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Comparative Antimicrobial Activity of O-Demethylfortimicin A, a Derivative of Fortimicin A

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The in vitro antimicrobial activity of O-demethylfortimicin A (ODMF), a derivative of fortimicin A, was compared with those of fortimicin A and gentamicin against a spectrum of 256 organisms. All three antibiotics were active in low concentrations against all strains of *Enterobacteriaceae*, *Acinetobacter* sp., and *Staphylococcus aureus*, with ODMF most active against *Proteus mirabilis*, indole-positive *Proteus*, and *Providencia* and gentamicin most active against other species. Activity of each of the antibiotics against group D streptococci was poor. The overall activity of ODMF was superior to that of fortimicin A for all groups of organisms examined and was most pronounced, approximately threefold, against strains of *Pseudomonas aeruginosa*. Both ODMF and fortimicin A were resistant to the action of several aminoglycoside-inactivating enzymes, with the exception of 3-N-acetyltransferase-I. ODMF and fortimicin A showed similar rapid bactericidal effects at multiples of the minimum inhibitory concentration and equivalent synergistic activity against enterococci when combined with penicillin G. The combination of carbenicillin with ODMF, fortimicin A, or gentamicin was synergistic for approximately 80% of the *P. aeruginosa* strains tested. Inactivation of ODMF and fortimicin A when combined with carbenicillin in vitro was minimal or absent, whereas gentamicin was substantially inactivated under similar conditions. ODMF, fortimicin A, and gentamicin exhibited protective activity in mice infected with *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *S. aureus*, or *P. aeruginosa*. Gentamicin was the most active, followed by ODMF and fortimicin A. The superior in vitro activity of ODMF compared with fortimicin A against *P. aeruginosa* was confirmed in vivo.

The fortimicins are a new class of pseudodisaccharide aminocyclitol antibiotics. Fortimicin A, the most active naturally occuring member of this class, has antimicrobial activity similar to that of amikacin, but lacks significant activity against *Pseudomonas aeruginosa* (3, 7, 12).

In an attempt to improve the activity and/or broaden the antimicrobial spectrum of this antibiotic, a number of modified fortimicins have been prepared (9). This study reports on the in vitro and in vivo antimicrobial properties of 3-O-demethylfortimicin A (ODMF), a derivative of fortimicin A with improved antibacterial activity (8).

MATERIALS AND METHODS

Organisms. The majority of organisms studied were randomly selected recent isolates from clinical material and were obtained from several hospital and public health laboratories.

Antibiotics. Antibiotics were supplied by the following manufacturers: ODMF sulfate and fortimicin A sulfate (Abbott Laboratories, North Chicago, Ill.); gentamicin sulfate (Schering Corp., Bloomfield, N.J.); amikacin sulfate (Bristol Laboratories, Syracuse, N.Y.); and carbenicillin disodium (Roerig, New York N.Y.).

Susceptibility testing. Antimicrobial activity was measured by agar dilution using a single lot of Mueller-Hinton agar. Minimum inhibitory concentrations (MICs) were determined by applying an inoculum of approximately 5×10^4 colony-forming units (CFU) to the agar surface with a Steers replicating device. Incubation was at 35°C for 18 h. The MIC was defined as the lowest concentration of antibiotic which inhibited development of visible growth. A slight haze or up to three colonies was ignored.

Synergy testing. Synergy of ODMF, fortimicin A, or gentamicin in combination with carbenicillin against *Pseudomonas aeruginosa* was determined by the checkerboard technique with microdilution procedures and Mueller-Hinton broth (MHB) supplemented with 50 μg of calcium and 20 μg of magnesium per ml. Carbenicillin was serially diluted in one direction, and the aminoglycoside was serially diluted in the perpendicular direction in the microdilution tray. Each tray contained a row of carbenicillin and a row of the aminoglycoside alone, from which the MIC was determined, plus a single well without antibiotic as a growth control. The inoculum was added by calibrated dropper to give a final count of approximately 5×10^5 CFU/ml. Plates were covered and incubated at 35°C for 18 h. The fractional inhibitory concentration was calculated for each antibiotic by dividing the MIC of the antibiotic in combination by the MIC of the antibiotic alone. The fractional inhibitory concentration

index is the sum of the fractional inhibitory concentrations of the individual antibiotics at the most effective combination, i.e., that for which the sum is minimal. Synergism is indicated by a fractional inhibitory concentration index of <0.6.

