A Triazine Dye, Cibacron Blue F3GA, Decreases Oxacillin Resistance Levels in Methicillin-Resistant Staphylococcus aureus

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Cibacron blue F3GA (CB) was found to reduce the MIC of oxacillin for methicillin-resistant Staphylococcus aureus (MRSA). This effect was not observed with methicillin-susceptible S. aureus. CB alters the resistance level of MRSA through a factor(s) other than mecA-related products, major autolysins, or femAB products. The exact target(s) of CB in causing the effect is unknown.

Cibacron blue F3GA (CB) is a triazinyl dye widely used as the affinity ligand for dye-ligand chromatography. CB is structurally similar to naturally occurring heterocycles, such as nucleoside phosphate, NAD1, coenzyme A, and folic acid (1–3, 8). It has been demonstrated that CB specifically binds to nucleotide binding sites of kinases and dehydrogenases and that some of the enzyme activities are inhibited by CB (1, 4, 6, 7). The aim of this study was to investigate the effect of CB on the in vitro susceptibility of methicillin-resistant Staphylococcus aureus (MRSA) to oxacillin. CB used in this study was from Sigma Chemical Co., St. Louis, Mo. (C 9534) and was formerly called reactive blue 2 (R 4502); it has an A-ring orthosulfonic acid (9). CB purified by reversed-phase high-pressure liquid chromatography according to the procedure described by Hanggi and Carr (9) behaved similarly to unpurified CB, and consequently CB was used without purification in this study. MICs were determined by a microdilution method (13), and population analysis was carried out as described elsewhere (12). CB alone was not inhibitory to staphylococcal strains when used at up to 2,500 µg/ml in the experiments. The effect of CB on in vitro susceptibility to oxacillin was evaluated with 28 MRSA and 10 methicillin-susceptible S. aureus (MSSA) strains. For all MRSA strains, the MIC of oxacillin was significantly reduced in the presence of CB concentrations of 39 µg/ml or higher (Fig. 1). Highly resistant MRSA strains appeared to be less susceptible to the sensitizing effect of CB, but 78 to 156 µg of CB per ml markedly reduced the MICs of oxacillin for those strains. We therefore employed 100 µg of CB per ml in further

FIG. 1. Effect of CB on the susceptibilities of MRSA isolates to oxacillin. The column height indicates the MIC of oxacillin.
TABLE 1. MICs of various antibiotics for MRSA in the presence and absence of CB

<table>
<thead>
<tr>
<th>MRSA strain</th>
<th>FOM</th>
<th>CS</th>
<th>VCM</th>
<th>BC</th>
<th>CP</th>
<th>PCG</th>
<th>DMPPC</th>
<th>MIPPC</th>
<th>CEZ</th>
<th>CCL</th>
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<tbody>
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<tr>
<td>MR1</td>
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<td>128</td>
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<td>64</td>
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<td>8</td>
<td>8</td>
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<tr>
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<td>&gt;512</td>
<td>128</td>
<td>64</td>
<td>4</td>
<td>4</td>
<td>64</td>
<td>128</td>
<td>4</td>
<td>&gt;512</td>
</tr>
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<td>32</td>
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<td>64</td>
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<td>2</td>
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<td>64</td>
<td>128</td>
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<td>128</td>
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<tr>
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<td>1</td>
<td>16</td>
<td>32</td>
<td>16</td>
<td>16</td>
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</table>

* MICs were tested in the absence (−) and presence (+) of CB. FOM, fosfomycin; CS, cycloserine; VCM, vancomycin; BC, bacitracin; CP, chloramphenicol; PCG, benzylpenicillin; DMPPC, methicillin; MIPPC, oxacillin; CEZ, cefazolin; CCL, cefadola.

studies, unless otherwise noted. On the other hand, MICs of oxacillin for MSSA did not change at all in the presence of CB (data not shown). A population analysis of 28 MRSA strains and 2 MSSA strains was carried out in the presence and absence of CB. In most cases, the population curve of homogeneously or heterogeneously oxacillin-resistant MRSA shifted to the left in the presence of CB. On the other hand, population curves of MSSA did not change at all in the presence of CB and confirmed the results of MIC analysis. CB alone also had no effect on the population curve. This sensitizing effect of CB and confirmed the results of MIC analysis. CB alone also had no effect on the population curve. This sensitizing effect of CB was observed only when β-lactam was used with CB (Table 1). Various triazinyl dyes related to CB were tested for an effect on the sensitivity of MRSA to oxacillin and were found to have a very weak effect on a limited number of strains (data not shown).

