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In Vitro Activities of Designed Antimicrobial Peptides against Multidrug-Resistant Cystic Fibrosis Pathogens

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The emergence of multidrug-resistant pathogens renders antibiotics ineffective in the treatment of lung infections in patients with cystic fibrosis (CF). Designed antimicrobial peptides (DAPs) are laboratory-synthesized peptide antibiotics that demonstrate a wide spectrum of antibacterial activity. Optimal conditions for susceptibility testing of these peptides have not yet been established. Medium composition is clearly a major factor influencing the results and reproducibilities of susceptibility tests. Using time-kill assays, we tested the effects of different media and buffers on the bactericidal activities of the peptides D2A21 and D4E1 on *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. Each peptide at 1 and 5 μM was incubated with bacteria in the different media and buffers. Both peptides were most active in Tris-HCl buffer against *S. aureus* and *P. aeruginosa*. Among the more complex media tested, modified RPMI medium was the medium in which the peptides demonstrated the highest activity, while it supported the growth of the bacteria. The broth microdilution technique was used to test the activities of D2A21 and D4E1 in modified RPMI medium against multidrug-resistant pathogens from patients with CF. The MICs of DAPs for methicillin-resistant *S. aureus* ranged from 0.25 to 4 μg/ml, those for multidrug-resistant *P. aeruginosa* ranged from 0.125 to 4 μg/ml, those for *Stenotrophomonas maltophilia* ranged from 0.5 to 32 μg/ml, and those for *Burkholderia cepacia* ranged from 32 to ≥64 μg/ml. When the activity of peptide D2A21 was compared with that of the tracheal antimicrobial peptide (TAP), D2A21 had greater potency than TAP against *P. aeruginosa*. In addition, no difference in the MICs of D2A21 was seen when it was tested in nutrient broth supplemented with NaCl at different concentrations. Thus, DAPs are a class of salt-insensitive antibiotics potentially useful in the treatment of CF patients harboring multidrug-resistant *P. aeruginosa*.

The emergence of multidrug-resistant pathogens increasingly renders antibiotics ineffective in the treatment of lung infections due to cystic fibrosis (CF), the most common fatal genetic disease in the United States. The past few years have brought dramatic advances in our knowledge of the molecular and cellular basis of CF lung disease (3, 8, 16). Several hypotheses have been proposed to explain the development of bronchitis due to an unusual mucoid phenotype of *Pseudomonas aeruginosa* based on the characteristic physiologic abnormality, the defective transport of chloride ions across the apical membrane of the airway epithelia (4, 14, 18, 19, 23). It has been proposed that the lack of Cl^- secretion promotes airway drying and mucus plugging, thus predisposing the airways to chronic colonization. Why mucus plugging should promote airway colonization predominantly with *P. aeruginosa* and not with other bacterial lung pathogens such as *Streptococcus pneumoniae*, as seen in non-CF patients, is not understood. An attempt has been made to prove a link between Cl^- secretion and infection by attributing airway infection to altered antimicrobial activity of salt-sensitive, epithelial cell-derived peptides in patients with CF (23). The investigators proposed that the defect in the CF gene product elevates NaCl levels in airway surface liquid and thereby inactivates antimicrobial molecules. Recently, an antimicrobial peptide, human β-defensin-1 (hBD-1), has been identified (6). It is expressed in human airway epithelial cells and shows broad-spectrum antimicrobial activity against gram-negative organisms. Furthermore, its antimicrobial activity may be salt dependent in airway surface fluids, although the point is controversial. The ion composition of airway surface fluid in the airways of healthy people and patients with CF is not yet defined (5, 9, 10).

The discovery of antibacterial epithelium-derived peptides suggests avenues for the development of innovative therapies, such as replacement of these peptides with salt-insensitive synthetic peptides which would restore the sterile airway environment and halt or slow the airway disease in patients with CF.

