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.
the effect of CB on the binding of 14C-labeled benzylpenicillin were not affected by the presence of CB in the culture. Next, Bucks, United Kingdom) (5). The amounts of PBP2
zylpenicillin (10 to 30 Ci/mmol) (Amersham International,
indicated that the Lyt
lysis of two Lyt
ucts), and of their parent, MRSA MR6 (13). Population anal-
virtually lack major autolysins of
S. aureus.
the effect of CB on the resistance levels of Lyt
 CB has been shown to inhibit bacteriolytic enzyme activities
CB and confirmed the results of MIC analysis. CB alone also
oxidation of MSSA did not change at all in the presence of CB
antibiotics to PBPs was investigated with 14C-labeled ben-
to exponentially growing MR15, the cells grew in clusters as
activity of oxacillin with
not shown).
We further assessed the effect of CB on the bactericidal
S. aureus. elucidate the molecular mechanism of methicillin resistance in
femAB
mecl-mecR
femAB
mecA
mecI
muramyl-L-alanine amidase,
sostaphin for the strains were identical.
absence of CB, the minimum bacteriolytic doses of
lysozyme and related dyes with nucleotide-requiring enzymes. Arch. Biochem. Bio-
purification by substrate elution chromatography from procion dye-polysac-
DNA sequencing and Southern blot hybridization of total DNA
be incorporated into the mRNA of the target bacteria.
Studies of the binding of cibacron blue F3GA to liver alcohol dehydrogenase.
1980. Intrinsic resistance to
In conclusion, our results suggested that CB alters the resis-
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purification by substrate elution chromatography from proline dye-polyas-
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studies of the binding of cibacron blue F3GA to liver alcohol dehydrogenase.