ANTISTAPHYLOCOCCAL ACTIVITY OF DX-619, ALONE AND IN COMBINATION WITH VANCOMYCIN, TEICOPLANIN AND LINEZOLID, BY TIME-KILL SYNERGY TESTING

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Running title: DX-619 antistaphylococcal synergy

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**ABSTRACT.** Time-kill synergy studies testing in vitro activity of DX-619 alone and with added vancomycin, teicoplanin or linezolid against 101 *S.aureus* strains showed synergy between DX-619 and teicoplanin between 12 and 24 h in 72 strains and between DX-619 with vancomycin in 28 strains. No synergy was found with linezolid and no antagonism was observed with any combination.
Emergence of methicillin- and quinolone-resistant, and recently glycopeptide-intermediate staphylococci, as well as the propensity of these organisms to cause serious systemic infections in immunocompromised hosts, necessitates other therapeutic modalities (10,12,16,17,21,23). During 2002, two clinical strains of vancomycin-resistant *Staphylococcus aureus* (VRSA) carrying *vanA*, one from Detroit, MI and one from our hospital, have been isolated. There are now a total of six VRSA strains: four from Michigan, one from Pennsylvania and one from New York (2,3,15,22, M. Rybak, personal communication).

By contrast, vancomycin intermediate strains (VISA), first described in Japan by Hiramatsu and co-workers (13), have been much more widely reported, and hospital colonization leading to systemic infections with these strains has recently been reported (9,18). Recent lowering of the vancomycin susceptibility breakpoint to $\leq 2 \mu g/ml$ (6) will doubtless result in an even higher report rate of these organisms whose incidence is almost certainly under-reported. It also seems that some VISA strains have higher daptomycin MICs than is the case with vancomycin susceptible strains (7).

The situation is further complicated by the emergence, both in the US and elsewhere, of virulent clonal (usually USA300 or 400) Panton-Valentine leukocidin associated community-acquired methicillin-resistant *S.aureus* strains (MRSA) which primarily cause abscesses but which have on occasion produced life-threatening and lethal diseases (1,10,11). Whether this toxin is the major virulence determinant in community-acquired MRSA disease is not clear at the present time (24).

Most methicillin-resistant staphylococci are also resistant to available quinolones such as ciprofloxacin, levofloxacin, moxifloxacin and gemifloxacin (10,14,19-21,23). Thus, the latter compounds may not be safely used in empiric therapy of patients with methicillin-resistant staphylococcal infections. DX-619 is a new des-F(6)-quinolone with excellent activity against Gram-positive organisms. In a recent study from our laboratory, DX-619 was shown to be potent and bactericidal against quinolone susceptible and resistant *S.aureus* strains, with a low propensity to develop resistance (2).

A previous preliminary study showed synergy between clinafloxacin and glycopeptides against *S.aureus* by time-kill methodology (5). In the current study, we used time-kill methodology to test the possible synergistic activity of DX-619 when combined with vancomycin, teicoplanin and linezolid against 101 *S.aureus* strains with varying phenotypes.
Bacterial strains used in this study were all non-duplicate recent clinical isolates (2002 to date) isolated at Hershey Medical Center and Southwestern Medical Center, Dallas, TX from wounds, blood and sputum identified by standard methods. The 101 organisms tested comprised i) Fifty (including 2 VISA) methicillin susceptible strains, of these 46 were quinolone susceptible and 4 were quinolone resistant; ii) Fifty-one MRSA strains; 37 MRSA strains were also quinolone resistant and included 4 VISA and 3 VRSA (1 VISA and 1 VRSA were isolated at Hershey Medical Center). Strains were stored frozen at -70°C in double-strength litmus milk (Difco, Inc., Detroit, Mi) until use. DX-619 powder was obtained from Daiichi Pharmaceutical Co., Ltd (Tokyo, Japan), and other compounds from their respective manufacturers.

MICs were predetermined by macrodilution in cation-adjusted Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md) according to standard methodology (6). All strains were tested by time-kill with each compound alone as described previously (5). Concentrations 1-2 dilutions below the MIC were chosen for synergy testing. Viability counts in synergy tests were performed at 0, 3, 6, 12 and 24 h in a shaking water bath at 35°C with final inocula between 5 x 10⁵ and 5 x 10⁶ cfu/ml. Drug carryover was addressed by dilution as described previously (5). Synergy was defined as a ≥2log₁₀ decrease in cfu/ml between the combination and its most active component after 3, 6, 12 and 24 h, the number of surviving organisms in the presence of the combination being ≥2log₁₀ below the starting inoculum at 0 h. At least one of the drugs had to be present in a concentration which did not significantly affect the growth curve of the test organism when used alone (5).

Compiled results are presented in Table 1 and data for individual strains as supplementary electronic data. MICs (µg/ml) of drugs alone were 0.004-2 (DX-619), 0.5->128 (vancomycin), 0.25-16 (teicoplanin), 1-4 (linezolid). Results of synergy time-kills are presented at 12 h and 24 h and only for DX-619 + vancomycin and DX-619 + teicoplanin, because all combinations at 3 h and 6 h, and all combinations with DX-619 + linezolid, were additive.

Eight of the 50 (16%) methicillin susceptible strains showed synergy between DX-619 + vancomycin at 12 h and 12 of 50 strains (24%) including 1 quinolone resistant strain showed synergy between DX-619 + vancomycin at 24 h. Ten of 50 methicillin susceptible strains (20%) showed synergy between DX-619 + teicoplanin at 12 h and 35 of 50 methicillin susceptible strains (70%) including 3
quinolone resistant strains showed synergy between DX-619 + teicoplanin at 24 h. The 2 methicillin susceptible VISA strains showed additive activity with all combinations.