We now report on the in vitro activities of designed antimicrobial peptides (DAPs), laboratory-synthesized peptides which are similar to the naturally occurring peptides such as tracheal antimicrobial peptide (TAP) (11), defensins (2, 12), and magainins (1). Unlike hBD-1, the antibacterial activities of DAPs are salt insensitive.

(These data were presented in part at the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, Canada, September 1997 [21].)

MATERIALS AND METHODS

Bacterial strains. The antibacterial activities of DAPs in different media against American Type Culture Collection (ATCC) strains *P. aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 were tested by time-kill assays. Clinical isolates of methicillin-resistant *S. aureus* (MRSA; $n = 21$), *P. aeruginosa* ($n = 32$), *Stenotrophomonas maltophilia* ($n = 16$), and *Burkholderia cepacia* ($n = 10$) were tested by the broth microdilution method.

DAPs. Seven DAPs (D2A21, D4E1, D2A22, D5C, D4E, D4B, and D5C1), whose amino sequences are presented in Fig. 1, were screened for their activities against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 in Mueller-Hinton broth (MHB; Remel, Lenexa, Kans., at a 1 μM (2.8- to 5.6-μg/ml) concentration.

Stock solutions of the peptides were prepared by dissolving 1 mg of each peptide in 1 ml or 500 μl of sterile distilled water. All subsequent dilutions were made in test medium and were prepared fresh for each experiment.

Naturally occurring peptide. TAP (kindly provided by Scott Randall, Cystic Fibrosis Center, University of North Carolina, Chapel Hill) was used in this study and was prepared as described above for the DAPs.

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D2A21	FAKKFAKKFKKFAKKFAKFAFAF
D4E1	FKLRAKIKVRLRAKIKL
D2A22	FARKFLKRFKKFVRKFIRFAFLF
D5C	KRKRAVKRVGRRLKKLARKIARLGVAF
D5C1	KRKRAVKRVGRRLKKLARKIARLGVAKLAGLRAVKLF
D4F	FKVKAKVKAKVKAKVKA
D4B	FKVKAKVKAKVKAKVKAKKKK

FIG. 1. Amino acid sequences of DAPs used in this study. The synthesis of the peptides was done by 9-fluorenylmethoxycarbonyl chemistry.

Media and buffers tested. The following media and buffers were tested for their effects on the antimicrobial activities of the different peptides studied: Trypticase soy broth (TSB; Becton Dickinson Microbiology Systems, Cockeysville, Md.), nutrient broth (NB; Becton Dickinson Microbiology Systems), chemically defined RPMI 1640 medium (15) with 20 mM HEPES and without sodium bicarbonate (catalog no. R7388; Sigma, St. Louis, Mo.), MHB, cation-adjusted MHB supplemented with 20 to 25 mg of Ca^{2+} per liter and 10 to 12.5 mg of Mg^{2+} per liter (adjMHB) (17), 0.1 M Tris-HCl buffer (pH 7.2 and 8.4), 0.01 M phosphate-buffered saline (PBS), 0.1 M phosphate buffer and citrate-phosphate buffer (pH 7.2 and 5.2), and unbuffered saline. All media and buffers were tested for their osmolarity (Vapro Pressure Osmometer) and, if necessary, were adjusted with NaCl to a physiologic osmolarity of 280 to 320 mosM. Fifty and 90 mM NaCl were added to Tris-HCl (pH 7.2 and 8.4, respectively), 40 mM NaCl was added to phosphate buffer, 50 and 70 mM were added to citrate-phosphate buffer (pH 7.2 and 5.2, respectively), 5 mM NaCl was added to RPMI 1640 medium, and 120 mM NaCl was added to NB. Furthermore, the pH of RPMI 1640 medium was standardized by adding an additional 10 mM HEPES (modified RPMI).

The effect of osmolarity on the antibacterial activities of the peptides was tested in NB (56 mosM; hypotonic), NB supplemented with 120 mM NaCl (290 mosM; isotonic), and NB supplemented with 170 mM NaCl (405 mosM; hypertonic).