We further assessed the effect of CB on the bactericidal activity of oxacillin with S. aureus MR15. When CB was added to exponentially growing MR15, the cells grew in clusters as described previously (20). Measurement of CFU of the culture after brief sonication to disperse clusters revealed that CB did not affect the growth of strain MR15. Oxacillin at a concentration of 16 µg/ml also did not significantly affect the growth of strain MR15. On the other hand, coinubation of CB with oxacillin (16 µg/ml) completely inhibited growth but did not reduce the number of CFU. These results suggest that the sensitization effect of CB on MRSA cells is bacteriostatic.

We studied the effect of CB on the synthesis of penicillin-binding proteins (PBPs) in strain MR6-2 (β-lactamase-free) (13). S. aureus cell membranes were prepared from cells grown in the presence or absence of CB. The binding of β-lactam antibiotics to PBPs was investigated with 14C-labeled benzylpenicillin (10 to 30 Ci/mmol) (Amersham International, Bucks, United Kingdom) (5). The amounts of PBP2’ and PBP2 were not affected by the presence of CB in the culture. Next, the effect of CB on the binding of 14C-labeled benzylpenicillin to PBPs was investigated. The binding study revealed that the kinetics also were not affected by CB.

CB has been shown to inhibit bacteriolytic enzyme activity of S. aureus (16, 19). To determine whether bacteriolytic enzymes are involved in the sensitizing effect of CB, we studied the effect of CB on the resistance levels of Lyt mutants, which virtually lack major autolysins of S. aureus (the αl gene products), and of their parent, MRSA MR6 (13). Population analysis of two Lyt mutants, Lyt-2 and Lyt-5, derived from MR6 indicated that the Lyt phenotype had no effect on the levels of resistance of these strains to oxacillin. Moreover, the mutants were as sensitive as the parent strains to the effect of CB on susceptibility to oxacillin. The mecA-mecR element and penicillinase plasmids were shown to affect the resistance level of MRSA (11, 14, 22). We studied the effect of CB on the resistance level of a prototype MRSA strain, N315, and its isogenic derivatives N315P (penicillinase negative) and N315-IR74 (mecI mecR::tet) (14). The population curve was shifted to the left irrespective of the status of the mecI-mecR element and the penicillinase plasmid. We studied whether CB could affect the structure of peptidoglycan as previously observed in studies demonstrating the lower sensitivity of femAB mutants to lysostaphin compared to that of the wild type (10, 15). We determined the susceptibilities of several MRSA strains grown in the presence or absence of CB to various bacteriolytic enzymes with different bond specificities, including 62-KDa N-acetylmuramyl-l-alanine amidase (18), 51-KDa endo-β-N-acetylmuramyl-l-alanine amidase (17), and lysostaphin, by zymography (12). Regardless of whether the cells were grown in the presence or absence of CB, the minimum bacteriolytic doses of N-acetylmuramyl-l-alanine amidase, N-acetylmuramyl-l-alanine amidase, and lysostaphin for the strains were identical.

In conclusion, our results suggested that CB alters the resistance level of MRSA through a factor(s) other than mecA-related products, major autolysins, or femAB products. Although the exact target(s) of CB in causing this sensitizing effect is not clear, it is likely that the target is involved in a critical metabolic pathway, given that PBP2’ is the only functional PBP. Further studies of the sensitization effect of CB may help to elucidate the molecular mechanism of methicillin resistance in S. aureus.

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REFERENCES


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