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NOTES

In Vitro Activities of Two Oxazolidinone Antimicrobial Agents, DuP 721 and DuP 105

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The antibacterial activities of DuP 105 and DuP 721, new oxazolidinone antimicrobial agents, were compared with those of beta-lactams and glycopeptides. Ninety percent of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates, including methicillin-resistant isolates, were inhibited by 4 μg of DuP 105 and 1 μg of DuP 721 per ml. The MICs for 90% of *Enterococcus faecalis* isolates were 16 μg of DuP 105 and 1 μg of DuP 721 per ml. DuP 721 inhibited hemolytic streptococcus groups A, B, C, F, and G at a concentration of ≤1 μg/ml, and it inhibited viridans group streptococci at a concentration of 2 μg/ml. Both agents inhibited *Listeria monocytogenes*, *Corynebacterium* group JK species, anaerobic cocci, and *Clostridium* spp. including *Clostridium difficile*. They did not inhibit members of the family *Enterobacteriaceae* or *Pseudomonas aeruginosa*, but the MIC for 90% of *Bacteroides fragilis* isolates was 8 μg of DuP 721 per ml.

The increase in gram-positive infections in hospitalized patients over the past few years has provoked interest in finding new and novel agents which inhibit gram-positive organisms, particularly the methicillin-resistant staphylococci (1, 2). DuP 105, *S-n*-[(3-(4-(methylsulfinyl)phenyl)-2-oxo-5-oxazolidinyl)methyl]-acetamide, and DuP 721, *S-n*-[(3-(4-acetylphenyl)-2-oxo-5-oxazolidinyl)methyl]acetamide, are synthetic compounds which do not belong to any existing antimicrobial class (4). We determined their in vitro activities and the effect of test conditions on their in vitro activities.

DuP 105 and DuP 721 were a gift from Martin Forbes of E.I. du Pont de Nemours & Co., Glenolden, Pa. Vancomycin and cephalexin were obtained from Lilly Research Laboratories, Indianapolis, Ind.; teicoplanin was from Merrell Dow Chemicals, Cincinnati, Ohio; and amoxicillin-clavulanate came from Beecham Laboratories, Bristol, Tenn.

Bacterial isolates were from patients hospitalized at The Presbyterian Hospital, New York, N.Y. Only one isolate from each patient was tested to avoid multiple copies of the same strain.

Antimicrobial susceptibility was measured by an agar dilution test with Mueller-Hinton agar unless otherwise specified. A replicating spot device applied 10^4 CFU, prepared by dilution of a fresh overnight broth. All drugs were tested simultaneously. Broth dilutions were performed with 5×10^5 CFU in 1-ml tubes for 10 isolates each of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*. Agar plates and tubes were incubated at 35°C for 18 h. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited the development of visible growth on agar or in the tubes. The MBC was determined by plating 0.01 ml from clear tubes to agar plates, and the criteria of Pearson et al. for 99.9% kill were used (3). Susceptibility tests with anaerobic bacteria were performed with brucella agar supplemented with hemin and vitamin K. Incubation was in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) at 35°C for 48 h. Susceptibility testing of streptococcal species was performed with Mueller-Hinton agar supplemented with 5% sheep blood, and tests of *Haemophilus* spp. and *Branhamella* spp. were performed with sheep chocolatized agar in the presence of 10% CO_2. The effect of ionic changes was determined by the addition of compounds to Mueller-Hinton medium to achieve the final concentrations noted.