Preparation of inoculum. Bacteria from frozen suspensions were subcultured onto sheep blood agar plates and were passaged twice prior to susceptibility testing. The bacteria were then grown in TSB for 3 to 5 h (exponential-phase cells) before adjusting their concentration to a 0.5 McFarland turbidity standard. The adjusted bacterial cultures were diluted to approximately 10^7 CFU/ml. For determination of the activities of the peptides in buffer, the bacteria were washed and were then incubated with the peptides in appropriate buffer. To verify the final inoculum size, the viable colonies in the inoculum were counted.

Killing curve method. To determine the effects of media and buffers on the activities of the peptides, time kill curve studies were performed as described in the guidelines of the National Committee for Clinical Laboratory Standards (17). Approximately 10^7 bacteria were incubated with 1 μM (4.3 μg/ml) and 5 μM (21.5 μg/ml) D2A21 and 1 μM (2.8 μg/ml) and 5 μM (14 μg/ml) D4E1 in the different media and buffers. Samples were removed at 0.5, 1, 2, and 4 h and the numbers of colonies were determined. For this purpose the samples were serially diluted in test tubes containing 4.5 ml of PBS to produce 10-fold dilutions. A total of 100 μl was inoculated in duplicate onto sheep blood agar plates (media base was Trypticase soy agar; Becton Dickinson Microbiology Systems) of each dilution tube and was spread by using sterile bent glass rods. After overnight incubation, the colonies were counted and average counts were determined. The percentage of bacteria killed was determined by the following equation: 100 × ($\log_{10}$ CFU per milliliter killed at end of incubation period with peptide)/($\log_{10}$ CFU per milliliter at end of incubation period without peptide). We defined killing of ≥99.9% of the final inoculum as 100% or total killing. All assays were repeated at least once, with the difference between assays being ≤1 $\log_{10}$ CFU/ml. The results presented in Fig. 2 and 3 are from a single representative experiment.

MIC determination by broth microdilution technique. In preliminary experiments the time-kill assay was compared to the broth microdilution technique. Five strains each of MRSA and *P. aeruginosa* were tested in modified RPMI. No differences in data between these two assays were seen. Thus, we used the broth microdilution technique to test the activities of the peptides against pathogens from patients with CF and compared their activities to the activity of the naturally occurring peptide TAP. In addition, the osmolarity effect on the antibacterial activity of D2A21 was tested.

MICs were determined by the broth microdilution method described by the National Committee for Clinical Laboratory Standards (17). Serial twofold dilutions of each peptide solution were prepared (final volume, 100 μl) in microtiter trays with appropriate medium. Each dilution series included control wells containing bacteria without peptide. A total of 100 μl of the adjusted inoculum (10^6 organisms) was added to each well, and then the trays were incubated at 35°C in ambient air overnight (18 to 24 h). The MIC of each peptide for each isolate was read as the lowest concentration of peptide that inhibited visible growth of the organism.

RESULTS

Screening of DAPs. Figure 2 demonstrates the bactericidal activities of the seven tested peptides after 1 h of incubation. D2A21, a linear, 23-amino-acid peptide, and D4E1, a linear, 17-amino-acid peptide, were the most active peptides against both bacterial species and were extensively tested in this study.

Antibacterial effects of media and buffers on the activities of D4E1 and D2A21. Modified RPMI was the medium in which the peptides demonstrated the highest activity against both *S. aureus* and *P. aeruginosa* (Table 1). *S. aureus* was killed within 2 h by both D2A21 and D4E1 at 5 μM. *P. aeruginosa* was more sensitive than *S. aureus*, with maximal killing by D2A21 at 1 and 5 μM occurring at 30 min and that by 5 μM D4E1