One of 36 (3%) quinolone resistant MRSA strains showed synergy between DX-619 + vancomycin at 12 h and 12 of 36 strains (33%) including 1 VISA and 1 Hershey VRSA showed synergy at 24 h between DX-619 and vancomycin. One of the 36 strains (a VRSA) was not tested with DX-619 and vancomycin (vancomycin MIC >128 µg/ml). Five of 37 (13%) quinolone resistant MRSA (including 2 VRSA, 1 being the Hershey strain) showed synergy between DX-619 + teicoplanin at 12 h and 22 of 37 quinolone resistant MRSA strains (59%) including the 2 previously mentioned VRSA showed synergy between DX-619 + teicoplanin at 24 h. The Hershey VISA did not show synergy.

One of 14 quinolone susceptible MRSAs (7%) showed synergy between DX-619 + vancomycin after 24 h. One of 14 (7%) quinolone susceptible MRSAs showed synergy between DX-619 + teicoplanin at 12 h and 12 of 14 (86%) quinolone susceptible MRSAs showed synergy between DX-619 and teicoplanin at 24 h. Synergy between DX-619 with both vancomycin and teicoplanin is depicted graphically in Fig. 1.

Additive results were obtained with the 5 VISA strains and 1 VRSA strain tested with all combinations at all time points. All other combinations (including all between DX-619 and linezolid) were additive and no antagonism with any combination was observed. The bacteriostatic nature of linezolid is well-known (4,8).

Results of this study confirm the potency of DX-619 against all S. aureus strains tested irrespective of phenotype (2). DX-619 + teicoplanin were synergistic at subMIC concentrations of DX-619 in 69 of the 101 strains (68%) after 24 h: these strains comprised 35 methicillin susceptible of these 3 were quinolone resistant, 12 MRSA and quinolone susceptible, and 22 MRSA and quinolone resistant (including 2 VRSA) strains.

By contrast, only 25 of 100 strains (25%) showed synergy between DX-619 and vancomycin after 24 h. We have no explanation why DX-619 was more synergistic in combination with teicoplanin than with vancomycin. Examination of data in the electronic supplement did not show any obvious correlation between strains showing synergy between DX-619 and one or both glycopeptides. Although clear in vitro synergy between DX-619 and teicoplanin was seen in most strains studied, antimicrobial interactions are
known to be strain-dependent, and generalizations based upon our results should be avoided, at least for the moment.

In summary, combination between DX-619 and teicoplanin and (in some cases) vancomycin showed in vitro synergy by time-kill against *S. aureus* strains of all resistotypes. The significance of these findings requires clinical confirmation.

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REFERENCES


Table 1. Combined results of MIC (µg/ml) and time-kill synergy tests.

<table>
<thead>
<tr>
<th>Strain</th>
<th>No.</th>
<th>DX 619 MIC Range</th>
<th>VANC MIC Range</th>
<th>TEIC MIC Range</th>
<th>DX 619 + VANC</th>
<th>DX 619 + TEIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Antagonistic</td>
<td>% Additive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Synergistic</td>
<td>% Antagonistic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Additive</td>
<td>% Synergistic</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Synergistic</td>
<td>% Antagonistic</td>
</tr>
<tr>
<td>MSSA</td>
<td>50b</td>
<td>0.004-.06</td>
<td>1-4</td>
<td>0.25-16</td>
<td>0%</td>
<td>76%</td>
</tr>
<tr>
<td>MRSA Quin S</td>
<td>14</td>
<td>0.008-0.016</td>
<td>1-2</td>
<td>0.25-2</td>
<td>0%</td>
<td>93%</td>
</tr>
<tr>
<td>MRSA Quin R</td>
<td>37c</td>
<td>0.06-2</td>
<td>0.5-&gt;128</td>
<td>0.25-16</td>
<td>0%</td>
<td>67%</td>
</tr>
<tr>
<td>VISA</td>
<td>6d</td>
<td>0.016-.125</td>
<td>4-8</td>
<td>4-16</td>
<td>0%</td>
<td>83%</td>
</tr>
<tr>
<td>VRSA</td>
<td>3e</td>
<td>0.125-0.5</td>
<td>32-&gt;128</td>
<td>1-16</td>
<td>0%</td>
<td>50%</td>
</tr>
</tbody>
</table>

a Percentage of strains at the 24-hr time point demonstrating the labeled effect.

b Includes 2 VISA strains 505 and 508. Strains 241, 242, 261 and 508 are quinolone resistant.

c Includes 4 VISA strains 504, 506, 507 and 555 and 3 VRSA strains 509, 510, and 512.

d Two isolates are included in MSSA strains 505 and 508 and 4 strains are included in MRSA Quinolone R (levofloxacin MICs ≥4 µg/ml)504, 506, 507, and 555.

e Three isolates are included in MRSA Quinolone resistant strains 509, 510 and 512. Isolate 512 was not tested with DX-619 + vancomycin combination. (Vancomycin MIC >128 µg/ml)
FIG.1. Time kill graphs. (A), DX-619/Vancomycin Quinolone Resistant MRSA strain #019, (B), DX-619/Teicoplanin Quinolone Resistant MRSA strain #019, (C), DX-619/Vancomycin VRSA strain #510, (D), DX-619/Teicoplanin VRSA strain #510.