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Oxazolidinones, a New Class of Synthetic Antibacterial Agents: In Vitro and In Vivo Activities of DuP 105 and DuP 721

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DuP 721 (*p*-acetylphenyloxooxazolidinylmethylacetamide) and DuP 105 (a methylsulfinyl derivative) are orally active representatives of the oxazolidinones, a new class of synthetic antibacterial agents. Their antibacterial spectrum includes staphylococci, streptococci, and *Bacteroides fragilis* strains. The compounds have equal activity against staphylococcal strains susceptible or resistant to β-lactam antibiotics, including methicillin-resistant strains. The MICs for 90% of the strains (MIC_{90}s) against staphylococcal isolates were 1 to 4 μg/ml for DuP 721 and 4 to 16 μg/ml for DuP 105, compared with 1 to 2 μg/ml for vancomycin, 0.5 μg/ml for ciprofloxacin, and 2 to >16 μg/ml for imipenem. The MIC_{90}s against group D streptococci were 4 μg/ml for DuP 721, 16 μg/ml for DuP 105, and 2 μg/ml for vancomycin, ciprofloxacin, and imipenem. MIC_{90}s against *B. fragilis* isolates were 4 μg/ml for DuP 721, 16 μg/ml for DuP 105, and 8 μg/ml for cefoxitin. DuP 721 and DuP 105 administered by either the oral or the parenteral route were protective against staphylococcal and streptococcal infections in mice. The 50% effective doses were 2 to 10 mg/kg for DuP 721, 9 to 23 mg/kg for DuP 105, and 2 to 12 mg/kg for vancomycin. These results indicate that further studies of compounds of the oxazolidinone series are warranted.

The antibacterial activity of compounds of the oxazolidinone series was initially identified in a screening program. The first members of this new synthetic class were primarily active against staphylococci and streptococci. Since infections produced by staphylococci resistant to available antibiotics are on the increase (1, 4, 6, 8, 9), efforts were made to develop analogs with potent activities and improved pharmacological properties.

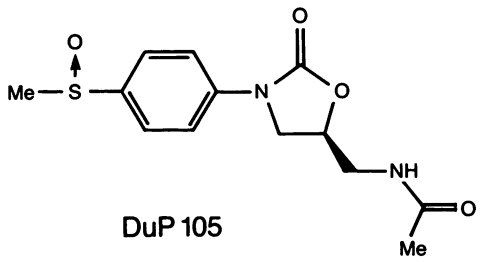
In this report, we describe the in vitro and in vivo activities of two new agents, DuP 721 (*p*-acetylphenyloxooxazolidinylmethylacetamide) and DuP 105 (a methylsulfinyl derivative), which are orally active representatives of the oxazolidinones (Fig. 1). The compounds were assessed, in comparison with other antibacterial agents, for activity against various gram-positive and gram-negative bacteria, including strains resistant to antibiotics in clinical use.

MATERIALS AND METHODS

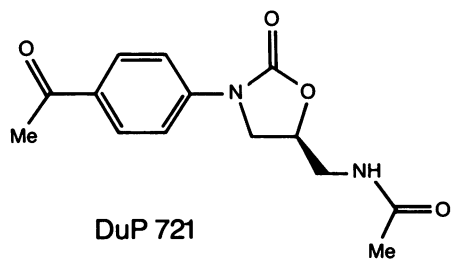
Organisms. The bacterial strains used were clinical isolates obtained from geographically separate medical centers in the United States and from the American Type Culture Collection. The yeast and fungal strains used were from the American Type Culture Collection.

Antibacterial agents. DuP 721 and DuP 105 were provided by the Medicinal Chemistry Section, Medical Products Department, du Pont Co., Wilmington, Del. Antibiotics used as reference drugs were obtained from the following sources: ampicillin, oxacillin, cephalexin, cefoxitin, cefotaxime, chloramphenicol, clindamycin, erythromycin, gentamicin, griseofulvin, tetracycline, vancomycin, and metronidazole from Sigma Chemical Co., St. Louis, Mo.; ciprofloxacin from Miles Pharmaceuticals, West Haven, Conn.; imipenem from Merck & Co., Inc., Rahway, N.J.; and amphotericin B from E. R. Squibb & Sons, Princeton, N.J.

For the in vitro tests, stock solutions were prepared by dissolving the compounds in water, except for ampicillin, which was dissolved in 0.1 M phosphate buffer (pH 8); DuP 721, chloramphenicol, and erythromycin, which were dissolved in dimethyl sulfoxide; ciprofloxacin, which was dissolved in 0.1 N NaOH solution; and griseofulvin, which was



DuP 105



DuP 721

FIG. 1. Structures of DuP 721 and DuP 105. DuP 721 is *S-n*-[(3-(4-acetylphenyl)-2-oxo-5-oxazolidinyl)methyl]acetamide. DuP 105 is *S-n*-[(3-(4-(methylsulfinyl)phenyl)-2-oxo-5-oxazolidinyl)methyl]-acetamide.

