Anti-VRE and Anti-MRSA Activities of New Quinolones and Their Synergism with Commercial Antibiotics. Part 2

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Anti-VRE and anti-MRSA activities of new quinolone derivatives [The two quinolone derivatives are 8-[(3-[(ethylamino)methyl]-1-pyrrodinyl]-7-fluoro-9,1-[(N-methylimino)methano]-5-oxo-5H-thiazolo[3,2-a]quinolone-4-carboxylic acid (compound A) and 7-fluoro-8-morpholino-9,1-[(N-methylimino)methano]-5-oxo-5H-thiazolo[3,2-a]quinolone-4-carboxylic acid (compound B)] and their synergism with commercial antibiotics were investigated. Compound A exhibited potent antibacterial activity against VRE and MRSA among the five new quinolone compounds tested, and showed superior activity to pefloxacin, ofloxacin and levofloxacin, which are clinically in use these days. With respect to the anti-VRE activity, compound A showed synergism with fosfomycin (FOM), and partial synergism with ampicillin (ABPC), gentamicin (GM), minocycline (MINO) and vancomycin hydrochloride (VCM). Partial synergism in anti-VRE activity was also observed between compound B and GM, MINO, FOM and VCM. Compound A also showed synergism with MINO and FOM in anti-MRSA activity. Partial synergism was observed with ABPC, GM and VCM. Synergism with ABPC was not detected in anti-MRSA activity. On the other hand, the synergism of compound B with FOM, and the partial synergism with ABPC, GM and MINO were also found against MRSA. No synergism with ABPC was found against MRSA. These results suggested that compound A and B could possibly reduce the daily administration dose of these antibiotics in the treatment of nosocomial infections, and also reduce the possibility of the occurrence of nosocomial infections caused by VRE and/or MRSA.

Key words: Antibacterial activity/New quinolones/VRE/MRSA/Synergism.

INTRODUCTION

Nosocomial infections caused by vancomycin-resistant enterococci (VRE) have long been one of the most serious problems in hospitals worldwide (Emori et al., 1993; Leclercq et al., 1997; Murry, 1997; Moellering, 1998; Timmers et al., 2002; Peta et al., 2006; Lucet et al., 2007). Although there have been many reports regarding the development of potential infection-control methods for VRE (Garner, 1996; Slaughter et al., 1996; Montecalvo et al., 1999; Nourse et al., 2000; Linden et al., 2002; Shaikh et al., 2002; Sakagami et al., 2002; Calfee et al., 2003; Kauffman, 2003; Muto et al., 2003; Kurup et al., 2008), further investigation is still needed to overcome and control VRE infection.

On the other hand, during the past two decades,
methicillin resistant *Staphylococcus aureus* (MRSA) has become an increasingly common microorganism in hospitals (Voss et al., 1994; Coolson, 1995; Cox et al., 1995; Working Party Report, 1998; Kotilainen et al., 2001; Kron et al., 2002; Samad et al., 2002; Shiojima et al., 2003; Burd et al., 2003; De Lucas-Villarrubia et al., 2003; Ellis et al., 2003; Jarvis et al., 2007; Millar et al., 2008). Thus, eradication of MRSA infection is becoming another serious target in clinical scenes worldwide.

In a previous paper, the authors investigated three kinds of new quinolone derivatives [compound (1), (2) and (3)], and clarified their inhibitory activities against VRE and MRSA (Sakagami et al., 2003). Synergism with commercial antibiotics with respect to the anti-VRE and anti-MRSA activities was also examined. Furthermore, we also performed the screening of other new quinolone derivatives which exhibited stronger anti-VRE and anti-MRSA activities.

In this paper, we present stronger novel quinolone compounds in order to reduce VRE and MRSA infections. This paper describes the antibacterial activities of two new quinolone compounds against the nosocomial infectious bacteria such as VRE and MRSA, and the synergism of these compounds with commercial antibiotics.

**MATERIALS AND METHODS**

**Bacterial strains**

VRE: Five strains of VRE (*Enterococcus faecalis* ATCC 51575, *E. faecalis* ATCC 51299, *E. faecium* ATCC 51559, *E. faecium* KIHC-237 and *E. gallinarum* KIHC-241) were used in this experiment. *E. faecium* KIHC-237 and *E. gallinarum* KIHC-241 were supplied by Kobe Prefectural Institute of Public Health, where these were isolated from imported poultry. Minimum Inhibitory Concentration (MIC) values of the 5 strains of VRE to vancomycin hydrochloride (VCM) were 250, 32, 200, 200 and 16 µg/mL, respectively. The genotypes of *E. faecalis* ATCC 51299, *E. faecium* KIHC-237 and *E. gallinarum* KIHC-241 were van B (+), van A (+) and van C1 (+), respectively. The genotypes of the other VRE such as *E. faecalis* ATCC 51575 and *E. faecium* ATCC 51559 were unknown.