The oxazolidinones DuP 721 and DuP 105 showed excellent activity against *S. aureus*, including methicillin-resistant strains with MICs for 90% of isolates of 1 μg of DuP 721 and 4 μg of DuP 105 per ml. (Table 1). DuP 721 was four- to eightfold more active than DuP 105. Both agents inhibited *S. epidermidis*, including methicillin-resistant isolates. Overall the activity of DuP 721 was comparable to the activities of vancomycin and teicoplanin. DuP 721 inhibited *E. faecalis* at a concentration of 2 μg/ml compared with a concentration of 16 μg/ml for DuP 105. This included strains for which vancomycin MICs were 8 μg/ml, but amoxicillin-clavulanate was the most active agent against the enterococci. In general, DuP 721 and DuP 105 inhibited 90% of hemolytic streptococci groups A, B, C, F, and G at concentrations of 1 and 4 μg/ml, respectively. DuP 721 also was very active against viridans group streptococci. In general, *Streptococcus pneumoniae* was less susceptible to DuP 721 and DuP 105 than to vancomycin, teicoplanin, and amoxicillin-clavulanate. DuP 721 inhibited *Listeria monocytogenes* at concentrations similar to those for vancomyin and teicoplanin. It also inhibited *Corynebacterium* group JK species at a concentration of 0.2 μg/ml, and it inhibited peptococcus species and *Clostridium* species including *Clostridium difficile*. The MIC of DuP 721 for 90% of *Bacteroides fragilis* isolates was 8 μg/ml.

At MICs of >128 μg/ml, neither agent inhibited *Esche-*

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TABLE 1. In vitro activities of DuP 721 and DuP 105 compared with those of other agents[a]

Organism (no. of isolates tested)	Agent[a]	MIC (μg/ml)[b]		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (30)	DuP 721	0.25–1	0.25	1
	DuP 105	2–8	2	4
	Teicoplanin	0.25–2	0.5	1
	Vancomycin	0.25–4	0.5	1
	Amox-clav	<0.25–2	0.25	1
	Cephalexin	0.25–8	2	4
Staphylococcus aureus, methicillin resistant (20)	DuP 721	0.125–0.5	0.25	0.5
	DuP 105	2–4	2	4
	Teicoplanin	0.25–1	0.5	1
	Vancomycin	0.5–1	0.5	1
	Amox-clav	>16	>16	>16
	Cephalexin	>64	>64	>64
Staphylococcus epidermidis, methicillin susceptible (25)	DuP 721	0.5–16	0.5	1
	DuP 105	1–32	2	4
	Teicoplanin	0.25–16	1	2
	Vancomycin	0.25–16	1	2
	Amox-clav	0.12–2	0.12	1
	Cephalexin	0.12–8	1	8
Staphylococcus epidermidis, methicillin resistant (20)	DuP 721	0.25–2	0.25	0.5
	DuP 105	2–8	4	8
	Teicoplanin	1–16	1	4
	Vancomycin	0.5–2	1	2
	Amox-clav	>16	>16	>16
	Cephalexin	>64	>64	>64
Enterococcus faecalis (30)	DuP 721	1–2	1	2
	DuP 105	4–16	8	16
	Teicoplanin	0.125–1	0.5	1
	Vancomycin	1–8	2	8
	Amox-clav	0.12	0.25	0.5
	Cephalexin	32–128	128	128
Streptococcus pyogenes (15)	DuP 721	0.5–2	0.5	1
	DuP 105	1–4	2	4
	Teicoplanin	0.06–0.5	0.125	0.25
	Vancomycin	0.25–0.5	0.25	0.5
	Amox-clav	<0.06–0.5	<0.06	0.12
	Cephalexin	0.12–8	0.25	2
Streptococcus agalactiae (21)	DuP 721	1–2	1	2
	DuP 105	2–8	2	4
	Teicoplanin	0.03–0.5	0.125	0.25
	Vancomycin	0.25–1	0.25	0.5
	Amox-clav	≤0.12–4	≤0.12	0.5
	Cephalexin	1–8	0.25	2
Streptococcus group C (12)	DuP 721	0.5–2	0.5	1
	DuP 105	1–8	2	4
	Teicoplanin	0.06–0.5	0.06	0.25
	Vancomycin	0.25–1	0.25	0.5
	Amox-clav	≤0.12–0.25	≤0.12	0.25
	Cephalexin	≤0.12–4	0.25	4
Streptococcus group F (19)	DuP 721	0.5–1	0.5	1
	DuP 105	2–16	2	8
	Teicoplanin	0.125–1	0.125	0.5
	Vancomycin	0.25–1	0.5	1
	Amox-clav	≤0.12–0.5	≤0.12	0.25
	Cephalexin	0.12–4	0.5	4