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TABLE 1. In vitro activity of DuP 721, DuP 105, and selected antibiotics against bacterial and fungal pathogens determined by the microtiter broth dilution method

Bacterium (no. of strains)	Drug	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (24)	DuP 721	1.0–4.0	2.0	4.0
	DuP 105	4.0–32.0	8.0	16.0
	Vancomycin	0.5–2.0	1.0	1.0
	Chloramphenicol	4.0–64.0	16.0	16.0
	Clindamycin	<0.12–>64	>64	>64
	Tetracycline	<0.12–>64	0.25	64.0
	Ciprofloxacin	0.12–1.0	0.5	0.5
	Penicillin	0.03–32	4.0	32.0
	Oxacillin	0.13–128	16.0	64.0
	Imipenem	<0.03–8.0	0.06	2.0
Staphylococcus epidermidis (20)	DuP 721	0.5–1.0	1.0	1.0
	DuP 105	4.0	4.0	4.0
	Vancomycin	2.0–4.0	2.0	2.0
	Chloramphenicol	4.0–>64	32.0	64.0
	Clindamycin	<0.12–>64	>64	>64
	Tetracycline	<0.12–>64	1.0	64.0
	Ciprofloxacin	0.12–0.25	0.12	0.25
	Penicillin	1.0–32.0	2.0	16.0
	Oxacillin	2.0–>128	8.0	>128
	Imipenem	1.0–>16	16	>16
Staphylococcus haemolyticus (16)	DuP 721	1.0–2.0	2.0	2.0
	DuP 105	8.0–16.0	8.0	8.0
	Vancomycin	1.0–4.0	2.0	4.0
	Chloramphenicol	4.0–>64	4.0	64.0
	Clindamycin	<0.12–>64	8.0	>64
	Tetracycline	1.0–>64	>64	>64
	Ciprofloxacin	0.12–0.25	0.25	0.25
	Imipenem	16.0–>16.0	>16	>16.0
Staphylococcus hominis (21)	DuP 721	1.0–4.0	2.0	2.0
	DuP 105	4.0–16.0	8.0	16.0
	Vancomycin	0.5–2.0	1.0	2.0
	Chloramphenicol	4.0–64.0	8.0	64.0
	Clindamycin	<0.12–>64	0.5	>64
	Tetracycline	<0.12–>64	0.5	64.0
	Ciprofloxacin	0.06–1.0	0.12	0.25
	Imipenem	<0.03–>16	<0.03	16.0
Streptococcus spp. group A (20)	DuP 721	0.5–2.0	1.0	1.0
	DuP 105	2.0–4.0	2.0	4.0
	Vancomycin	0.25–1.0	0.5	0.5
	Chloramphenicol	2.0–4.0	2.0	4.0
	Clindamycin	<0.06	<0.06	<0.06
	Erythromycin	<0.06–8.0	<0.06	<0.06
	Ciprofloxacin	0.25–2.0	0.5	0.5
	Penicillin	0.13	0.13	0.13
	Imipenem	<0.015	<0.015	<0.015
Streptococcus spp. group B (20)	DuP 721	0.5–2.0	1.0	2.0
	DuP 105	2.0–4.0	4.0	4.0
	Vancomycin	0.25–0.5	0.5	0.5
	Chloramphenicol	2.0	2.0	2.0
	Clindamycin	<0.12	<0.12	<0.12
	Erythromycin	<0.12	<0.12	<0.12
	Ciprofloxacin	0.5–1.0	0.5	1.0
	Imipenem	<0.03	<0.03	<0.03
Streptococcus spp. group D (20) (including *S. faecalis*)	DuP 721	2.0–4.0	2.0	4.0
	DuP 105	4.0–16.0	8.0	16.0
	Vancomycin	1.0–4.0	2.0	2.0
	Chloramphenicol	4.0–64.0	8.0	64.0
	Clindamycin	8.0–>64	16.0	>64
	Erythromycin	<0.12–>64	2.0	>64
	Ciprofloxacin	0.5–4.0	1.0	2.0
	Ampicillin	0.5–>16	1.0	1.0
	Imipenem	0.5–>16	0.5	2.0

