して有意に高いものであった（$P<0.001$）。STFXと対照キノロン系抗菌薬との殺菌効果の差が，野生型より一変異型*S. pneumoniae*で大きくなる傾向を確認するために，*S. pneumoniae* ATCC49619（野生型）と本株から実験的に作製された*parC*一変異株を用いて同様の検討を実施した。その結果，STFXは両株に対して同等の高い初期殺菌効果を示したが，対照キノロン系抗菌薬では，一変異型*S. pneumoniae*に対する初期殺菌効果が顕著に減少した（$P<0.05$）。

以上の結果より，STFXは野生型及び一変異型*S. pneumoniae*に対して最も高い初期殺菌効果を示すことが明らかとなった。さらに，STFXの殺菌効果はLVFX, MFLX, 及びGRNXと比較して，標的酵素の一変異の影響を受けにくいことが明らかになった。