THE INACTIVATION OF GENTAMICIN AND NETILMICIN BY CARBENICILLIN: ITS EFFECT ON *SERRATIA MARCESCENS*

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(Received for publication April 25, 1978)

Ten clinical isolates of *Serratia marcescens* were tested on MUELLER-HINTON agar containing gentamicin or netilmicin with carbenicillin. The isolates grew on plates where inactivation occurred, at higher antibiotic concentrations, but failed at lower concentrations. This growth response was individualistic and not closely related to the minimum inhibitory concentrations.

Carbenicillin has been shown to inactivate gentamicin.[1] Since inactivation can occur *in vivo*,[2,3] it could also lead to decreased activity against certain pathogens *via* reduced aminoglycoside concentration. The question therefore occurs, when inactivation takes place, how does it affect the pathogen?

Materials and Methods

Antimicrobials:

Gentamicin (GM) and netilmicin (N) powders were obtained from Schering Corporation while carbenicillin (CB) powder was supplied by Beecham Laboratories.

Organisms:

Ten clinical isolates of *Serratia marcescens* were tested. They were previously tested[4] and their corresponding numbers are: 3A1=isolate #1, 3B1=2, 3C1=3, 3D1=4, 3E1=5, 3F1=6, 3G1=7, 3H1=8 and 3A2=9 and 3B2=10.

Susceptibility test methods: Minimal inhibitory concentrations (MICs) with individual drugs were done by a broth microdilution method[5] using MUELLER-HINTON broth (Difco).

Inactivation studies:

(a) Inactivation versus organisms. Agar dilution was the method used to test individual and combined antibiotics. Inactivation was characterized by growth of isolates on plates with antibiotic concentrations exceeding those of corresponding individual antibiotic MICs.

(b) Inactivation versus antagonism. Inactivation was distinguished from antagonism by assaying the MUELLER-HINTON agar for aminoglycoside concentration in the absence and presence of carbenicillin. The assay was done following removal of MUELLER-HINTON agar cylinder plugs with the large-diameter end of a Pasteur pipette. Agar cylinders were then placed on Antibiotic Medium #5 (Difco) seeded with *Staphylococcus epidermidis* ATCC 27626 and incubated 18 hours at 35°C. This indicator strain is susceptible to the aminoglycosides but resistant (MIC=512 μg/ml) to carbenicillin. Zones of inhibition were measured, a standard curve plotted on semilogarithmic paper and the unknown concentrations calculated from the curve. All unknowns and standards were run in triplicate on a 150 mm Petri plate. A reduction in aminoglycoside concentration in the presence of carbenicillin was indicative of inactivation.

(c) Inactivation versus age of medium. An experiment was devised to determine how long

* This investigation was supported by a grant from Schering Corporation. Please address requests for reprints to D. J. FLOURNOY, Veterans Administration Hospital (113), 921 NE 13th St., Oklahoma City, Oklahoma 73104, U.S.A.

MUELLER-HINTON agar plates containing GM-CB or N-CB could be stored without affecting inactivation. Plates contained carbenicillin at two-fold dilutions (8,000~62.5 μg/ml) and either gentamicin (160 μg/ml) or netilmicin (1.25 μg/ml). *Serratia marcescens* #8 (3H1) was tested on the plates immediately after preparation, 24 and 48 hours later. The plates were stored at 4°C. A STEER's replicator was used to inoculate the plates. All testing was done in duplicate.

Results

Broth microdilution MICs of the isolates are noted in Table 1. All isolates were resistant to gentamicin and carbenicillin. Isolate #10 was somewhat resistant to netilmicin while the others were susceptible.

Inactivation of gentamicin (320 μg/ml) by carbenicillin (8,000 μg/ml) resulted in the growth of all 10 isolates as seen in Table 2. Table 3 shows growth responses to netilmicin inactivation by carbenicillin. No correlations could be established among individual antibiotic MICs, growth response to gentamicin or netilmicin inactivation and concentration or ratio of aminoglycoside to carbenicillin. In essence, this phenomenon is apparently individualistic for each isolate in this study.

MUELLER-HINTON agar plates with gentamicin or netilmicin, at 10 μg/ml, and carbenicillin, at 500 μg/ml, were assayed for aminoglycoside concentration before and after incubation at 35°C for 18 hours. The gentamicin and netilmicin concentrations were reduced 43% and 28% respectively by carbenicillin. No aminoglycoside concentration reduction was seen in the absence of carbenicillin.

Serratia marcescens #8 was used to determine if the degree of inactivation changed when MUELLER-HINTON agar plates, containing gentamicin or netilmicin and carbenicillin, where stored at 4°C for up to 48 hours. There was no detectable change in the degree of inactivation when plates from the same batch of medium were tested immediately, 24 or 48 hours follow-

Table 1. MICs of gentamicin (GM), netilmicin (N) and carbenicillin (CB)

Isolate	MIC (μg/ml)		
	GM	N	CB
1	160	1.25	62,500
2	160	0.62	125,000
3	40	0.62	31,250
4	80	2.50	62,500
5	80	2.50	62,500
6	40	0.62	31,250
7	40	0.62	62,500
8	80	0.62	62,500
9	80	0.62	31,250
10	80	10.00	62,500