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TABLE 1—*Continued*

Bacterium (no. of strains)	Drug	MIC (μg/ml)		
		Range	50%	90%
Bacteroides fragilis (10)	DuP 721	4.0	4.0	4.0
	DuP 105	16.0–32.0	16.0	16.0
	Cefoxitin	4.0–16.0	8.0	8.0
Bacteroides spp. (10) (including *B. melaninogenicus*, *B. corrodens*, *B. oralis*, and *B. distasonis*)	DuP 721	0.5–4.0	4.0	4.0
	DuP 105	4.0–32.0	16.0	16.0
	Cefoxitin	4.0–>100	>100	>100
Fusobacterium spp. (10)	DuP 721	0.5–1.0	0.5	1.0
	DuP 105	2.0–16.0	8.0	8.0
	Cefotaxime	2.0–8.0	4.0	8.0
Peptostreptococcus spp. (10)	DuP 721	0.25–1.0	0.5	1.0
	DuP 105	2.0–16.0	4.0	8.0
	Cefoxitin	0.13–4	2	4
Escherichia coli (21)	DuP 721	128–>128	>128	>128
	DuP 105	128–>128	>128	>128
	Gentamicin	0.25–16.0	1.0	1.0
	Cefotaxime	<0.03–0.25	0.13	0.13
Klebsiella spp. (10)	DuP 721	64–>128	>128	>128
	DuP 105	>128	>128	>128
	Gentamicin	0.5–2.0	0.5	1.0
	Cefotaxime	0.06–0.25	0.25	1.25
Enterobacter spp. (10)	DuP 721	>128	>128	>128
	DuP 105	>128	>128	>128
	Gentamicin	0.5–2.0	0.5	1.0
	Cefotaxime	0.6–>16	16	>16
Proteus spp. (10)	DuP 721	128	128	128
	DuP 105	128–>128	128	128
	Gentamicin	0.5–4.0	2.0	4.0
	Cefotaxime	0.25	0.25	0.25
Serratia spp. (10)	Dup 721	>128	>128	>128
	DuP 105	>128	>128	>128
	Gentamicin	1.0–16.0	8.0	16.0
	Cefotaxime	0.5–>16	1.0	2.0
Candida albicans ATCC 11651[a]	DuP 105	>100		
	Amphotericin B	0.1		
Candida albicans ATCC 10231[a]	DuP 105	>100		
	Amphotericin B	0.2		
Microsporum gypseum ATCC E9083[a]	DuP 105	>100		
	Griseofulvin	2.5		
Trichophyton rubrum ATCC 18756[a]	DuP 105	>100		
	Griseofulvin	1.3		

[a] MIC determined by the agar dilution method.

dissolved in acetone. For the in vivo tests, DuP 721, DuP 105, and the reference drugs were suspended in 0.25% Methocel (Dow Chemical Co., Midland, Mich.) in water and the suspensions were sonicated in a water bath at room temperature immediately prior to use. The desired dose was administered in a 0.2-ml volume by gavage or subcutaneous injection.

Susceptibility tests. MICs of the compounds for the various bacterial strains were determined by a standard agar dilution or a microtiter broth dilution assay (7). In the agar dilution test, Mueller-Hinton (MH) agar (BBL Microbiology Systems, Cockeysville, Md.) was used for the facultative anaerobic organisms; it was supplemented with 5% lysed horse blood to support the growth of beta-hemolytic streptococci. Wilkens Chalgren agar (Difco Laboratories, Detroit, Mich.) was used for the obligate anaerobic bacteria. A multispot inoculating device (Mast Lab Ltd., Bootley, Lancashire, United Kingdom) was used to inoculate 10^4 CFU per spot on agar plates containing twofold dilutions of the test compounds. The plates were incubated aerobically for 24 h at 35°C for the facultative anaerobic organisms and anaerobically (Coy chamber; Coy Laboratory Products, Ann Arbor,

TABLE 2. Effect of bacterial inoculum size on the in vitro activity of DuP 721, DuP 105, and three other antibacterial agents determined by the agar dilution method

Bacterium	Inoculum (CFU/ml)	MIC (μg/ml)				
		DuP 721	DuP 105	Oxacillin	Vancomycin	Ciprofloxacin
Staphylococcus aureus ATCC 29213	10^3	2.0	8.0	0.25	1.0	0.5
	10^5	4.0	32.0	0.25	1.0	16.0
	10^7	>64	>64	64.0	64.0	>64.0
Staphylococcus epidermidis (methicillin resistant)	10^3	1.0	2.0	>64	1.0	0.25
	10^5	4.0	16.0	>64	1.0	16.0
	10^7	>64	>64	>64	64.0	>16
Streptococcus faecalis	10^3	2.0	8.0	8.0	1.0	0.5
	10^5	4.0	16.0	16.0	2.0	16.0
	10^7	>64	>64	>64	>64	>16

Mich.) for 48 h at 37°C for the obligate anaerobic organisms. The MIC is defined as the lowest concentration of the compound which prevented visible growth.