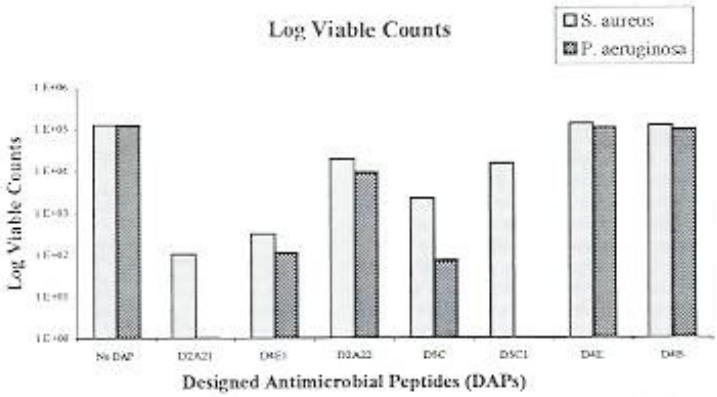

FIG. 2. Bactericidal activities of DAPs against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853. DAPs at a concentration of 1 μM were incubated with bacteria in MHB for 1 h. Samples were removed, serially diluted, and plated onto agar plates. After overnight incubation, the colonies were counted and the percentage of bacteria killed was determined.

TABLE 1. Effects of media and buffers on bactericidal activities of DAPs against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853

Medium or buffer	mosM	pH	Peptide treatment (concn [μM])[a]	% Organisms killed at indicated incubation times:							
				S. aureus				*P. aeruginosa*			
				30 min	1 h	2 h	4 h	30 min	1 h	2 h	4 h
Media											
TSB	324	7.2	D2A21 (1)	0	0	4.6	1.7	20	34	40.6	74.7
			D2A21 (5)	25.8	25	39.8	40	30.4	66	100[b]	100
			D4E1 (1)	0	3.6	0.8	10.9	0	0	1.9	16.4
			D4E1 (5)	2.3	4.2	5.3	15.8	20	56.4	75.1	54.9
NB plus 125 mM NaCl	290	7.2	D2A21 (1)	13.7	16	35.9	56.3	78.5	71.1	72.8	87
			D2A21 (5)	31.1	36	52.8	73.6	100	100	100	100
			D4E1 (1)	0	3.1	7	7.1	57.8	51.4	43.4	50
			D4E1 (5)	38.5	42.6	56.1	46.1	100	100	100	100
adjMHB	285	7.2	D2A21 (1)	1.7	11.4	3.9	0.3	17.4	19.4	19.2	18.2
			D2A21 (5)	37.3	50.4	60.4	73.1	85.4	100	100	100
			D4E1 (1)	3.7	5.4	6.2	12.1	1.5	2	11.1	11
			D4E1 (5)	6.7	12.2	22.2	44	36.1	50.6	60.2	60.7
MHB	297	7.2	D2A21 (1)	14.6	16.7	23.5	43.1	89.6	89.8	84	71.4
			D2A21 (5)	47.9	52.1	74.5	77.6	100	100	100	100
			D4E1 (1)	10.4	16.7	7.8	19	12.2	18.1	35.3	48.5
			D4E1 (5)	43.8	45.8	68.6	86.2	29.9	28.8	53.1	73.2
Modified RPMI	285	7.2	D2A21 (1)	30.5	43.3	62.4	100	100	100	100	100
			D2A21 (5)	84.9	85.2	100	100	100	100	100	100
			D4E1 (1)	9.7	9.7	11.9	19	46.4	29.5	42.6	50.3
			D4E1 (5)	49.7	75.4	100	100	68.1	100	100	100
Buffers											
PBS	312	7.2	D2A21 (1)	4.7	9.3	12.6	37.5	100	100	100	100
			D2A21 (5)	26.3	34.4	64	100	100	100	100	100
			D4E1 (1)	1.1	0	11.8	74.8	46.6	57.6	70.7	71.9
			D4E1 (5)	9	11.1	83.6	100	100	100	100	100
Tris-NaCl buffer	276	7.2	D2A21 (1)	89.2	75.6	100	100	100	100	100	100
			D2A21 (5)	100	100	100	100	100	100	100	100
			D4E1 (1)	11.4	19	30.7	41.6	6.9	40.7	50.7	55.4
			D4E1 (5)	83.4	100	100	100	43.3	100	100	100
Tris-NaCl buffer	285	8.4	D2A21 (1)	100	100	100	100	100	100	100	100
			D2A21 (5)	100	100	100	100	100	100	100	100
			D4E1 (1)	100	100	100	100	100	100	100	100
			D4E1 (5)	100	100	100	100	100	100	100	100
Citrate-phosphate buffer	309	7	D2A21 (1)	28.3	31	36.1	47.1	82.3	100	100	100
			D2A21 (5)	26.1	77.6	100	100	100	100	100	100
			D4E1 (1)	6.9	8.8	10.4	22.7	46.4	61.9	63.3	59.6
			D4E1 (5)	54.3	59.5	62.2	65.2	63.3	69.7	70	85.3
Citrate-phosphate buffer	298	5	D2A21 (1)	5.2	2.3	1.3	23.1	86.8	100	100	100
			D2A21 (5)	20.1	19.3	15.8	23.1	100	100	100	100
			D4E1 (1)	5.9	3.8	1.1	22.7	100	100	100	100
			D4E1 (5)	88.7	100	100	100	100	100	100	100
Saline	281	5.6	D2A21 (1)	30.5	32.5	42.3	57.7	100	100	100	100
			D2A21 (5)	71.2	62	62.3	79.8	100	100	100	100
			D4E1 (1)	13.1	59.8	100	100	17	34.8	57.1	79.6
			D4E1 (5)	100	100	100	100	57.4	67.4	83.7	100
Phosphate buffer	309	7.2	D2A21 (1)	10.8	10	16.5	15.4	100	100	100	100
			D2A21 (5)	28.7	52	41.6	67.7	100	100	100	100
			D4E1 (1)	0	0	1.4	0	39.1	38.8	39.8	63.6
			D4E1 (5)	12.4	18.3	32.9	42.5	74.2	100	100	100