Table 2. Response of isolates to gentamicin inactivation by carbenicillin

μg/ml		Ratio (GM:CB)	Growth of isolates
Gentamicin	Carbenicillin		
320	8,000	1 : 25	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
320	4,000	1 : 12	1, 2, 4, 8
320	2,000	1 : 6	1
160	8,000	1 : 50	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
160	4,000	1 : 25	1, 2, 3, 4, 5, 6, 7, 8, 9
160	2,000	1 : 12	1, 2, 4, 8
160	1,000	1 : 6	1, 2
160	500	1 : 3	1
80	8,000	1 : 100	3, 4, 5, 6, 7, 8, 9, 10
80	4,000	1 : 50	3, 4, 5, 6, 7, 8, 9, 10
80	2,000	1 : 25	3, 4, 5, 6, 7, 8, 9
80	1,000	1 : 12	4, 5, 6, 8
40	8,000	1 : 200	3, 4, 5, 6, 7, 8, 9, 10
40	4,000	1 : 100	3, 5, 6, 7, 9, 10
40	2,000	1 : 50	3, 5, 6, 7, 9, 10
40	1,000	1 : 25	3, 5, 6, 7, 9
40	500	1 : 12	5, 6, 9
40	250	1 : 6	5, 6
20	8,000	1 : 400	3, 10
20	4,000	1 : 200	3, 10
20	2,000	1 : 100	3, 10
20	1,000	1 : 50	3
20	500	1 : 25	3

ing preparation.

Discussion

Results of this study indicate an individualistic growth response of *Serratia marcescens* isolates to inactivation. No correlations could be made among individual antibiotic MICs and growth response to inactivation. MICs by broth microdilution agreed to within a two-fold dilution of previously reported agar dilution MICs on the same isolates.[4] Of special interest is the fact that some of these isolates were inhibited by certain low (synergistic) combined antibiotic concentrations[4] yet grew at higher concentrations where inactivation occurred (this study). Theoretically this could occur *in vivo* where combined antibiotic concentrations and ratios are in a continuous state of flux.

Therapeutic serum ranges for gentamicin (4~8 μg/ml) and carbenicillin (100~400 μg/ml) are considerably lower than many of the concentrations where inactivation occurred in this study. Is the growth of these isolates during inactivation relevant to a clinical setting? Only 2/10 of the isolates (#5, 6) grew at concentrations in the therapeutic range for GM/CB; while 2/10 of the isolates (#9, 10) grew at therapeutic levels of N/CB. The wide variability of growth responses to inactivation in a very small number of clinical isolates seems to be the significant finding in relation to the above question.

WAITZ *et al.* also noted an unusual response by *Pseudomonas* strains to certain combinations of gentamicin and carbenicillin. They attributed this to inactivation.

Inactivation could be confused with antagonism. NOONE and PATTISON discussed proper terminology of the words.[7] Inactivation is indeed a separate physico-chemical interaction which occurs irregardless of bacterial growth, but since the end result can affect and be observed by the growth of bacteria it could be more closely related to antagonism than was previously believed. The exact mechanisms of synergy and antagonism are not fully understood as evidenced by the unpredictability of effects by certain combined antibiotics. As far as this study was concerned, however, inactivation was a reduction of aminoglycoside concentration in the presence, and not the absence, of carbenicillin. WAITZ noted a reduction in gentamicin concentration in the presence of carbenicillin in MUELLER-HINTON broth.[6]

Table 3. Response of isolates to netilmicin inactivation by carbenicillin

μg/ml		Ratio (N : CB)	Growth of isolates
Netilmicin	Carbenicillin		
10	31,250	1 : 3,125	1
10	15,625	1 : 1,563	1
5	31,250	1 : 6,250	1, 2
5	15,625	1 : 3,125	1, 2, 4, 7, 8
5	7,812	1 : 1,563	1
2.5	31,250	1 : 12,500	1, 2, 7, 8
2.5	15,625	1 : 6,250	1, 2, 7, 8
2.5	7,812	1 : 3,125	1
1.25	31,250	1 : 25,000	1, 2, 7
1.25	15,625	1 : 12,500	1, 2, 4, 6, 7, 8
1.25	7,812	1 : 6,250	1, 2, 4, 5, 6, 7, 8
1.25	3,906	1 : 3,125	1, 2, 4, 5
1.25	1,953	1 : 1,563	5
1.25	976	1 : 781	5
0.62	31,250	1 : 50,403	1, 2, 7, 8
0.62	15,625	1 : 25,201	1, 2, 3, 4, 6, 7, 8
0.62	7,812	1 : 12,600	1, 2, 3, 4, 6, 7, 8, 9
0.62	3,906	1 : 6,300	1, 2, 3, 4, 6, 7, 8
0.62	1,953	1 : 3,150	1, 2, 4, 8, 10
0.62	976	1 : 1,574	1, 2, 4, 8
0.62	488	1 : 787	4
0.31	15,625	1 : 50,403	3, 6
0.31	7,812	1 : 25,200	3, 6, 9
0.31	3,906	1 : 12,600	3, 6, 9, 10
0.31	1,953	1 : 6,300	3, 6, 9, 10
0.31	976	1 : 3,148	3, 6, 9, 10
0.31	488	1 : 1,574	9, 10
0.31	244	1 : 787	9, 10
0.31	122	1 : 394	9, 10
0.31	61	1 : 197	9, 10
0.31	30	1 : 97	9, 10
0.31	15	1 : 48	10

Attention has been called to the use of plates, containing gentamicin and carbenicillin, on the day of preparation to prevent interference of results *via* inactivation. Studies here indicate that plates can be held up to 48 hours at 4°C without a change in degree of inactivation. This applies to GM/CB and N/CB but not to tobramycin/carbenicillin since tobramycin can be inactivated to a greater degree.[8]

Although inactivation may be regarded lightly by some, it undoubtedly could influence patient

care by allowing certain organisms to grow *in vivo*.

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