In the microtiter broth dilution assay, an automated diluter (Pro/Pette; Cetus Instrument Systems, Emeryville, Calif.) was used to deliver serial twofold dilutions of the test compounds in MH broth in 96-well microtiter plates. An inoculum of 5×10^4 CFU was added to all wells; the final volume in each well was 100 μl. The microtiter plates were incubated for 24 h at 35°C, after which bacterial growth was assessed by using an immunoplate reader (MCC; Flow Laboratories, Inc., McLean, Va.) and the MICs were determined.

The effect of changes in the culture conditions on the activities of the oxazolidinones was assessed by determining their MICs for test organisms at different pHs in the presence of added serum (human, calf, or mouse; GIBCO Diagnostics, Madison, Wis.) and in various types of medium, including MH agar, Trypticase soy agar, yeast beef agar, neomycin assay agar, antibiotic assay agar no. 3, brain heart infusion agar, polymyxin seed agar (all except MH from BBL), and Sensitest agar (Oxoid, Columbia, Md.).

Effect of inoculum size. The effect of the inoculum size on the antibacterial activity of the compounds was evaluated by the agar dilution method by using inocula of 10^7, 10^5, and 10^3 CFU per spot.

Time-kill studies. The rates of killing of bacteria by the test compounds were examined by determination of the number of viable cells in cultures of *Staphylococcus aureus* and *Streptococcus faecalis* as a function of the time of exposure to the test compounds. At intervals, samples were withdrawn from the test cultures and 1-ml amounts of these or 1-ml amounts of serial dilutions in MH broth were spread on triplicate MH agar plates. After incubation at 35°C for 24 h, the numbers of CFU per milliliter in the cultures were determined. The highest concentration of the oxazolidinones in the test cultures was fourfold the MIC for the organism. The largest carry-over of compound occurred when 1-ml samples of these cultures were spread on the plates containing 20 to 22 g of MH agar. This resulted in a concentration of oxazolidinone of about 1/5 × MIC per g of agar, which did not inhibit bacterial growth. The lowest number of CFU in the test culture that could be detected was 30 CFU/ml.

Development of resistance. The propensity of selected gram-positive cocci to develop rapid resistance to DuP 721 or DuP 105 was evaluated by the method of Tenney et al. (10). The inoculum (10^9 CFU/ml) from MH agar, along with the test compounds at concentrations ranging from the MIC to fourfold the MIC, was added to tubes containing MH agar maintained at 45°C. After being mixed, the agar was poured into standard petri dishes and incubated for 24 h at 35°C. The plates were stained with a solution (0.05% in water) of 2-(*p*-iodophenyl)-3-*p*-nitrophenyl-5 phenyltetrazolium chloride (Sigma) to facilitate the detection of any small colonies, and the plates were incubated for a further 24 h at 35°C. After the second incubation, the number of bacterial colonies was determined.

Test for antifungal activity. MICs of DuP 105 and the reference drugs for strains of *Candida* and dermatophytic fungi were determined by an agar dilution assay. Suspensions of the test compounds were added to 20-ml portions of molten Eagle minimum essential medium agar (GIBCO) maintained at 50°C to achieve final concentrations of the compounds in the medium ranging from 100 to 0.04 μg/ml. After being mixed, the portions of agar medium were poured into petri dishes, and the agar was allowed to cool at room temperature. The agar plates were then inoculated with 10^4 spores of the various test organisms per spot by using the multispot inoculating device. The plates inoculated with the *Candida* strains were incubated at 30°C; the plates inoculated with the dermatophytic fungi were incubated at 20°C. The cultures with *Candida* strains and those with the dermatophytes were examined for growth at 2 and 7 days, respectively. The MIC is defined as the lowest concentration of the compound which prevented visible growth.

Acute lethal infections in mice. CF-1 female mice (Charles River Breeding Laboratories, Inc., Kingston, N.Y.) weighing 20 ± 2 g were used in all tests. The infecting inoculum was prepared from an overnight culture; the bacteria were washed with saline and resuspended in saline containing 5% porcine gastric mucin (Pflatz and Bauer, Stamford, Conn.). An inoculum of 2×10^8 CFU in 0.2 ml, representing fivefold the number required to kill all nontreated mice in 24 h, was injected intraperitoneally in each mouse. Graded doses of the test compounds were administered by the subcutaneous or oral route at 1 and 5 h postinfection; the infected nontreated control mice received injections of the vehicle (0.25% Methocel in water). A group of 10 mice was used for each dose level. The number of survivors in each group on day 7 after infection was used in the calculation of the drug dose that protects 50% of the mice (ED_{50}; 5).

