Effects of Cerulenin on Antibiotic-Induced Lysis of Streptococcus faecalis (S. faecium)

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Addition of the antibiotic cerulenin to cultures lowered the minimal effective concentration of penicillin G or methicillin required to produce bacterial lysis and killing. This effect was most pronounced at subinhibitory antibiotic concentrations. Cerulenin had no significant effects on lysis or killing induced in the presence of d-cycloserine, fosfomycin, bacitracin, or vancomycin.

Bacterial autolytic enzymes are thought to be active in cell surface growth and division (6, 9), as well as in the lytic response which follows exposure to inhibitors of cell wall biosynthesis (6, 10). In Streptococcus faecalis ATCC 9790 evidence has accumulated indicating that certain lipids or lipid derivatives such as lipoteichoic acids may regulate the expression of autolysins at a cellular level (2-4). Fatty acids are constituents of both lipids and lipoteichoic acid, and the presence of intact fatty acids is essential for the inhibition by these compounds of S. faecalis autolytic activity (2-4). In the present study we have examined the effects of cerulenin, an inhibitor of fatty acid biosynthesis (11), on the lytic and bacteriocidal effects of six antibiotic inhibitors of cell wall biosynthesis. If the lytic response of S. faecalis is triggered directly by a mechanism mediated by lipids or lipoteichoic acid or both, then addition of cerulenin should have a uniform effect on bacterial lytic processes. However, the results of this study indicate that addition of cerulenin can stimulate lysis and killing at low concentrations of penicillin and methicillin, but has no substantial effect on lysis or killing due to bacitracin, cycloserine, fosfomycin, or vancomycin.

The minimal concentration of cerulenin required to inhibit lipid and lipoteichoic acid synthesis, without a significant effect on deoxyribonucleic acid, ribonucleic acid, protein, or peptidoglycan synthesis, was found to be 5 μg/ml (1) when added to cultures at a cell density of about 150 μg (dry weight) per ml. In the present experiments it was desirable to follow the cultures for prolonged periods of time (Fig. 1), and cerulenin was added at a culture density of 40 to 50 μg (dry weight) per ml. Figure 2 shows that incorporation of leucine into protein, and of uracil into ribonucleic acid continues at normal rates for 80 and 60 min after cerulenin addition at a concentration of 2 μg/ml. At a 5-μg/ml cerulenin concentration, these macromolecular biosynthetic processes deviated from the expected exponential rate after 40 and 20 min for protein and ribonucleic acid synthesis, respectively (Fig. 2). After exposure of the low-density cultures to cerulenin (2 μg/ml), the incorporation of [14C]glycerol into lipid and lipoteichoic acid, determined as described previously (1), was inhibited 92.9 and 79.6%, respectively. Thus, for cultures exposed to cerulenin at a low cell density (40 to 50 μg/ml), 2 μg of the antibiotic per ml produced the selective effects observed previously (1) when cultures were exposed to 5 μg of cerulenin per ml at a density of 150 μg (dry weight) per ml.

When cultures exposed to both cerulenin (2 μg/ml) and penicillin were compared to those exposed to penicillin alone, the most striking effects of cerulenin occurred at very low penicillin concentrations (Fig. 1). At a penicillin concentration of 2 μg/ml, below the penicillin minimal inhibitory or minimal bactericidal concentration for this organism (5 μg/ml; ref. 5), cultures lysed in the presence and not in the absence of cerulenin.

At 2 μg/ml, cerulenin induced a lytic response and stimulated the rate of lysis at concentrations of penicillin G or methicillin below and just above their respective minimal inhibitory or minimal bactericidal concentrations (ref. 5; Fig. 3A and B). Cerulenin had no observable effects on the rates of lysis obtained on addition of a broad range of cycloserine, bacitracin, fosfomycin, or vancomycin concentrations (Fig. 3C, D, E, and F).

A culture of cells growing exponentially was
FIG. 1. Effect of a series of concentrations of benzylpenicillin on cultures of S. faecalis growing exponentially in a chemically defined (8) growth medium at 37°C in the absence (A) and presence (B) of cerulenin (2 µg/ml). Cells were grown for at least six exponential mass doublings in a chemically defined medium (8) at 37°C. At 0 time antibiotics were added, and the turbidity of the cultures was determined for up to 5 h. Here, as well as in Fig. 3, rates of lysis were determined from the most rapid exponential portions of similar curves (7).

FIG. 2. Effects of cerulenin concentrations on the incorporation of [3H]leucine into protein (A), and of [14C]uracil into ribonucleic acid (B), given as disintegrations per minute (dpm) into 10% trichloroacetic acid-precipitable material (I). Controls (●●), and cerulenin at 1 µg/ml (○○), 2 µg/ml (■■), and 5 µg/ml (□□).

split into 12 samples which were exposed to concentrations of the various antibiotics selected to give minimal lytic rates in the absence of cerulenin (Fig. 3). Each sample was exposed to the antibiotics for 120 min in the presence and absence of cerulenin (2 µg/ml). The addition of cerulenin alone for 120 min gave no change in viable counts compared to the 0 time value (Table 1, line 1). In the penicillin- and methicillin-treated cultures, cerulenin addition caused a reduction in viable counts to about 0.4 and 0.1% of the 0 time value, respectively. At the concentrations used here, penicillin and methicillin alone had no significant bactericidal effect, although bacterial cell division appeared to be inhibited (Table 1). The addition of cerulenin to fosfomycin- or vancomycin-treated cultures had no detectable effects on the viable counts, which
remained around the 0 time level (Table 1). In the
cycloserine-treated cultures, viable counts
were reduced by essentially the same amount in
the presence or absence of cerulenin. Cerulenin
did appear to have a small effect on viability in
bacitracin-treated cultures (Table 1).

The results of these experiments are consis-
tent with the view that restrictions in the rate of
fatty acid biosynthesis (which in turn decreases
 cellular content of both lipids and lipoteichoic
acid) has stimulatory effects on the rates of lysis
and killing induced by low concentrations of
penicillin and methicillin. It is also clear that in
these experiments cerulenin had no significant
effects on the rates of lysis or on killing induced
by the four non-β-lactam antibiotics examined.
Thus, it would appear that inhibition of the de
novo synthesis of lipids or lipoteichoic acid or
both can enhance lysis and killing due to some,
but not all, inhibitors of wall biosynthesis.

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**TABLE 1. Effects of cerulenin (2 μg/ml) on viable counts**

<table>
<thead>
<tr>
<th>Antibiotic added</th>
<th>Viable cells/ml × 10⁶</th>
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<tbody>
<tr>
<td></td>
<td>Cerulenin + Cerulenin</td>
</tr>
<tr>
<td>No wall antibiotic added</td>
<td>4,000</td>
</tr>
<tr>
<td>Penicillin G (2.5 μg/ml)</td>
<td>550</td>
</tr>
<tr>
<td>Methicillin (600 μg/ml)</td>
<td>260</td>
</tr>
<tr>
<td>Bacitracin (1.5 μg/ml)</td>
<td>190</td>
</tr>
<tr>
<td>Fosfomycin (100 μg/ml)</td>
<td>2,700</td>
</tr>
<tr>
<td>Vancomycin (0.3 μg/ml)</td>
<td>5,800</td>
</tr>
<tr>
<td>Cycloserine (40 μg/ml)</td>
<td>50</td>
</tr>
</tbody>
</table>

* All counts determined after 120 min. Counts are the mean of duplicate plates.

* The 0 time count was 480 × 10⁶ cells per ml.