[a] Concentrations of 1 and 5 μM D2A21 correspond to 4.3 and 21.5 μg/mL respectively, and concentrations of 1 and 5 μM D4E1 correspond to 2.8 and 14 μg/mL respectively.

[b] Killing of ≥99.9% of the final inoculum was defined as 100%.

occurring at 1 h. Furthermore, no growth of either species was detected at 24 h after incubation with the peptides.

In TSB, NB, MHB, and adjMHB, the peptides were less active against staphylococci. The highest reduction in staphylococcal counts was seen in MHB for both peptides at a concentration of 5 μM; however, total killing did not occur. In contrast, *P. aeruginosa* was killed by 5 μM D2A21 within 0.5 to 2 h in all four media tested and by 5 μM D4E1 within 30 min in NB (Table 1).

In the neutral buffers, D2A21 was highly active against *P. aeruginosa*, as indicated in Table 1. One micromolar D2A21 killed all organisms within 30 to 60 min. D4E1 at the same concentration was less active, killing only up to 71.9% of all *Pseudomonas* organisms after an incubation of 4 h. Both peptides killed *P. aeruginosa* more effectively than they killed *S. aureus*. Total killing of *S. aureus* by both peptides at 5 μM occurred in Tris-NaCl buffer and PBS. A low level of activity was observed in phosphate buffer. Only 67.7 and 42.5% of staphylococci were killed by 5 μM D2A21 and D4E1, respectively.