Immunosuppressed mouse infection model. The mice were made neutropenic by the intraperitoneal administration of 100 mg of cyclophosphamide per kg on days −5, −3, and 0 (the day of infection). The extent of immunosuppression was assessed by using a standard hematological technique to determine leukocyte counts (Unopette; Becton Dickinson Vacutainer Systems, Rutherford, N.J.). In all experiments, the leukocyte count in the cyclophosphamide-treated mice was less than 15% of that in nontreated mice from day 0 through day 7. On day 0, the mice were injected intraperitoneally with a bacterial inoculum suspended in 0.2 ml of

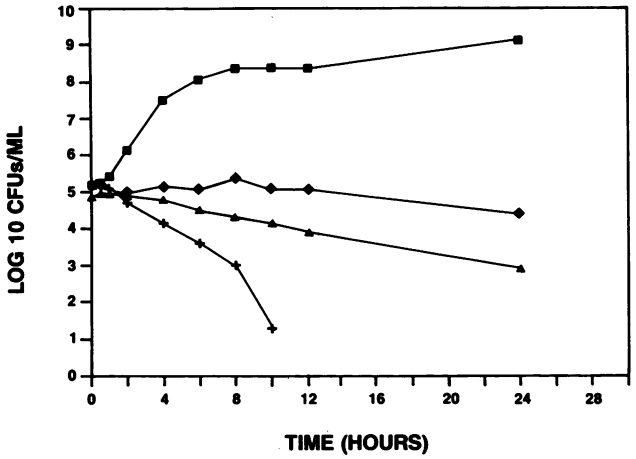

FIG. 2. Evaluation of the bactericidal activity of oxazolidinones against *S. aureus* 1A. ■, Control (no drug); ◆, DuP 721 at 8 μg/ml (4× MIC); ▲, DuP 105 at 32 μg/ml (4× MIC); +, vancomycin at 2 μg/ml (2× MIC).

saline containing 5% gastric porcine mucin. This inoculum (2×10^4 CFU) was fivefold that required to kill all the nontreated immunosuppressed mice in 48 h. Graded doses of the test compounds were administered by the subcutaneous or oral route at 1 and 4 h after infection. In parallel with the immunosuppressed mice, groups of nonimmunosuppressed mice were infected, as described above, and treated with the antibacterial drugs. The number of survivors on day 7 in each group was used to calculate the ED_{50} values of the test compounds.

Nonlethal kidney infection model. Mice were infected by the intravenous injection of a 0.1-ml suspension of 2×10^7 CFU of *S. aureus* 1A. Starting 1 h after infection, graded doses of the test compounds and reference drugs were administered by gavage or subcutaneous injection twice daily for 4 days. On day 5, the animals were sacrificed and the kidneys were removed. The kidneys were trimmed of fat tissue, weighed, and macerated in Tissue Mizers (Tekmar, Cincinnati, Ohio). The ground tissues were suspended in saline, and the suspensions were agitated (Vortexer 2; VWR Scientific, Philadelphia, Pa.) vigorously. Samples (0.1 ml) of dilutions of the suspensions were inoculated on Trypticase soy agar medium. The plates were incubated for 24 h at 35°C, and the CFUs were counted.

Disk implant model. The procedure of Grappel et al. (3) was used to assess the activity of the oxazolidinones against *Bacteroides fragilis* in mice. The CF-1 mice, female and weighing 20 ± 2 g, were immunosuppressed with cyclophosphamide as described above. Paper disks (5-mm diameter) inoculated with 10^7 CFU of *B. fragilis* derived from an actively growing culture of strain 65 and suspended in 0.5% carrageenan (Sigma) were implanted subcutaneously in the ventral region. After implantation of the disk, the incision was closed with surgical staples. Graded doses of the test compounds were administered starting at 1 and 5 h on the day of infection and twice daily for 4 consecutive days thereafter. On day 6, the animals were sacrificed, and the disks were removed and placed in 2 ml of MH broth. The suspensions were agitated vigorously (Vortexer 2), and samples (0.1 ml) of various dilutions were plated on Trypticase soy agar containing 5% sheep blood. The plates were incubated anaerobically (Coy anaerobic chamber) for 24 h at 35°C, and the number of CFU was determined. Drug efficacy was assessed by comparing the number of organisms on disks from treated mice with the number on disks from nontreated infected mice.

Statistical analysis of data. Differences between treatment groups in the kidney infection and disk implant models were evaluated by analysis of variance-randomized design. Statistical evaluations were performed by using the PC Statistician Program (Human Systems Dynamics, Northridge, Calif.).

