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Effect of Clavulanic Acid on the In Vitro Synergism Between Carbenicillin and Gentamicin Against *Serratia marcescens*

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Clavulanic acid, a potent β-lactamase inhibitor, reduced the minimum inhibitory concentrations of carbenicillin for 13 of 15 clinical isolates of *Serratia marcescens* by at least eightfold. The combination of clavulanic acid and carbenicillin also exhibited synergistic activity with gentamicin against 11 of 15 isolates tested, including 9 of 10 strains which were highly resistant to carbenicillin (minimum inhibitory concentration ≥ 8,000 μg/ml) and had previously been found resistant to the synergistic activity between carbenicillin and gentamicin.

The prevalence of plasmids responsible for resistance to aminoglycoside and β-lactam antibiotics and the selective pressure of these widely used antibiotics suggest that infection with *Serratia marcescens* resistant to both groups may become an increasingly frequent problem. This situation and the low toxic-therapeutic ratio of aminoglycoside antibiotics prompted the evaluation in our laboratory of the synergistic activity between carbenicillin and various aminoglycoside antibiotics against this organism (3). Of the 55 clinical isolates evaluated, 21 were highly resistant to carbenicillin, with minimum bactericidal concentrations (MBCs) ≥ 8,000 μg/ml. No synergistic activity between carbenicillin and any of the three aminoglycoside antibiotics tested was demonstrated against these 21 isolates, in contrast to the synergistic effects of carbenicillin with an aminoglycoside against isolates more susceptible to carbenicillin. Clavulanic acid, a potent irreversible β-lactamase inhibitor, has been found to reduce markedly the minimum inhibitory concentration (MIC) of β-lactam antibiotics, including carbenicillin, for a wide spectrum of β-lactamase-producing clinical isolates (7, 8). This study was undertaken to examine the effect of clavulanic acid on synergism between carbenicillin and gentamicin against clinical isolates of *S. marcescens*, especially those highly resistant to carbenicillin.

Stock solutions of gentamicin and of carbenicillin in distilled water and of clavulanic acid in 0.1 M potassium phosphate buffer, pH 7.0, were kept at −20°C. Carbenicillin and clavulanic acid stock solutions were used within 1 week of preparation. All stock solutions were diluted in Trypticase soy broth (Baltimore Biological Laboratory) for use in susceptibility testing.

The effect of clavulanic acid on the synergistic activity of carbenicillin and gentamicin against *S. marcescens* was tested by the checkerboard twofold dilution method (6). For each isolate tested, two checkerboards, one with and one without clavulanic acid, were set up in a single experiment. The final titer of *Serratia* was approximately 10^6 bacteria per ml, and the final concentration of clavulanic acid, when present, was 5 μg/ml. The MIC and MBC were as defined previously (3).

Beta-lactamase production of the isolates was detected with a chromogenic cephalosporin, nitrocefin (2).

Fifteen clinical isolates of *S. marcescens* obtained from the Clinical Microbiology Laboratory of the University of Chicago Hospitals and Clinics were studied (Table 1). They were chosen for resistance to both carbenicillin and gentamicin from among isolates whose susceptibility to carbenicillin and gentamicin alone and in combination had been studied previously (3). The MBCs of carbenicillin and gentamicin for all isolates were equal to or more than 250 and 12.5 μg/ml, respectively. To determine whether these 15 isolates represented a diversified population of *S. marcescens*, they were grouped into strains according to biotype and serotype (5). There were at least 11 different strains by these criteria. Nine strains had one isolate each. The three isolates of strain R came from widely different areas of the hospital. Furthermore, the data on susceptibility to carbenicillin shown in Table 1 suggest that isolate 83040-1 differs from the other two isolates (88695 and 78854) of strain

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TABLE 1. *Susceptibility of S. marcescens isolates to carbenicillin and gentamicin in the presence and absence of clavulanic acid*

Strain	Isolate	Carbenicillin MBC(=MIC)[a]	Carbenicillin MBC(MIC) with 5 μg/ml of clavulanic acid	Gentamicin MBC	Gentamicin MBC with subinhibitory level of carbenicillin[b]	Gentamicin MBC with subinhibitory level of carbenicillin[b] and 5 μg of clavulanic acid/ml	Effect of clavulanic acid (reduction of gentamicin MBC)[c]
CC	88894	≥32,000	8,000 (4,000)	200	400	3.12	128
D	77294	≥32,000	1,000 (1,000)	400	400	50	8
EE	80904	≥32,000	1,000 (1,000)	≥800	≥400	25	≥16
H	82006	≥32,000	500 (500)	≥800	≥800	100	≥8
H	74710-1	≥32,000	1,000 (1,000)	400	800	50	16
H	91157-1	≥32,000	4,000 (1,000)	400	≥200[d]	50[d]	≥4
P	73380	≥32,000	500 (500)	400	≥400	6.25	≥64
Q	US3N	≥32,000	1,000 (1,000)	400	≥800	≤6.25	≥128
A	72332	4,000	125 (125)	12.5	6.25	0.39	16
R	83040-1	8,000	125 (125)	25	12.5	≥25	No effect[e]
Z	83040-2	16,000	500 (500)	50	50	12.5	4
R	88695	250	31.2 (15.6)	12.5	6.25	3.12	2
R	78854	500	31.2 (31.2)	25	≥25	1.56	≥16
GG	82851	250	250 (250)	12.5	3.12	6.25	No effect
V	75259	250	250 (250)	25	1.56	3.12	No effect

[a] MBCs and MICs are in micrograms per milliliter.