Killing curves. The kinetics of bactericidal activity for ODMF and fortimicin A were studied by the killing curve technique. Both antibiotics were tested singly at MIC multiples against strains of *Escherichia coli* and *P. aeruginosa* and in combination with penicillin G against strains of *Streptococcus faecalis*. A 0.1-ml portion of a 10^{-2} dilution of an 18-h MHB culture was added to 9.9 ml of MHB (supplemented with 50 μg of calcium and 25 μg of magnesium per ml for *P. aeruginosa*) containing the desired concentration of antibiotic(s) to yield an inoculum level of 5×10^4 to 1×10^5 CFU/ml. Final antibiotic concentrations in the combination studies were 8 μg/ml for ODMF and fortimicin A and 20 μg/ml for penicillin G. A control tube contained no antibiotic(s). Incubation was at 35°C in a water bath. Samples were removed at various times, and viable CFU per milliliter were determined by standard plate count techniques using Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). Synergy, in this method, was defined as a greater than 1 log decrease in CFU after 4 h with the combination of antibiotics as compared with the count obtained with the most active single agent (6).

Stability of aminoglycosides in combination with carbenicillin. Mixtures of ODMF (30 μg/ml), fortimicin A (30 μg/ml), or gentamicin (10 μg/ml) plus carbenicillin (50 or 500 μg/ml) were prepared in distilled water or MHB and incubated at 35°C for 2 or 18 h. Appropriate controls were included consisting of each antibiotic alone in each diluent for the desired time. The concentration of each aminoglycoside was determined at the end of each time period by an agar diffusion assay utilizing *Bacillus subtilis* 10707 and streptomycin assay agar with yeast extract (BBL). Carbenicillin was inactivated by addition of penicillinase (10^6 U/liter of assay medium) to eliminate interference with the assay.

In vivo efficacy studies. Female Swiss albino mice, weighing 18 to 20 g, were infected intraperitoneally with approximately 100 times the number of organisms needed to kill 50% of the untreated animals. The bacterial suspensions used to infect mice consisted of appropriate dilutions in brain heart infusion broth containing 5% aqueous hog gastric mucin (American Laboratories, Inc.). Serial twofold dilutions of the test substances were administered by the subcutaneous route to groups of 10 mice at 1 and 6 h postinfection. The animals were observed for 7 days, and mortality was recorded. The total dose of antibiotic which protected 50% of the infected animals and the 95% confidence limits were calculated.

RESULTS

The comparative antimicrobial activities of ODMF, fortimicin A, and gentamicin for a spectrum of 256 organisms are presented in Table 1. On a weight basis, ODMF was the most active compound against *Proteus mirabilis*, indole-positive *Proteus*, and *Providencia* sp. ODMF and gentamicin were equally active against *Shigella* sp. Against other species gentamicin was most active. All three antibiotics were effective at low concentrations against all isolates except for certain strains of group D streptococci and *P. aeruginosa*. Three strains of *Providencia* sp. were resistant to gentamicin, which resulted in an unusually high geometric mean MIC for this antibiotic. Geometric mean MICs of ODMF were 3.1 μg/ml or less for all species of *Enterobacteriaceae*, *Acinetobacter* sp., and *Staphylococcus aureus*. Fortimicin A geometric mean MICs were slightly higher for these organisms. Against *P. aeruginosa*, ODMF demonstrated a geometric mean MIC of 9.2 μg/ml, a nearly threefold gain in activity compared with fortimicin A.