Biological assay for DuP 105 and DuP 721. To determine levels in serum of the oxazolidinones, blood samples were collected by cardiac puncture at 5, 10, 15, 30, 45, 60, 120, 180, and 240 min following the administration of the compounds to nontreated CF-1 mice. The levels in serum were determined by a standard diffusion method (2) using stainless steel cylinders, antibiotic medium 11 (BBL), and *Micrococcus luteus* ATCC 9341 as the indicator organism. The concentration of the compound in serum was determined by use of a standard curve which relates compound concentration to the size (millimeters) of the zone of inhibition. All tests were conducted in triplicate, and the limits of detection were 8 μg/ml for DuP 105 and 1 μg/ml for DuP 721.

RESULTS

DuP 721 at concentrations of 1 to 4 μg/ml and DuP 105 at concentrations of 4 to 16 μg/ml inhibited the growth of all clinical isolates of staphylococci, streptococci, and *B. fragilis* isolates, including strains resistant to antibacterial drugs in clinical use. The compounds were equally potent against staphylococcal strains susceptible or resistant to β-lactam antibiotics. The oxazolidinones were more potent than penicillin, oxacillin, clindamycin, tetracycline, and chloramphenicol but less potent than vancomycin and ciprofloxacin against these bacteria.

Both oxazolidinones at concentrations of 1 to 4 μg/ml inhibited the growth of streptococci belonging to Lancefield

TABLE 3. In vitro activity of DuP 721, DuP 105, and selected antibiotics against bacteria used in mouse infection studies determined by the microtiter broth dilution method

Bacterium	MIC (μg/ml)						
	DuP 721	DuP 105	Vancomycin	Gentamicin	Cephalexin	Cefoxitin	Metronidazole
Staphylococcus aureus							
1A	2	8	1	0.25	8		
46A	2	8	1				
267	4	16	2				
148	4	16	1				
Streptococcus faecalis 19	2	8	1				
Bacteroides fragilis 65	4	16				8	0.25

TABLE 4. In vivo activity of DuP 721, DuP 105, and vancomycin against selected strains of staphylococci and streptococci in an acute lethal mouse model

Bacterium	ED_{50} (mg/kg)[a] (95% confidence limits)				
	DuP 721		DuP 105		Vancomycin, s.c.
	s.c.	p.o.	s.c.	p.o.	
Staphylococcus aureus					
1A	3.8 (2.9–4.5)	5.1 (3.6–7.6)	9.0 (6.6–12.0)	16.6 (12.0–22.6)	3.3 (2.5–4.3)
46A (methicillin resistant)	2.2 (1.3–3.2)	2.8 (1.7–4.1)	11.9 (8.3–20.7)	12.7 (9.1–19.9)	4.9 (3.5–6.3)
267 (Smith)	6.1 (4.3–8.6)	6.6 (5.0–8.6)	16.3 (11.0–25.5)	15.1 (11.6–19.9)	1.2 (0.9–1.6)
148	10.0 (5.6–13.3)	5.8 (4.3–7.7)	17.3 (13.03–22.37)	22.8 (16.7–33.5)	6.8 (5.5–9.07)
Streptococcus faecalis STCO 19	2.5 (1.6–3.5)	3.1 (2.3–4.1)	6.3 (3.0–8.8)	14.1 (11.0–18.1)	11.8 (5.0–15.9)

[a] Dose given at 1 and 5 h. s.c., Subcutaneous; p.o., oral.

groups A and B, and at concentrations of 4 to 16 μg/ml, they both inhibited group D streptococci, including *S. faecalis* strains. Against these organisms, DuP 721 and DuP 105 were more potent than erythromycin, clindamycin, and chloramphenicol; they were less potent than ampicillin, imipenem, vancomycin, and ciprofloxacin.

DuP 721 at concentrations of 0.5 to 4 μg/ml and DuP 105 at concentrations of 4 to 32 μg/ml inhibited strains of *Peptostreptococcus*, *Fusobacterium*, and *Bacteroides* spp. The compounds were not active against gram-negative facultative anaerobic bacteria or against strains of *Candida* and dermatophytic fungi (Table 1).

The effect of changes in culture conditions on the antibacterial activity of the oxazolidinones was determined. The type of medium used, the addition of 25% human, mouse, or calf serum to MH medium, and variations in the pH of the medium between pH 5 and 8 had no significant effect on the antibacterial activity (MICs) of DuP 721 or DuP 105 against four strains of *S. aureus* and one of *S. faecalis*.