MRSA: Each of the following institutions kindly donated 3 strains (total: 9 strains) of methicillin resistant *Staphylococcus aureus* (MRSA) in 1997: Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka National Hospital and Kitano Hospital. MIC values of methicillin (DMPMC) against these 9 strains of MRSA were 12.5, 400, 25, 12.5, 400, 1600, 25, 12.5 and 400 µg/mL, respectively.

**Quinolone derivatives and antibiotics**

The two new quinolone derivatives, 8-[3-[(ethylamino) methyl]-1-pyrroldinyl]-7-fluoro-9,1-[(N-methylimino) methano]-5-oxo-5H-thiazolo [3,2-a] quinolone-4-carboxylic acid (compound A, Mol wt: 429.51), 7-fluoro-8-morpholino-9,1-[(N-methylimino) methano]-5-oxo-5H-thiazolo [3,2-a] quinolone-4-carboxylic acid (compound B, Mol wt: 388.41) and the former compound (Sakagami et al., 2003) of 7-fluoro-9,1-[(N-methylimino)methano]-8-(4,3-dimethyl-1-piperazinyl)-5-oxo-5H-thiazolo [3,2-a] quinolone-4-carboxylic acid (compound 1, Mol wt: 416.47) shown in chart 1 were prepared by the previously re-

![Chart 1](image-url)

**CHART 1.** Structural formula of new quinolone derivatives tested in this experiment.
ported methods (Jinbo et al., 1993a; Jinbo et al., 1993b; Lowe et al., 1994). Pefloxacin (PFLX), as a standard reagent, was donated from Organon Co., Ltd., Japan, and ofloxacin (OFLX) and levofloxacin (LVFX), also as standards, were donated from Daiichi Sankyo Pharmaceutical Co., Ltd.


Medium
Soybean Casein Digest (SCD) broth (Nihon Pharm. Co., Ltd., Osaka, Japan) was used to preincubate the test strains and Muller-Hinton (MH) agar (Difco Co., Ltd.) was used to determine minimum inhibitory concentration (MIC) values.

<table>
<thead>
<tr>
<th>TABLE 1. Antibacterial activities of 6 test compounds against 5 strains of vancomycin resistant Enterococci (VRE)</th>
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<tr>
<td>Test bacteria</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 51299 (VRE)</td>
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<tr>
<td>Enterococcus faecalis ATCC 51755 (VRE)</td>
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<tr>
<td>Enterococcus faecium ATCC 51599 (VRE)</td>
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<tr>
<td>Enterococcus faecium KIHC-237 (VRE)*</td>
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<tr>
<td>Enterococcus gallininarum KIHC-241 (VRE)*</td>
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</table>

a) E. faecium KIHC-237 and E. gallinarum KIHC-241 were isolated from imported poultry and supplied by Kobe Prefectural Institute of Public Health in Japan.
b) Compound (1) has been presented in a previous paper (Sakagami et al., Biocontrol Science, 8, 159-166, 2003).

c) Compound (1) has been presented in a previous paper (Sakagami et al., Biocontrol Science, 8, 159-166, 2003).
alone) were poured into the above petri dishes and mixed. After cooling the dishes, the MICs of the test compounds, the antibiotic alone and the test compound combined with each antibiotic, were examined. Throughout this experiment, the fraction inhibitory concentration (FIC) indices were calculated by the method used by Didry et al. (1993), and interactive effects between the test compound and antibiotics were examined.

FIC indices were evaluated as follows. FIC index ≤ 0.5: Synergetic effect, 0.5 < FIC index < 1.0: Partially synergetic effect, FIC index ≥ 1.0: No synergetic effect and FIC index ≥ 2.0: Antagonistic effect.

RESULTS

MIC values of the novel quinolone compounds against VRE and MRSA

Table 1 and 2 show the MIC values of the novel quinolones against VRE and MRSA. The MIC values of compound A against 5 strains of VRE were 0.013–0.78 μg/mL, and those of compound B were 0.025–3.13 μg/mL. On the other hand, the MIC values of compound (1), PFLX, OFLX and LVFX against 5 strains of VRE were 0.2–1.56 μg/mL, 1.56–12.5 μg/mL, 1.56–12.5 μg/mL and 0.78–6.25 μg/mL, respectively. The MIC values of compound A against VRE were considerably superior to those of the compound (1), PFLX, OFLX and LVFX.

The MIC values of compound A against 9 strains of MRSA were 0.2–1.56 μg/mL, and those of compound B were 0.05–3.13 μg/mL. On the other hand, the MIC values of compound (1), PFLX, OFLX and LVFX against 9 strains of MRSA were 0.2–6.25 μg/mL, 6.25–25 μg/mL, 3.13–25 μg/mL and 1.56–25 μg/mL, respectively. The MIC values of compound A against MRSA were also superior to those of compound (1), PFLX, OFLX and LVFX.