Table 2 presents the activity of ODMF, fortimicin A, gentamicin, and amikacin against 13 organisms known to possess aminoglycoside-inactivating enzymes or to be permeability mutants. ODMF and fortimicin A were active against organisms possessing 2″-aminoglycoside adenyltransferase [AAD(2″)], 6′-*N*-acetyltransferase [AAC(6′)], or 3-*N*-acetyltransferase-III [AAC(3)-III]. As previously reported (12, 15), only 3-*N*-acetyltransferase-I [ACC(3)-I] inactivated fortimicin A. ODMF was similarly affected. The two permeability mutants were resistant to all four antibiotics.

The comparative bactericidal activities of one-, two-, and fourfold the MIC of ODMF and fortimicin A for strains of *E. coli* and *P. aeruginosa* are shown in Fig. 1. For each organism, an equivalent multiple of the MIC of either antibiotic resulted in an approximately equal effect. At an MIC multiple of fourfold, the bactericidal activity was rapid, and no viable organisms were detected after 4 h. At double the MIC of each antibiotic, the population of both organisms was reduced from 10^5 CFU/ml at 0 time to less than 10^1 CFU/ml (≥99.9%) after 4 h. Results with these two organisms were representative of similar results for two additional strains of *E. coli* and one of *P. aeruginosa*.

Table 3 presents the comparative synergistic activity of ODMF, fortimicin A, or gentamicin in combination with carbenicillin against 22 strains of *P. aeruginosa*. As seen from the fractional inhibitory concentration indexes, the three combinations are approximately equal in overall synergistic activity. The ODMF-carbenicillin and fortimicin A-carbenicillin combinations were each synergistic against 17 of 22 strains, and the gentamicin-carbenicillin combination was synergistic for 19 of 22 strains.

Figure 2 shows the synergistic effect of the combination of ODMF or fortimicin A with penicillin G against two strains of enterococci. After 4 h, both combinations produced an additional

TABLE 1. *Antimicrobial activity of ODMF, fortimicin A (Fort), and gentamicin (Gent)*

Isolate (no.)	Test agent	MIC (μg/ml)		
		Range	90%	Geometric mean
E. coli (30)	ODMF	0.8–6.2	3.1	1.8
	Fort	1.6–6.2	3.1	2.4
	Gent	0.8–3.1	3.1	1.5
Klebsiella pneumoniae (20)	ODMF	0.8–6.2	3.1	2.5
	Fort	0.4–12.5	6.2	3.8
	Gent	≤0.2–0.8	0.8	0.7
Enterobacter sp. (20)	ODMF	1.6–3.1	3.1	2.4
	Fort	0.8–6.2	6.2	3.2
	Gent	≤0.2–1.6	1.6	0.7
Serratia marcescens (10)	ODMF	1.6–6.2	3.1	2.9
	Fort	1.6–6.2	6.2	3.8
	Gent	0.4–6.2	3.1	1.3
Shigella sp. (10)	ODMF	1.6–3.1	1.6	2.0
	Fort	3.1–6.2	3.1	3.3
	Gent	0.8–3.1	3.1	2.0
Salmonella sp. (10)	ODMF	1.6–3.1	3.1	2.9
	Fort	3.1	3.1	3.1
	Gent	0.8–1.6	1.6	1.3
P. mirabilis (8)	ODMF	1.6–6.2	6.2	2.1
	Fort	1.6–6.2	6.2	4.0
	Gent	3.1–6.2	6.2	4.0
Proteus, indole positive (20)	ODMF	0.4–3.1	3.1	1.4
	Fort	0.8–6.2	3.1	2.3
	Gent	0.8–6.2	6.2	2.5
Providencia sp. (4)	ODMF	1.6–6.2	6.2	3.1
	Fort	6.2–12.5	12.5	7.4
	Gent	6.2–>100	>100	29.7
P. aeruginosa (88)	ODMF	0.8–100	25	9.2
	Fort	0.8–>100	50	25.6
	Gent	≤0.2–25	6.2	3.0
S. aureus (19)	ODMF	0.8	0.8	0.8
	Fort	0.8–1.6	1.6	1.1
	Gent	≤0.2–0.4	0.4	0.4
Streptococcus sp. (group D) (9)	ODMF	6.2–25	25	15.8
	Fort	12.5–50	50	25.0
	Gent	3.1–12.5	12.5	8.5
Acinetobacter sp. (8)	ODMF	1.6–6.2	6.2	2.4
	Fort	3.1–12.5	12.5	5.2
	Gent	0.8–3.1	3.1	1.6

2 to 3 log reduction in viable count from that obtained with the most active single drug.