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TABLE 1—*Continued*

Organism (no. of isolates tested)	Agent[a]	MIC (μg/ml)[b]		
		Range	50%	90%
Streptococcus group G (19)	DuP 721	0.5–1	0.5	1
	DuP 105	2–8	2	4
	Teicoplanin	0.125–0.5	0.125	0.5
	Vancomycin	0.25–1	0.25	0.5
	Amox-clav	≤0.12–4	≤0.12	0.5
	Cephalexin	0.06–2	0.5	2
Viridans group streptococci (20)	DuP 721	0.5–2	1	2
	DuP 105	1–16	4	16
	Teicoplanin	0.125–1	0.5	1
	Vancomycin	0.25–2	0.5	1
	Amox-clav	≤0.12–4	0.12	1
	Cephalexin	0.12–8	0.5	8
Streptococcus bovis (10)	DuP 721	0.5–2	0.5	1
	DuP 105	2–16	4	8
	Teicoplanin	0.25–1	0.25	0.5
	Vancomycin	0.25–2	0.25	1
	Amox-clav	≤0.12–1	≤0.12	1
	Cephalexin	0.06–2	0.5	2
Streptococcus pneumoniae (20)	DuP 721	0.5–2	1	2
	DuP 105	4–8	4	8
	Teicoplanin	≤0.15–0.25	0.06	0.12
	Vancomycin	0.25–0.5	0.25	0.5
	Amox-clav	≤0.015–0.2	≤0.015	0.12
	Cephalexin	1–8	2	4
Listeria monocytogenes (20)	DuP 721	1–2	1	2
	DuP 105	16	16	16
	Teicoplanin	0.25–1	0.05	1
	Vancomycin	0.5–4	1	2
	Amox-clav	0.25–0.5	0.25	0.25
	Cephalexin	16–128	64	128
Corynebacterium group JK species (18)	DuP 721	0.25	0.25	0.25
	DuP 105	2–8	2	8
	Teicoplanin	0.25–1	0.25	1
	Vancomycin	0.5	0.5	0.5
	Amox-clav	≤0.12–16	4	16
Propionibacterium spp. (18)	DuP 721	0.25–1	0.25	0.5
	DuP 105	0.5–2	1	2
	Teicoplanin	0.06–0.5	0.25	0.5
	Vancomycin	0.25–0.5	0.25	0.5
Peptococcus spp. (16)	DuP 721	0.25–2	0.5	2
	DuP 105	2–8	4	8
	Teicoplanin	0.06–2	0.125	0.25
	Vancomycin	0.06–1	0.06	0.125
Clostridium spp.[c] (20)	DuP 721	1–8	2	4
	DuP 105	4–64	8	32
	Teicoplanin	≤0.015	0.125	1
	Vancomycin	0.25–1	0.25	1
	Amox-clav	≤0.12–0.5	≤0.12	0.25
	Cephalexin	2–16	16	>16
Bacteroides fragilis (10)	DuP 721	4–8	8	8
	DuP 105	32	32	32
Haemophilus influenzae (13)	DuP 721	32	32	32
	DuP 105	64	64	64
Branhamella catarrhalis (6)	DuP 721	8		
	DuP 105	8–32		

[a] Amox-clav, Amoxicillin-clavulanate. The drugs were combined in a ratio of 2:1.
[b] 50% and 90%, MIC for 50 and 90% of isolates tested, respectively.
[c] Includes *Clostridium perfringens*, *C. difficile*, and *C. septicum*.