As shown in Table 1, the potencies of the peptides increased as the pH was increased from neutral to alkaline in Tris-HCl buffer. Both D2A21 and D4E1 at 1 μM concentrations killed *S. aureus* and *P. aeruginosa* within 30 min at pH 8.4. In contrast, at pH 7.2, a 2-h incubation with 1 μM D2A21 was required to kill all organisms. Only 41.6% of the organisms were killed after an incubation period of 4 h. Interestingly, D4E1 seemed to become more active at pHs below the neutral range. In citrate-phosphate buffer at pH 5.0, total killing of *P. aeruginosa* and *S. aureus* by 5 μM D4E1 occurred within 0.5 and 1 h, respectively, whereas in neutral citrate-phosphate buffer only 85.3% of *Pseudomonas* bacteria and 65.2% of staphylococci bacteria were killed. In contrast, the antistaphylococcal activity of D2A21 was reduced in citrate-phosphate buffer at pH 5.0. Only 23.1% of staphylococci were killed by 5 μM D2A21, whereas 100% of staphylococci were killed in NB. However, the activity of D2A21 against *P. aeruginosa* was not affected by the change in pH. In unbuffered saline, the antistaphylococcal activity of D2A21 was slightly improved compared to the activity in acidic citrate-phosphate buffer. However, the activity of D4E1 against *S. aureus* but not against *P. aeruginosa* increased significantly. Within 2 h all staphylococci were killed by 1 μM D4E1, whereas a 4-h incubation period was required to kill all *P. aeruginosa* bacteria with 5 μM D4E1. Unbuffered saline was the only environment in which *S. aureus* was more susceptible to D4E1 than *P. aeruginosa*.

Antibacterial activities of D4E1 and D2A21 against multidrug-resistant pathogens. The MIC data for both peptides and for each strain are shown in Fig. 3. D2A21 was slightly more active than D4E1 against MRSA and multidrug-resistant *P. aeruginosa*. The D2A21 MICs for MRSA ranged between 0.25 and 4 μg/ml, and those for multidrug-resistant *P. aeruginosa* ranged between 0.125 and 4 μg/ml. The MICs at which 50% of strains are inhibited (MIC_{50}s) for both species were 1 μg/ml. The D4E1 MICs for all MRSA and multidrug-resistant *P. aeruginosa* strains tested ranged between 0.5 and 4 μg/ml, with an MIC_{50} of 2 μg/ml.

The peptides were less active against *S. maltophilia* and *B. cepacia*. The 16 isolates of *S. maltophilia* showed various levels of sensitivity, with MICs ranging between 0.5 and 16 μg/ml for D2A21 (MIC_{50}, 2 μg/ml) and 1 to 32 μg/ml for D4E1 (MIC_{50}, 4 μg/ml). The *B. cepacia* isolates were much less sensitive (D2A21 and D4E1 MICs, 32 and ≥64 μg/ml, respectively).

Comparison of activities of DAPs with that of the naturally occurring peptide TAP. As shown in Table 2, DAPs have

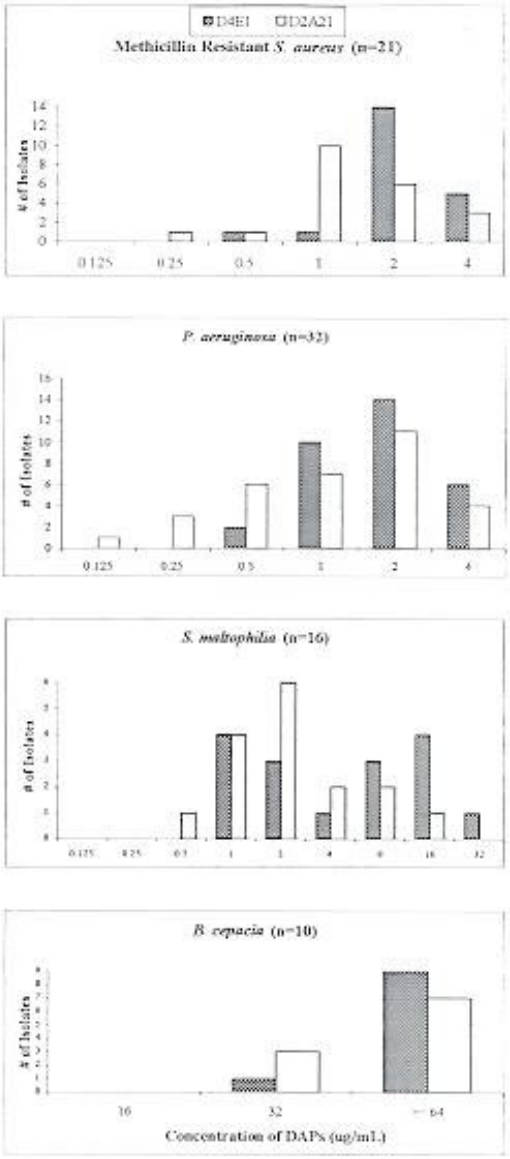

FIG. 3. Determination of MICs of DAPs for multidrug-resistant pathogens from patients with CF. MICs were determined by the broth microdilution technique.