The stability of ODMF, fortimicin A, or gentamicin in the presence of carbenicillin was examined in both water and MHB. ODMF was slightly inactivated only after long incubation in the presence of a high concentration of carbenicillin in water (Table 4). Fortimicin A was completely stable under these conditions. Gentamicin, on the other hand, was inactivated to a substantial degree by carbenicillin after 18 h in both water and broth and within only 2 h in water.

The in vivo efficacy of ODMF compared with

TABLE 2. *Antimicrobial activity of ODMF, fortimicin A (Fort), gentamicin (Gent), and amikacin (Amik) against organisms with known resistance mechanisms*

Organism	Strain	Resistance mechanism	MIC (μg/ml)			
			ODMF	Fort	Gent	Amik
E. coli	76-2	AAD (2″)	1.6	1.6	50	3.1
K. pneumoniae	Ky 4262	AAD (2″)	3.1	6.2	25	1.6
E. coli	R19	AAC (3)-I	>100	>100	12.5	0.8
P. aeruginosa	Ky 8511	AAC (3)-I	>100	>100	>100	6.2
P. aeruginosa	Ao 8606	AAC (3)-I	>100	>100	>100	12.5
P. aeruginosa	PST-1	AAC (3)-III	6.2	12.5	3.1	3.1
P. aeruginosa	A08034	AAC (3)-III	12.5	25	25	3.1
P. aeruginosa	Ky 8516	AAC (6′)	12.5	50	25	>100
P. aeruginosa	Ky 8510	AAC (6′)	12.5	50	12.5	100
P. aeruginosa	3796	AAC (6′)	100	>100	>100	25
S. marcescens	Ky 4249	AAC (6′)	3.1	3.1	3.1	25
E. coli	9624	Permeability	25	25	12.5	50
E. coli	20948	Permeability	25	50	12.5	50

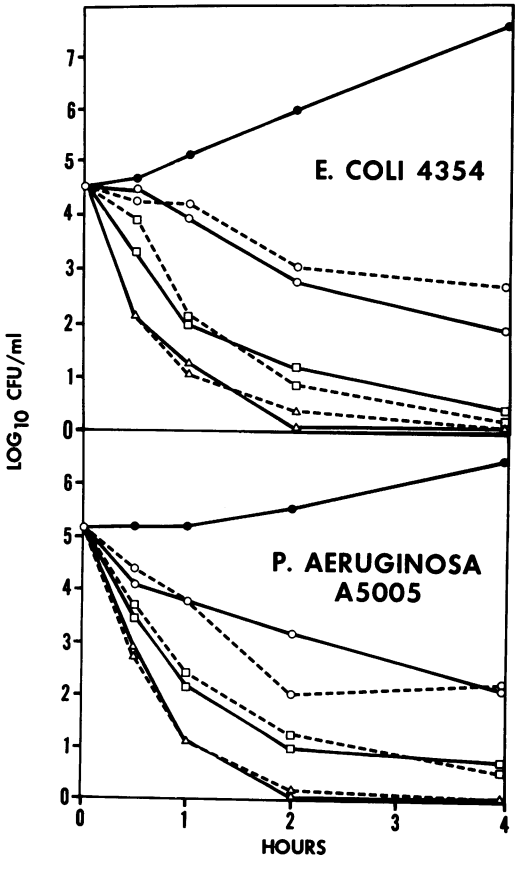

FIG. 1. *Comparative bactericidal activity of ODMF (- - -) and fortimicin A (——) at the MIC (○), twofold the MIC (□), and fourfold the MIC (△). Control (●).*

fortimicin A and gentamicin against several *Enterobacteriaceae*, *S. aureus*, and *P. aeruginosa* is presented in Table 5. All three compounds exhibited protective activity against all infections, with gentamicin most active, followed by ODMF and then by fortimicin A. These results were in general agreement with the MICs obtained with these organisms.