TABLE 2. Comparison of MICs and MBCs of DuP 721 and DuP 105

Organism[a]	Agent	Geometric mean concn (μg/ml)	
		MIC	MBC
S. aureus	DuP 721	0.7	5.3
	DuP 105	5.2	42.2
S. epidermidis	DuP 721	0.2	1.5
	DuP 105	1.6	4
E. faecalis	DuP 721	1.7	64
	DuP 105	8	>128

[a] Ten isolates tested for each species.

richia coli, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Salmonella* spp., or *Shigella* spp. (five isolates of each). The MICs for 90% of *Haemophilus influenzae* isolates were 32 μg of DuP 721 and 64 μg of DuP 105 per ml.

There was a marked increase in MBCs for *S. aureus*, *S. epidermidis*, and *E. faecalis* (Table 2). Increasing the inoculum size from 10^5 to 10^7 CFU caused a fourfold increase in MICs for 70% of *S. aureus* and 80% of *E. faecalis* (10 isolates of each were tested). There was an eightfold increase in MIC at 10^7 CFU for only 1 of 10 *E. faecalis* isolates, whereas there was an eightfold increase in MIC for 2 of 10 *S. aureus* isolates.

The effects of various growth conditions on MICs for *S. aureus*, *S. epidermidis*, and *E. faecalis* are shown in Table 3. Five isolates of each species were tested. The addition of 3% NaCl, increased calcium or magnesium, and 50% normal human serum did not alter the MICs. Similarly, the addition of glucose 6-phosphate to the medium did not affect the MICs. Determination of activity under anaerobic conditions did not affect the MICs by more than twofold. The activities of DuP 721 and DuP 105 at pH 5.5 to 7.4 were similar for *S. aureus* and *E. faecalis*.

To determine whether repeated subculture of organisms in DuP 721 resulted in development of resistant isolates, one isolate each of *S. aureus*, methicillin-resistant *S. aureus*, *S. epidermidis*, methicillin-resistant *S. epidermidis*, and *E. faecalis* was transferred daily for 14 days in the presence of twofold-increasing concentrations of DuP 721. The MICs did not differ by more than twofold for any organism during or at the end of 14 days.

The activities of DuP 721 and DuP 105 were restricted to gram-positive species. Both agents inhibited staphylococci and streptococci but in general were slightly less active than the glycopeptides and considerably less active than amoxicillin-clavulanate against streptococcal species. Both agents showed a moderate inoculum effect and a difference between MICs and MBCs, but their activities were not adversely affected by anaerobic conditions, cations, or human serum. Our in vitro results are similar to those of Slee et al. (4). Further studies are needed to determine the levels of these agents in serum and tissue before more definitive statements can be made about their potential utility in the treatment of gram-positive infections.

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TABLE 3. Effect of various growth conditions on the activities of DuP 721 and DuP 105

Organism	Agent	MIC (μg/ml) on:						
		Mueller-Hinton agar						G6P[a]
		Alone	+3% NaCl	+3 mM Ca^{2+}	+3 mM Mg^{2+}	+9 mM Mg^{2+}	+50% serum	
S. aureus 1008	DuP 721	0.5	0.5	1	1	0.5	1	0.5
	DuP 105	4	2	4	4	4	2	2
S. aureus 1107	DuP 721	2	2	1	1	0.5	2	1
	DuP 105	8	4	8	4	8	4	4
S. epidermidis 664	DuP 721	0.5	0.25	0.25	0.5	0.5	0.5	0.25
	DuP 105	2	1	2	2	2	2	2
S. epidermidis 636	DuP 721	0.12	0.12	0.12	0.12	0.12	0.5	0.1
	DuP 105	0.5	0.5	0.5	1	1	0.5	0.5
E. faecalis 10039	DuP 721	2	1	2	2	2	2	1
	DuP 105	8	4	8	4	8	8	8
E. faecalis 10040	DuP 721	2	1	2	2	1	4	1
	DuP 105	8	4	8	8	8	8	8

[a] GGP, Glucose 6-phosphate at 25 μg/ml.