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In Vitro Activity of Fusidic Acid (CEM-102, Sodium Fusidate) against *Staphylococcus aureus* Isolates from Cystic Fibrosis Patients and Its Effect on the Activities of Tobramycin and Amikacin against *Pseudomonas aeruginosa* and *Burkholderia cepacia*[▽]

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We tested the MICs of fusidic acid (CEM-102) plus other agents against 40 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from cystic fibrosis patients and the activities of fusidic acid with or without tobramycin or amikacin against *Pseudomonas aeruginosa*, MRSA, and *Burkholderia cepacia* isolates from cystic fibrosis patients in a 24-h time-kill study. Fusidic acid was potent (MICs, 0.125 to 0.5 μg/ml; a single 500-mg dose of fusidic acid at 8 h averaged 8 to 12. 5 μg/ml with 91 to 97% protein binding) against all MRSA strains. No antagonism was observed; synergy occurred for one MRSA strain treated with fusidic acid plus tobramycin.

Cystic fibrosis (CF) patients suffer from recurrent bouts of pneumonia caused by *Pseudomonas aeruginosa* (often mucoid), *Burkholderia cepacia*, and methicillin-resistant *Staphylococcus aureus* (MRSA). The recurrent nature of these infections leads to multidrug-resistant organisms and sometimes to panresistance to all available drugs, with combination antimicrobial therapy the only therapeutic option (6, 14). There is a dearth of new experimental agents active against resistant Gram-negative and -positive strains in general and CF strains in particular (7). Fusidic acid (CEM-102, sodium fusidate [17]) is an oral antibiotic with an established record outside the United States of treating staphylococcal infections, including those due to MRSA, since the 1960s and is being developed in a new front-loading dosing regimen (17). It is effective against all staphylococci, including hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA). Sodium fusidate has been successfully used in Europe to decolonize MRSA from the lungs of CF patients (6, 15). Overall resistance to fusidic acid among *S. aureus* isolates from CF patients has been reported at 1.2% (9) and among isolates from non-CF patients at 1.7% (2).

Amikacin and tobramycin are aminoglycosides, bactericidal agents that inactivate translation by binding to conserved sequences within 16S rRNA of the 30S ribosomal subunit (12). In contrast, fusidic acid is bacteriostatic, blocking bacterial protein synthesis (translation) by binding to ribosomal elongation factor G (8). Fusidic acid, amikacin, and tobramycin are commonly used in CF patients, and the chance that they will be used simultaneously in the CF patient is great. Their synergy activities have not been investigated before, to our knowledge.

We tested the activity of fusidic acid (i) against 40 MRSA strains from CF patients by microdilution MICs and compared it to those of vancomycin, teicoplanin, daptomycin, tigecycline, azithromycin, clarithromycin, linezolid, quinupristin-dalfopristin, and trimethoprim-sulfamethoxazole; (ii) against *P. aeruginosa*, MRSA, and *B. cepacia* from CF patients alone and in combination with amikacin or tobramycin by time-kill analysis; and (iii) against two *S. aureus* strains by single-step passage analysis.

Forty MRSA strains isolated within the past 12 months from patients at our CF clinic were tested for resistance by determining the MIC of fusidic acid for each strain. Only one strain per patient was tested, as determined by multiple-locus variable-number tandem-repeat typing (MLVF; formerly MLVA) (13, 15). For time-kill synergy testing, two strains each of mucoid *P. aeruginosa*, *B. cepacia*, and MRSA were used. All strains were identified by standard methods. Fusidic acid powder was obtained from Cempra Holdings, LLC (Chapel Hill, NC), and other antimicrobial agents were obtained from their respective manufacturers. The MICs of fusidic acid and other comparators for each of 40 MRSA strains were tested by CLSI microdilution methodology (3) in 96-well trays with a 100-μl final volume of bacterial broth. Trays were obtained from Trek, Inc. (Cleveland, OH) or prepared in-house. For time-kill analyses, macrobroth MIC dilution (3, 4) was performed according to CLSI methodology for all synergy testing in glass tubes (5-ml final volume of bacterial broth).