DISCUSSION

The removal of the methyl group from the 3-*O* position of fortimicin A to give ODMF results in improvement in geometric mean MICs for all of the genera examined, particularly against *P. aeruginosa*. Improved antibacterial activity is also obtained after removal of the 3-*O*-methyl group from other fortimicin derivatives (9).

Additional in vitro comparisons of ODMF and fortimicin A showed similar antibacterial activity. Both compounds demonstrated rapid lethal action against strains of *E. coli* and *P. aeruginosa*. The combination of either ODMF or fortimicin A with penicillin G resulted in excellent synergistic activity against enterococci, similar to that reported for the combination of gentamicin and penicillin G (11). The combination of an aminoglycoside and carbenicillin has been reported to be synergistic against strains of *P. aeruginosa* both in vitro (1, 4, 5) and in vivo (2). In this study, the in vitro combination of carbenicillin with ODMF, fortimicin A, or gentamicin was equally synergistic for the strain of *P. aeruginosa*.

It is generally recognized that gentamicin is inactivated in the presence of carbenicillin in vitro (10, 13, 14, 16). This inactivation has been ascribed to the formation of a conjugate linked

TABLE 3. *Synergistic activity of ODMF, fortimicin A (Fort), or gentamicin (Gent) in combination with carbenicillin (Carb) against P. aeruginosa*

P. aeruginosa strain	MIC (μg/ml)				Fractional inhibitory concentration index		
	ODMF	Fort	Gent	Carb	ODMF-Carb	Fort-Carb	Gent-Carb
A-5000	12.5	25	3.1	50	0.50	0.31	0.37
A-5002	6.2	25	1.6	50–100[a]	0.50	0.50	0.18
A-5005	12.5	25	1.6	50	0.37	0.37	0.37
A-5007	6.2	25	3.1	25–50	0.24	0.50	0.37
A-5010	25	25	6.2	100	0.37	0.50	0.24
A-5012	12.5	25	3.1	50–100	0.75	0.37	0.37
A-5016	6.2	12.5	3.1	50	0.50	0.62	0.37
A-5022	12.5	25	3.1	50	0.50	0.50	0.37
A-5025	6.2	12.5	3.1	50	0.50	0.37	0.31
A-5029	6.2	12.5	3.1	50	0.28	0.37	0.37
A-5180	12.5	50	3.1	50	0.50	0.37	0.53
A-5188	12.5	50	3.1	50–100	0.24	0.37	0.24
A-5191	6.2	25	1.6	25–50	0.37	0.31	0.62
A-5196	6.2	25	3.1	50	0.50	0.31	0.37
U566-1	3.1	12.5	1.6	25	0.62	0.62	0.50
24302	25	100	6.2	200	0.50	0.37	0.50
C23	12.5	50	6.2	100	0.50	0.50	0.37
EMC 22	25	100	6.2	100	0.31	0.28	0.50
W19	12.5	12.5	200	25–50	0.62	0.62	0.62
D7	12.5	25	200	50	0.50	0.37	0.37
W2	6.2	12.5	200	25	1	0.75	0.75
BL15352	12.5	12.5	100	50	0.75	0.62	0.51

[a] Carbenicillin MIC varied in tests of different combinations.