higher potencies than the naturally occurring peptide TAP against the clinical bacterial isolates tested. The MICs of TAP ranged between 32 and ≥64 μg/ml for *P. aeruginosa* and MRSA, whereas the MICs of DAPs were ≤4 μg/ml.

Effect of osmolarity on activities of DAPs. NB has a much lower osmolarity, ionic strength, and concentration of sodium

TABLE 2. Activities of DAPs compared with that of TAP in modified RPMI medium

Isolate[a]	MIC (μg/ml)		
	D2A21	D4E1	TAP
ATCC 27853	2	4	64
PAE-2	1	1	16
PAE-5	1	1	64
PAE-7	1	1	64
PAE-8	2	2	64
PAE-9	1	1	64
PAE-10	1	2	64
PAE-11	0.5	1	64
PAE-12	1	1	64
PAE-26	2	2	≥64
PAE-30	1	2	>64
ATCC 29213	2	4	64
MRSA-1	2	2	32
MRSA-2	1	2	>64
MRSA-4	1	1	64
MRSA-5	1	2	64
MRSA-6	1	2	64
MRSA-23	1	2	≥64
MRSA-24	1	4	>64
MRSA-29	1	2	64
MRSA-32	1	2	≥64
MRSA-33	2	2	>64
SM-1	1	4	≥64
SM-4	1	1	≥64
SM-6	2	8	>64
SM-12	8	16	≥64
SM-16	4	8	≥64
BC-1	≥64	>64	≥64
BC-8	≥64	≥64	≥64
BC-10	>64	>64	≥64
BC-11	≥64	≥64	>64
BC-12	≥64	≥64	≥64

[a] PAE, *P. aeruginosa*; SM, *S. maltophilia*; BC, *B. cepacia*.

and chloride. However, increasing the unusually low concentration of NaCl in NB to the levels in isotonic and hypertonic NB did not affect the activity of D2A21 or TAP. The D2A21 MICs in the three media with various osmolarities did not differ more than 1 dilution interval from each other. The MICs for only two MRSA strains exhibited twofold increases when the strains were tested in hypertonic NB (Table 3). The MICs of TAP were ≥64 μg/ml in all three media tested (data not shown).

DISCUSSION

DAPs are synthetic peptides which are similar to naturally occurring peptides (1, 2, 11, 12). They show antimicrobial activity against a wide spectrum of bacteria. Although the modes of action of these peptides are not fully understood, it is believed that peptide-lipid interactions rather than receptor-mediated recognition processes play a major role in their function. Thus, it may be more difficult for bacteria to develop resistance to these peptides than to existing antibacterial agents.

Optimal in vitro conditions for susceptibility testing of cationic peptides have not yet been established. Since these peptides are highly charged, protocols such as those used for presently available antibiotics may not be applicable. For example, DAPs loose their antibacterial activity when they are incorporated in agar (7), perhaps due to extensive peptide binding to complex carbohydrates found in agar. It is also reported that the activities of synthetic peptides of lysosomal cathepsin G against *P. aeruginosa* is inhibited by calcium and magnesium (22), whereas the opposite is true for aminoglycosides, which require specific concentrations of Ca^{2+} (20 to 25 mg/liter) and Mg^{2+} (10 to 12.5 mg/liter) to demonstrate optimal antipseudomonal activity (17). We therefore investigated the effects of several different media on the antibacterial activities of DAPs. Modified RPMI was the medium in which the peptides were most active. In TSB, NB, MHB, and adjMHB, the peptides were less active. This decrease in activity could be due to peptide binding to complex carbohydrates such as starch or to proteins or could be due to interference of cations with the DAPs. An interference of cations with MSI-78, an analog of the frog skin antibiotic magainin, was recently reported (13). We also observed a decrease in the antibacterial activities of both peptides when they were tested in cation-adjusted MHB. Further studies are planned to determine which medium components affect the activities of the peptides. That pH has an effect on DAPs is demonstrated by the increase in peptide activity in Tris-HCl buffer seen at pH 8.4 compared to that seen at pH 7, as well as by the increased activity of D4E1 in acidic buffers.