The kill kinetics of each drug was tested alone by incubating an initial inoculum of 5×10^5 to 5×10^6 CFU/ml of a MRSA strain with drug concentrations at the MIC, three dilutions above the MIC, and three dilutions below the MIC (1/2 × MIC, 1/4 × MIC, and 1/8 × MIC). Viability counts were performed after 0, 3, 6, 12, and 24 h of incubation at 37°C in a shaking water bath by plating the bacterial suspensions onto Trypticase soy–5% sheep blood agar (SB-TSA) plates (BD Diagnostics, Sparks, MD). After the initial time-kill assays with single agents were completed, fusidic acid was combined with

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TABLE 1. Broth microdilution MICs of all compounds against 40 MRSA strains from CF patients

Drug or drug combination	Range of MICs (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Fusidic acid	0.125 to 0.5	0.125	0.25
Vancomycin	0.5 to 1	0.5	1
Teicoplanin	0.25 to 1	0.5	1
Daptomycin	0.5 to 1	0.5	1
Tigecycline	0.125 to 0.25	0.125	0.25
Azithromycin	1 to ≥32	≥32	≥32
Clarithromycin	0.25 to ≥32	≥32	≥32
Linezolid	1 to 4	2	2
Quinupristin-dalfopristin	0.25 to 1	0.5	1
Trimethoprim-sulfamethoxazole	≤0.5 and ≤9.5	≤0.5 and ≤9.5	≤0.5 and ≤9.5

amikacin or tobramycin. Combinations were tested 1 to 2 dilutions below the MIC (1/2 × MIC and 1/4 × MIC) of each drug. Inoculum and time-kill methodologies were performed as described above. The concentrations used in the synergy time-kill tests were selected such that one of the two drugs yielded a growth curve similar to that of the drug-free control, while the other drug was more active. MICs were assayed by standard methodology (3). Synergy and antagonism were defined as previously described (11).

The frequencies of spontaneous single-passage mutations were tested for two *S. aureus* isolates as described previously (1, 10).

All MRSA strains tested for the determination of the MICs of different drugs and drug combinations were individual clones. The MICs (μg/ml) are listed in Table 1. CEM-102 had potent activity, with MICs between 0.125 and 0.5, against all strains tested. Vancomycin and teicoplanin were also active at MICs of 0.25 to 1 μg/ml, linezolid at MICs of 1 to 4 μg/ml, quinupristin-dalfopristin at MICs of 0.25 to 1 μg/ml, and trimethoprim-sulfamethoxazole at MICs of ≤0.5 μg/ml for trimethoprim and ≤9.5 μg/ml for sulfamethoxazole. Most strains (38 of 40) were resistant (MICs of >32 μg/ml) to both azithromycin and clarithromycin.

Macrobroth MICs for the four Gram-negative rods and two MRSA isolates selected for time-kill synergy studies and synergy time-kill data are shown in Tables 2 and 3. Synergy

TABLE 2. Interactions in *in vitro* time-kill analysis using fusidic acid combined with amikacin or tobramycin[a]

Interaction	No. of strains interacting with fusidic acid-tobramycin combination at indicated time points (h)				No. of strains interacting with fusidic acid-amikacin combination at indicated time points (h)			
	3	6	12	24	3	6	12	24
Synergy	0	0	1	2	0	0	0	2
Indifference	4	4	3	2	5	5	5	3
Antagonism	0	0	0	0	0	0	0	0

[a] A total of 4 strains were tested in fusidic acid-tobramycin; a total of 5 strains were tested in fusidic acid-amikacin. One strain (PSAR 461) was not tested in either drug combination (fusidic acid MIC of >512 μg/ml). One strain (MRSA 2232) was not tested in fusidic acid-tobramycin (fusidic acid MIC of >512 μg/ml).

TABLE 3. Results of *in vitro* time-kill analyses of fusidic acid with amikacin or tobramycin for all strains tested

Strain	Initial MIC for indicated drug (μg/ml)[a]			Interaction[b] with fusidic acid-tobramycin at indicated time point (h)				Interaction[b] with fusidic acid-amikacin at indicated time point (h)			
	Fusidic acid	Tobramycin	Amikacin	3	6	12	24	3	6	12	24
S. aureus HMC2230	0.5	4.0	32.0	IND	IND	IND	SYN (0.125, 1)	IND	IND	IND	IND
S. aureus HMC2232	0.25	>512	64.0	NT	NT	NT	NT	IND	IND	IND	IND
P. aeruginosa HMC461	>512	2.0	8.0	NT	NT	NT	NT	NT	NT	NT	NT
P. aeruginosa HMC468	256	1.0	4.0	IND	IND	IND	IND	IND	IND	IND	IND
B. cepacia HMC953	512	128	512	IND	IND	SYN (256, 64)	SYN (256, 32)	IND	IND	IND	SYN (128, 128)
B. cepacia HMC954	512	128	256	IND	IND	IND	IND	IND	IND	IND	SYN (128, 128)