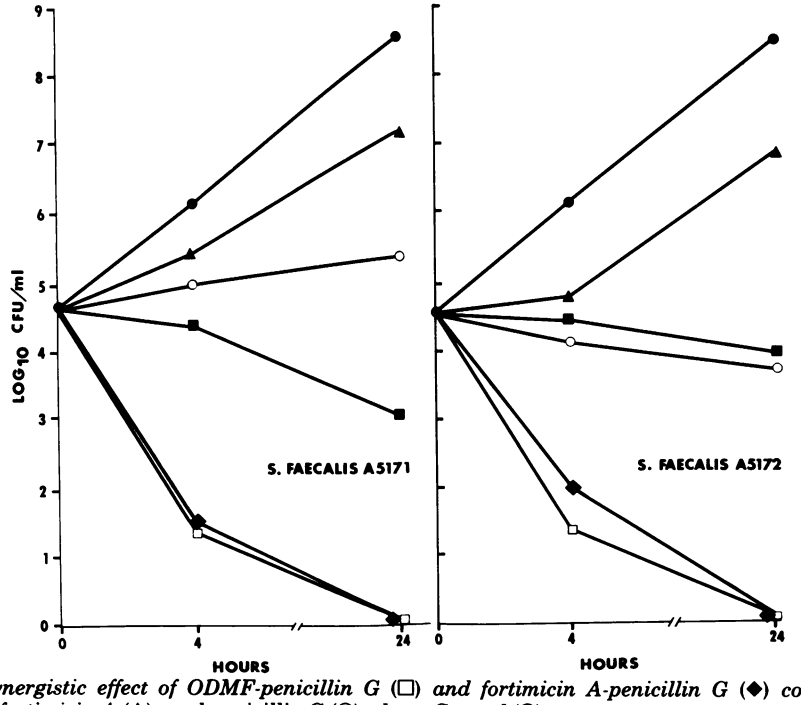

FIG. 2. *Synergistic effect of ODMF-penicillin G (□) and fortimicin A-penicillin G (◆) compared with ODMF (■), fortimicin A (▲), and penicillin G (○) alone. Control (●).*

between the amino groups in the sugars of gentamicin and the β-lactam ring of the penicillin (14). The lack of substantial inactivation of ODMF or fortimicin A under conditions in which gentamicin is almost completely inactivated demonstrates the fundamental structural differences between the fortimicins and the gentamicin complex.

TABLE 4. *Inactivation of ODMF (30 μg/ml), fortimicin A (Fort, 30 μg/ml), and gentamicin (Gent, 10 μg/ml) when in combination with carbenicillin (50 or 500 μg/ml) in water or MHB*

Carbenicillin concn (μg/ml)	% Inactivated											
	ODMF				Fort				Gent			
	Water		MHB		Water		MHB		Water		MHB	
	2 h	18 h	2 h	18 h	2 h	18 h	2 h	18 h	2 h	18 h	2 h	18 h
50	0	5.8	0	0	0	0	0	0	22.8	67.5	0	18.7
500	0	19.2	0	0	0	0	0	0	63.1	>92	21.2	78.2

TABLE 5. *In vivo efficacy of ODMF, fortimicin A (Fort), and gentamicin (Gent)*

Organism	Strain	ODMF		Fort		Gent	
		MIC (μg/ml)	CD_{50}[a] (mg/kg)	MIC (μg/ml)	CD_{50} (mg/kg)	MIC (μg/ml)	CD_{50} (mg/kg)
E. coli	Juhl	3.1	3.5 (2.8–4.4)	3.1	4.6 (3.8–5.6)	0.8	1.5 (0.7–3.1)
K. pneumoniae	4508	0.8	2.6	0.4	6.0	≤0.2	1.9 (1.3–3.3)
Proteus vulgaris	JJ	1.6	4.3 (3.7–4.9)	3.1	20.0 (14–34)	0.8	1.5 (1.3–1.8)
S. aureus	Smith	0.8	0.42 (0.3–0.5)	0.8	0.8	0.8	0.2 (0.1–0.3)
P. aeruginosa	VA 1316	3.1	45 (37–56)	12.5	167 (140–216)	1.6	24 (21–32)
P. aeruginosa	A-5000	6.2	48 (38–68)	25	154 (124–211)	3.1	18 (15–22)

[a] CD_{50}, Total dose of antibiotic which protected 50% of the infected animals. The 95% confidence limits, when calculable, are within parentheses.

The improved in vitro antimicrobial activity of ODMF compared with fortimicin A was also seen in vivo. Both antibiotics protected mice against all of the infections examined, but the amount of antibiotic required was much greater for infections with *P. aeruginosa* than for the *Enterobacteriaceae*. With fortimicin A the differential averaged 23-fold, whereas the average with ODMF was only 13-fold, approximately the same as that seen with gentamicin.

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