Synergism between the compounds (A and B) and commercial antibiotics against VRE and MRSA

The results are shown in Figure 1 and Figure 2. Compound A and B exhibited more effective anti-VRE and anti-MRSA activities than any other quinolone derivatives described in the previous paper (Sakagami et al., 2003).

The synergism between these compounds (A, B) and commercial antibiotics such as ABPC, GM, MINO, FOM and VCM were also investigated. Against VRE, the average and standard deviation of FIC indices between compound A and ABPC, GM, MINO, FOM and VCM were 0.5386 ± 0.1633, 0.5198 ± 0.3803, 0.8886 ± 0.4371, 0.4633 ± 0.1633 and 0.5222 ± 0.3392, respectively. Synergism with FOM and the partial synergism with ABPC, GM, MINO and VCM were observed. Against MRSA, the averages and standard deviation of FIC indices between compound A and ABPC, GM, MINO, FOM and VCM were 0.4340 ± 0.1764, 0.6801 ± 0.2946, 0.3597 ± 0.2000, 0.2294 ± 0.1513 and 0.7016 ± 0.4290, respectively. Synergism with ABPC, MINO and FOM, and the partial synergism with GM and VCM were found (Figure 1).

On the other hand, the average and standard deviation of FIC indices against VRE between compound B and ABPC, GM, MINO, FOM and VCM were 1.4192 ± 0.3958, 0.7172 ± 0.2661, 0.9206 ± 0.6370, 0.6275 ± 0.1219 and 0.7141 ± 0.5770, respectively. Partial synergism with GM, MINO, FOM and VCM was observed.

Against MRSA, the average and standard deviation of FIC indices between compound B and ABPC, GM, MINO, FOM and VCM were 0.9167 ± 0.2998, 0.9476 ± 0.4228, 0.5834 ± 0.4881, 0.3815 ± 0.2463 and 1.3542 ± 0.3094, respectively. Synergism with FOM, and the partial synergism with ABPC, GM and MINO were found. No synergism with VCM was found.
DISCUSSION

The development of effective anti-VRE and/or anti-MRSA drugs is one of the most important and urgent matters to prevent the current serious infections caused by these two deadly infectious bacteria. The authors previously developed a general and efficient method to synthesize novel quinolone derivatives, some of which were found to be potentially effective in inhibiting the growth of a variety of infectious bacteria such as VRE and MRSA (Sakagami et al., 2003). Among them, three new quinolone derivatives (1, 2 and 3) were found to exhibit potential anti-VRE and anti-MRSA activities, and synergism with some commercial antibiotics was also observed (Sakagami et al., 2003). Further study on the structure-activity relationships of the quinolone derivatives enabled us to find more effective derivatives against these two infectious bacteria.

Thus, compound A showed the highest activity against both VRE and MRSA among the 8 quinolone derivatives which involved the three compounds previously tested, PFLX, OFLX and LVFX (Sakagami et al., 2003). The synergism with FOM against VRE, and the synergism with ABPC, MINO and FOM against MRSA were also observed. Partial synergism was recognized with ABPC, GM, MINO and VCM against VRE, and with GM and VCM against MRSA, respectively.

Compound B also showed superior activity to the three previously tested compounds against VRE and MRSA, but not as much as compound A. Synergism with FOM was recognized against MRSA. Partial synergism with GM, MINO, FOM and VCM against VRE, and partial synergism with ABPC, GM and MINO against MRSA were also recognized.

Concerning the sensitivities of compound A and B to the tested bacteria, no differences between VRE and vancomycin-sensitive enterococci (VSE) were observed. On the other hand, the sensitivities of these two compounds to methicillin-sensitive Staphylococcus aureus (MSSA) was greater than that to MRSA (data not shown).

The authors previously demonstrated that sophoraflavanone G (SFG), a flavanone derivative isolated from Sophora species (Leguminosae), sustained significant anti-MRSA activity. The synergism of SFG had been observed with VCM and also with FOM, and partial synergism with methicillin, cefzonam, GM and LVFX (Sakagami et al., 1998).

Thus, the two new quinolones A and B showed high anti-VRE and anti-MRSA activities among the quinolone derivatives reported so far, and the synergism with the commercial antibiotics is considerable practical. Only a few papers have described the antibacterial activity of quinolone compounds against VRE and/or MRSA (Shetty et al, 2000; Sakagami et al., 2003).

Novel antibacterial agents which possess synergistic effects in combination with commercial antibiotics would reduce the daily administration dose of the commercial antibiotics for the cure of nosocomial infection, contributing to the reduction of the ratio of nosocomial infections caused by VRE and MRSA. Therefore, the present results suggest the value of studying the effect of this type of new quinolone derivatives on nosocomial infection.

Unfortunately, the poor solubility of compounds A and B in water, like that of the former compound (1), would not allow practical use. However, it would be worth synthesizing further derivatives of compounds A and B to improve their poor solubility, their anti-VRE and anti-MRSA activities, and their synergism with commercial antibiotics.
REFERENCES


Announcement from the Editor of

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Sincerely yours