Increases in the size of the inoculum from 10^3 to 10^5 CFU/ml caused two- to fourfold and four- to eightfold increases in the MICs of DuP 721 and DuP 105, respectively, against strains of staphylococci and *S. faecalis*. When the inoculum size was increased from 10^5 to 10^7 CFU/ml, the activities of both DuP 721 and DuP 105 were significantly reduced; this decrease of activity also occurred with other antibacterial agents (Table 2).

In time-kill studies with *S. aureus* 1A, DuP 721 at a concentration of 8 μg/ml (fourfold the MIC) or DuP 105 at a concentration of 32 μg/ml (fourfold the MIC) caused a slight decrease in the bacterial population from 10^5 to 10^4 CFU/ml in a period of 24 h. Vancomycin at a concentration of 2 μg/ml (twofold the MIC) caused a decrease from 10^5 to 10^2 CFU/ml in 8 h (Fig. 2). Time-kill studies were also conducted with DuP 721 and DuP 105 against two additional strains of *S. aureus* (strains 46A and 148), against one strain of *Staphylococcus epidermidis* (SFCO 315) and two strains of *S. faecalis* (STCO 19 and STCO 140). The results show that the compounds were bacteriostatic.

Attempts were made to select mutants resistant to the oxazolidinones in a single-step selection process. No resistant colonies emerged when inocula of 10^9 CFU of several staphylococcal strains were applied to MH agar plates containing graded concentrations (1×, 2×, and 4× the MIC) of DuP 721 or DuP 105. The strains tested included five clinical isolates of *S. aureus*, six isolates of *S. epidermidis*, and six isolates of *Staphylococcus haemolyticus*.

In acute lethal mouse protection tests, both DuP 721 and DuP 105 were active against infections caused by strains of staphylococci and streptococci. The MICs of the test compounds against the strains are shown in Table 3. Against infections produced by *S. aureus*, including strains susceptible or resistant to β-lactam antibiotics, and by group D streptococci, the ED_{50}s of DuP 721 and DuP 105 were of the same order as those of vancomycin (Table 4).

DuP 721 and DuP 105 were also effective against infections produced by *S. aureus* in immunosuppressed mice, although the ED_{50}s of the compounds were about three to four times larger in the immunosuppressed than in the normal mice. With vancomycin, the ED_{50} in immunosuppressed mice was the same as that in immunocompetent mice (Table 5).

In the nonlethal *S. aureus* mouse kidney infection model, the administration of DuP 721 or DuP 105 resulted in a reduction in the number of organisms in the kidneys compared with that in the nontreated infected control mice. The reduction in the number of organisms achieved by DuP 721 or DuP 105 was similar to that achieved by vancomycin or cephalexin but less than that produced by treatment with gentamicin (Table 6).

In the *B. fragilis* disk implant mouse model, DuP 721 at doses of 50 mg/kg significantly reduced the bacterial population on the disks compared with the numbers of bacteria on the disks from nontreated control mice. DuP 721 was more effective than cefoxitin, which had little efficacy in reducing the bacterial population. Metronidazole at a dose of 25 mg/kg achieved a significant reduction in the number of bacteria on the implanted disks; DuP 105 was not effective at doses up to 100 mg/kg (Table 7).

Pharmacokinetic determinations in mice indicated that the peak drug levels in serum were achieved at 30 min after oral administration of the oxazolidinones. Doses of 50 mg of DuP 721 or DuP 105 per kg resulted in a peak level in serum of 30 μg/ml; this concentration is in excess of the MIC for 90% of the *Staphylococcus* and *Streptococcus* strains (Table 8).

TABLE 5. In vivo activity of DuP 721, DuP 105, and vancomycin against *S. aureus* 1A in an acute lethal mouse model with immunocompetent and immunosuppressed mice

Drug	Route of administration[a]	ED_{50} (mg/kg)[b] (95% confidence limits)	
		Immunocompetent	Immunosuppressed
DuP 721	s.c.	3.8 (2.9–4.5)	17.0 (11.0–25.2)
DuP 721	p.o.	5.1 (3.6–7.6)	17.6 (12.6–25.2)
DuP 105	s.c.	9.0 (6.6–12.0)	37.5 (21.5–109.3)
DuP 105	p.o.	16.6 (12.0–22.6)	32.7 (14.8–46.8)
Vancomycin	s.c.	3.3 (2.5–4.3)	3.1 (2.2–4.3)

[a] s.c., Subcutaneous; p.o., oral.

[b] Immunocompetent mice were infected with 10^8 CFU per mouse, and immunosuppressed mice were infected with 10^4 CFU per mouse.