[b] The subinhibitory level of carbenicillin equals one-fourth of the MIC of carbenicillin in the presence of clavulanic acid (column 4), unless otherwise indicated.

[c] Ratio of MBC in column 6 divided by MBC in column 7.

[d] Carbenicillin concentration, 125 μg/ml.

[e] ≤1.

R. Two of the isolates of strain H (74710-1 and 91157-1) were from the same ward in the hospital. Thus, 14 of the 15 isolates were either of different strains or from different hospital wards.

Of the 15 isolates studied (Table 1), 8 were highly resistant to both carbenicillin and gentamicin (MBC of carbenicillin, ≥32,000 μg/ml and that of gentamicin, ≥200 μg/ml). The MBC of carbenicillin was 4,000 to 16,000 μg/ml for three other isolates which moreover were moderately resistant to gentamicin (MBC, 12.5 to 50 μg/ml). Four were moderately resistant to both carbenicillin (MBC, 250 to 500 μg/ml) and gentamicin (MBC, 12.5 to 25 μg/ml).

The addition of clavulanic acid to carbenicillin (column 4) reduced the MIC of carbenicillin at least eightfold from the level with carbenicillin alone (column 3) for all but two isolates. One of these (75259) was the only isolate that lacked demonstrable β-lactamase, and therefore it failed to respond to the β-lactamase inhibitor. The β-lactamase of the other isolate (82851) might be inhibited to a lesser degree by clavulanic acid than the enzymes of more highly protected strains (4, 7). Alternatively, this strain might be less permeable to clavulanic acid (7). Although clavulanic acid had a dramatic effect on susceptibility to carbenicillin, the initial MICs of carbenicillin for most isolates were so high that they were lowered to clinically achievable levels (≤125 μg/ml) for only four isolates (72332, 83040-1, 88695, and 78854). As expected, the addition of clavulanic acid alone did not alter by more than one dilution the MIC or MBC of gentamicin for any of the isolates (data not shown).

The addition of a subinhibitory concentration of carbenicillin (one-fourth the MIC of carbenicillin when in the presence of 5 μg of clavulanic acid per ml) (column 6) had no appreciable effect on the MBC of gentamicin for all isolates but 82851 and 75259. Except for these two isolates and 83040-1 and 88695, the further addition of clavulanic acid to the carbenicillin and gentamicin (column 7) lowered the MBC of gentamicin synergistically, 4- to 128-fold (column 8). For five isolates this combination of clavulanic acid and a subinhibitory level of carbenicillin reduced the MBC of gentamicin to a clinically achievable level (≤6.25 μg/ml). For three of these (73380, 72332, and 78854), the subinhibitory concentration of carbenicillin used in synergistic killing with clavulanic acid and gentamicin was also clinically achievable (≤125 μg/ml).

For 10 of the 15 isolates tested in this study, the MBC of carbenicillin was ≥8,000 μg/ml. In a previous study (3), none of these ten isolates was killed synergistically by a combination of carbenicillin and gentamicin with concentrations of each equal to one-fourth of the individual

MBCs. The simple addition of clavulanic acid to an even lower concentration of carbenicillin in this study resulted in a profound decrease in the MBC of gentamicin from 4- to 128-fold in nine of these isolates. In addition, two of the other five isolates, which were killed synergistically by carbenicillin and gentamicin (3), showed at least fourfold reduction of the gentamicin MBC when clavulanic acid was added to a subinhibitory concentration of carbenicillin. Two of the remaining three isolates were unaffected by clavulanic acid in their susceptibility to carbenicillin. It is not surprising that clavulanic acid failed to enhance the synergism in these cases.

Although clavulanic acid has a profound effect on the synergistic potential of gentamicin with β-lactam antibiotics such as carbenicillin, with only three isolates did it lower the synergistic concentrations of both carbenicillin and gentamicin to clinically attainable levels. With the other eight isolates against which clavulanic acid facilitated synergism, the initial levels of resistance were so high that, even with the profound enhancement of killing, the concentrations of both carbenicillin and gentamicin that acted synergistically were not clinically achievable. Increase of the concentration of clavulanic acid above 5 μg/ml, use of more active aminoglycosides or β-lactam antibiotics (1), and/or development of a β-lactamase inhibitor which also has significant antibacterial activity might permit synergistic killing with clinically achievable concentrations of the antibiotics.

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