However, under optimal conditions as defined by these studies, DAPs have been shown to be highly potent against multidrug-resistant and mucoid forms of *P. aeruginosa* and MRSA. Less activity was seen against *S. maltophilia* and *B. cepacia*. Although the two extensively tested peptides D2A21 and D4E1 did not show activity against *B. cepacia* isolates, D2A22,

TABLE 3. Effect of osmolarity on MICs of D2A21 in NB for multidrug-resistant pathogens from patients with CF

Isolate[a]	MIC (μg/ml) of D2A21 in NB at the following osmolarity:		
	56 mosM	290 mosM	805 mosM
ATCC 27853	4	4	4
PAE-2	2	≤1	≤1
PAE-5	2	2	2
PAE-7	≤1	≤1	≤1
PAE-8	4	4	4
PAE-9	4	2	2
PAE-10	≤1	≤1	≤1
PAE-11	≤1	≤1	≤1
PAE-12	≤1	≤1	≤1
PAE-26	4	2	4
PAE-30	≤1	≤1	≤1
ATCC 29213	8	4	64
MRSA-1	2	2	4
MRSA-2	4	4	8
MRSA-4	4	8	8
MRSA-5	4	8	8
MRSA-6	4	8	16
MRSA-23	4	4	16
MRSA-24	2	4	4
MRSA-29	2	4	4
MRSA-32	4	4	8
MRSA-33	4	4	8
SM-1	4	2	4
SM-4	≤1	≤1	≤1
SM-6	16	16	16
SM-12	8	16	16
SM-16	8	16	16
BC-1	16	32	16
BC-8	>64	>64	>64
BC-10	≥64	≥64	≥64
BC-11	≥64	≥64	≥64
BC-12	>64	>64	>64

[a] PAE, *P. aeruginosa*; SM, *S. maltophilia*; BC, *B. cepacia*.

a peptide with less activity against *S. aureus* and *P. aeruginosa*, showed a twofold increase in activity (20). These data are promising, and on the basis of these results Demegen Inc. has developed other peptides which will be tested for their anti-*B. cepacia* activities.

Unlike the DAPs, the natural occurring peptide TAP was not active against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 or multidrug-resistant pathogens from patients with CF in modified RPMI. In contrast, Lawyer et al. (11) showed that TAP in sodium phosphate buffer (pH 7.4) has antipseudomonal activity, highlighting again the need to standardize the conditions for peptide susceptibility testing. There are no reports regarding the activity of TAP against gram-positive bacteria in any system.

DAPs are particularly attractive as therapeutic agents for patients with CF because their activities do not appear to be diminished over a wide range of osmolarities since hyper- or hypotonic NB did not change the activities of the peptides. In contrast, Goldman et al. (6) observed a significant loss of activity of hBD-1 as the salt concentration increased from 50 mM to the more physiologic range of 125 mM. We increased the NaCl concentration in NB to as high as 250 mM, and no change in the MICs of D2A21 for *P. aeruginosa* and MRSA was observed. The slight increase in the MICs of D2A21 for two MRSA isolates may be due to their more rapid growth in this medium.

Since aerosolized drug delivery is a strategy used in the treatment of CF and other airway diseases, the effect of DAPs on a culture of human airway epithelium (157 HTB; ATCC) has been preliminarily investigated. At antibacterial concentrations, no toxic effects have been detected, emphasizing that this new class of antibiotics can be used in the treatment of CF patients. These results support the further development of DAPs as potentially useful antistaphylococcal and antipseudomonal therapies.

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