[a] MIC read at 24 h.
[b] SYN, synergy (antimicrobial concentrations in μg/ml); IND, indifference; NT, not tested (MIC of >512 μg/ml).

was found with one fusidic acid-plus-tobramycin combination (0.125 μg/ml for fusidic acid and 1 μg/ml for tobramycin) at 24 h for one MRSA strain (HMC2230). We prolonged the time-kill analysis for 48 h; synergy was still present at 0.125 μg/ml for fusidic acid and 2 μg/ml for tobramycin. All other time points and combinations were indifferent with both MRSA strains. One strain of MRSA was not tested with tobramycin in combination because of a very high MIC (>512 μg/ml). All time points and combinations were indifferent with the two *P. aeruginosa* strains tested. One *P. aeruginosa* strain was not tested with fusidic acid due to a very high MIC (>512 μg/ml). One *B. cepacia* strain showed synergy at 12 and 24 h with fusidic acid plus tobramycin at concentrations of 256 μg/ml of fusidic acid and 64 μg/ml of tobramycin and 256 μg/ml of fusidic acid and 32 μg/ml of tobramycin, respectively. Both *B. cepacia* strains showed synergy with fusidic acid plus amikacin at concentrations of 128 μg/ml of fusidic acid and128 μg/ml of amikacin. All other time points and combinations were indifferent with the two *B. cepacia* strains. No antagonism was noted in any organism with any antibiotic combination.

The resistance frequencies of two *S. aureus* strains were 7.8×10^{-7} (SA2230) and 1.1×10^{-6} (SA2232) at 2 times the MIC and 4.6×10^{-7} (SA2230) and 4.1×10^{-7} (SA2232) at 4 times the MIC after 48 h.

Fusidic acid has been shown to be a potent agent against MRSA isolates. More than 20% of CF patients are colonized with *S. aureus* (5). In a recent multicenter study of survival analysis in 19,833 CF patients, MRSA was associated with shortened survival, with the relative risk of death 1.27 times higher if the patient was colonized with MRSA (5). Fusidic acid was very potent against all strains of MRSA isolated from CF patients in our clinic.

In CF patients, *P. aeruginosa* and *B. cepacia* are the most frequently isolated pathogens (7, 16). Amikacin and tobramycin are the most common agents used to treat *P. aeruginosa* and *B. cepacia* in CF patients (7). To test for possible antibiotic interactions with fusidic acid, combinations with these two aminoglycosides were evaluated by time-kill analysis. No antagonism was found. Although some instances of synergy were found, MICs in synergistic combinations were not clinically achievable for the Gram-negative rods. For MRSA, clinically achievable synergy was observed with strain *S. aureus* HMC2230 with a combination of fusidic acid and tobramycin. It has been described that after a single 500-mg dose, levels present at 8 h (8 to 12.5 μg/ml) and at 12 h (7.5 to 10 μg/ml) appear to exceed typical MICs (<1 μg/ml) for pathogens such as MRSA (18). With fusidic acid protein binding averaging 96% (17), the concentration of unbound fusidic acid is >0.125 μg/ml. Prolonged incubation in time-kill testing showed no regrowth in bacterial suspensions, and synergy was observed at drug concentrations similar to those at 24 h. Single-step mutation frequencies for fusidic acid against *S. aureus* strains were similar to those reported previously (10).

In summary, fusidic acid had potent activity against MRSA strains isolated from CF patients and could provide an oral-treatment alternative for CF patients infected with MRSA. No antagonism was found with the aminoglycosides commonly used to treat the Gram-negative bacteria commonly found in the lungs of CF patients. Other fusidic acid combinations need to be tested for synergy against these Gram-negative rods. Our results require confirmation with additional *in vitro* and clinical studies.

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