TABLE 6. In vivo activity of DuP 721, DuP 105, gentamicin, vancomycin and cephalexin in the *S. aureus* 1A nonlethal mouse kidney infection model

Drug	Dose (mg/kg)[a]	Route of administration[b]	No. of bacteria in kidneys (log_{10} CFU/g)[c]
None			9.95 ± 0.6
DuP 721	50	s.c.	7.8 ± 0.4[d]
	12.5	s.c.	8.0 ± 0.1[d]
	50	p.o.	7.9 ± 0.1[d]
	12.5	p.o.	8.3 ± 0.1[d]
DuP 105	100	s.c.	8.4 ± 0.5[d]
	100	p.o.	8.1 ± 0.4[d]
Gentamicin	50	s.c.	5.5 ± 0.1[d]
	12.5	s.c.	7.5 ± 0.1[d]
Vancomycin	50	s.c.	7.7 ± 0.1[d]
Cephalexin	50	p.o.	7.4[d]

[a] Dose was given at 1 and 5 h on day 0 and twice daily for a total of 4 days.
[b] s.c., Subcutaneous; p.o., oral.
[c] Numbers were determined or day 5 after infection.
[d] Significantly different from controls at $P \leq 0.05$ by analysis of variance-randomized t test.

DISCUSSION

DuP 105 and DuP 721 are members of a new class of synthetic antibacterial agents, the oxazolidinones. Their spectrum of activity includes primarily gram-positive bacteria and gram-negative anaerobic organisms. DuP 721 at concentrations of 0.5 to 4 μg/ml and DuP 105 at 4 to 32 μg/ml inhibited all clinical isolates tested, including strains of *S. aureus*, *S. epidermidis*, *Streptococcus* groups A, B, and D, and *B. fragilis*. The compounds were active against strains resistant to available antibiotics, including β-lactamase-positive and methicillin-resistant staphylococci. The oxazolidinones are bacteriostatic, and preliminary studies indicate that their primary action is on protein synthesis. The compounds appear to act by a novel mechanism, since unlike most clinically used antibacterial agents that inhibit protein synthesis, the oxazolidinones do not inhibit the peptide elongation step (D. C. Eustice and A. M. Slee, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 246, 1987). Attempts to select mutants resistant to DuP 105 or DuP 721 by a single-step selection process indicate that bacteria resistant to these oxazolidinones occurred at a frequency of less than 1 in 10^9 bacteria. The oxazolidinoneswere as effective as vancomycin in protecting mice against acute lethal infections produced by *S. aureus* strains susceptible or resistant to penicillin or by *S. faecalis* strains. The compounds were also effective against infections in immunosuppressed mice, although the doses of compounds required were three- to fourfold greater than those required to protect the immunocompetent mice. Preliminary pharmacokinetic studies in mice indicate that drug levels in blood that exceed the MICs for staphylococci, streptococci, and *B. fragilis* can readily be achieved. A dose of 50 mg of DuP 721 or DuP 105 per kg provides a peak drug level in serum of 30 μg/ml, which for DuP 721 is at least eightfold and for DuP 105 about twofold the MIC_{90}s for staphylococci and streptococci (I. Zajac, G. N. Lam, H. E. Hoffman, and A. M. Slee, 27th ICAAC, abstr. no. 247, 1987). Infections caused by staphylococci resistant to available antibiotics are on the increase, and there is a need for new agents. The studies reported here suggest that members of the new oxazolidinone series may have potential utility in the control of infections caused by certain gram-positive pathogens, especially those resistant to clinically used antibacterial agents.

TABLE 7. Activity of DuP 721, DuP 105, cefoxitin, and metronidazole in a *B. fragilis* disk implant mouse model

Drug	Dose (mg/kg)[a]	Route administration[b]	No. of bacteria on disk (log_{10} CFU/disk)[c]
None			8.0 ± 0.1
DuP 721	50	s.c.	5.5 ± 0.1[d]
DuP 105	100	s.c.	7.2 ± 0.1
Cefoxitin	50	s.c.	7.3 ± 0.2
Metronidazole	25	p.o.	2.5 ± 0.1[d]

[a] Dose given at 1 and 5 h on day 0 and twice daily for a total of 4 days.
[b] s.c., Subcutaneous; p.o., oral.
[c] Numbers were determined on day 5 after infection. Inoculum, 10^7 bacteria per disk.
[d] Significantly different from nontreated controls at $P < 0.05$ by analysis of variance-randomized t test.

TABLE 8. Pharmacokinetic profile of DuP 721 and DuP 105 in mice

Drug	Oral dose (mg/kg)	Peak concn in serum (μg/ml) at 30 min	$t_{1/2}$ (min)[a]
DuP 721	100	50	
	50	30	50
	12.5	5	
DuP 105	100	64	30
	50	32	

[a] $t_{1/2}$, Half-life.

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