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In Vitro Activities of Oxazolidinones U-100592 and U-100766 against Penicillin-Resistant and Cephalosporin-Resistant Strains of *Streptococcus pneumoniae*

EDWARD O. MASON, JR.,[1,2,3]* LINDA B. LAMBERTH,[3] AND SHELDON L. KAPLAN[1,3]

Departments of Pediatrics[1] and Microbiology and Immunology,[2] Baylor College of Medicine, and Infectious Disease Laboratory, Texas Children's Hospital,[3] Houston, Texas

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Two oxazolidinones and ceftriaxone, imipenem, rifampin, and vancomycin were tested against 162 penicillin-intermediate and 68 penicillin-resistant strains of pneumococci. U-100592 is two- to fourfold more active than U-100766 against penicillin-resistant pneumococci. The MICs of U-100592 at which 90% of the isolates were inhibited were 0.25 and 0.5 μg/ml for penicillin-intermediate and -resistant strains, respectively, and 0.5 μg/ml for ceftriaxone-susceptible, -intermediate, and -resistant strains. U-100592 MICs for 7 of 230 strains (2 from blood, 3 from middle-ear fluid, and 2 from the upper respiratory tract) were 1 μg/ml.

Streptococcus pneumoniae resistance to penicillin and other antimicrobial agents, including the extended-spectrum cephalosporins, is a recognized therapeutic problem worldwide (7). Optimal therapy of infections caused by *S. pneumoniae* now requires confirmation of susceptibility by quantitative methods rather than past assumptions of universal susceptibility (10). It is becoming increasingly common in many areas to include vancomycin in the initial empiric treatment of meningitis, usually an extended-spectrum cephalosporin such as cefotaxime or ceftriaxone (5, 6, 8). Further, isolates from the middle ears of children with chronic otitis media are often resistant to all but parenterally administered antibiotics, making outpatient treatment of these infections difficult (1, 3). Currently, vancomycin is the only available antibiotic universally active against multiple-antibiotic-resistant *S. pneumoniae*. Thus, identifying antibiotics with activity against *S. pneumoniae* remains particularly important. A new class of antibiotics, the oxazolidinones, appear to be potentially effective agents against many of the gram-positive bacteria (2). We investigated the activities of U-100592 and U-100766 in comparison with those of several other antibiotics against penicillin- and cephalosporin-resistant *S. pneumoniae* strains isolated from children.

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Two hundred thirty strains of *S. pneumoniae* isolated from children at Texas Children's Hospital between October 1987 and November 1994 were identified as resistant to penicillin. Penicillin-intermediate strains (162 strains) were defined as those for which the penicillin MIC was between 0.1 and 1.0 μg/ml. Highly penicillin-resistant strains (68 strains) were defined as those for which the penicillin MIC was ≥2.0 μg/ml. Strains were defined as susceptible to ceftriaxone if the MIC was ≤0.5 μg/ml, as intermediate if the MIC was 1.0 μg/ml, and resistant if the MIC was >2.0 μg/ml (11).

MICs were determined by the agar dilution method by a modification of the methodology of the National Committee for Clinical Laboratory Standards (11). Dilutions of antibiotics were incorporated into plates containing cation-supplemented Mueller Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 3% lysed horse blood (final concentration). The inoculum was prepared from overnight growth on sheep blood agar and adjusted to a turbidity equal to a 0.5 McFarland standard. A 1:10 dilution of this preparation with Mueller-Hinton broth reliably resulted in an inoculum of 3×10^4 to 6×10^4 CFU per replicator spot, as determined by quantitative culture. Plates were incubated at 35°C for 20 to 22 h without CO_2. *S. pneumoniae* ATCC 49619, for which the penicillin MIC was 0.25 μg/ml, was used as a control.

Strains were tested for susceptibility to penicillin (Bristol-Myers Squibb Co., Evansville, Ind.), ceftriaxone (Roche Laboratories, Nutley, N.J.), imipenem (Merck, Sharpe & Dohme, West Point, Pa.), rifampin (Marion Merrel Dow, Kansas City, Mo.), vancomycin (Eli Lilly & Co., Indianapolis, Ind.), and the two oxazolidinones U-100592 and U-100766 (Pharmacia & Upjohn, Kalamazoo, Mich.). Standard powders provided by the manufacturers were used to prepare doubling dilutions from 0.06 to 32 μg/ml.

The oxazolidinone U-100592 was two- to fourfold more active than U-100766 against intermediately and fully resistant pneumococci in vitro. The MIC of U-100592 ranged between 0.06 and 1.0 μg/ml for strains intermediately resistant to penicillin and between 0.125 and 1.0 μg/ml for strains for which penicillin MICs were ≥2.0 μg/ml (Table 1). The MICs at which 90% of the isolates were inhibited (MIC_{90}s) for intermediately penicillin-resistant strains were 0.25 and 1.0 μg/ml for U-100592 and U-100766, respectively. The MIC_{90}s for penicil-

TABLE 1. MIC_{50}, MIC_{90}, and MIC range for intermediate and highly penicillin-resistant *S. pneumoniae* strains

Antibiotic	MIC (μg/ml) for strains:					
	Penicillin intermediate (n = 162)			Penicillin resistant (n = 68)		
	50%	90%	Range	50%	90%	Range
U-100592	0.25	0.25	0.06–1.0	0.25	0.50	0.125–1.0
U-100766	0.50	1.00	0.06–2.0	1.00	1.00	0.25–4.0
Ceftriaxone	0.25	0.50	0.06–1.0	1.00	2.00	0.25–8.0
Imipenem	0.125	0.25	0.06–1.0	0.50	2.00	0.06–2.0
Rifampin	0.06	0.06	0.06	0.06	0.06	0.06
Vancomycin	0.25	0.50	0.06–1.0	0.25	0.50	0.25–1.0

* Corresponding author. Mailing address: Infectious Diseases MC 3-2371, Texas Children's Hospital, 6621 Fannin, Houston, TX 77030.

TABLE 2. MIC_{50}, MIC_{90}, and MIC range for susceptible, intermediate, and highly ceftriaxone-resistant *S. pneumoniae* strains

Antibiotic	MIC (μg/ml) for strains:								
	Ceftriaxone susceptible (n = 177)			Ceftriaxone intermediate (n = 37)			Ceftriaxone resistant (n = 16)		
	50%	90%	Range	50%	90%	Range	50%	90%	Range
U-100592	0.25	0.50	0.06–1.0	0.25	0.50	0.125–1.0	0.25	0.50	0.125–0.5
U-100766	0.50	1.00	0.06–4.0	1.00	1.00	0.25–2.0	0.50	1.00	0.25–1.0
Imipenem	0.125	0.50	0.06–2.0	0.25	1.00	0.06–2.0	1.00	2.00	0.125–2.0
Rifampin	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Vancomycin	0.25	0.50	0.06–1.0	0.25	0.50	0.25–0.5	0.50	0.50	0.25–0.5

lin-resistant strains were essentially identical for the two compounds (MIC_{90} = 0.5 μg/ml for U-100592 and MIC_{90} = 1.0 μg/ml for U-100766). Both intermediate and resistant strains were susceptible to rifampin and vancomycin. Ceftriaxone MICs ranged from 0.06 to 1.0 μg/ml (MIC_{90} = 0.5 μg/ml) for penicillin-intermediate strains and between 0.25 and 8.0 μg/ml (MIC_{90} = 2.0 μg/ml) for penicillin-resistant strains.

Table 2 shows the susceptibilities to the various antibiotics when the strains were categorized by ceftriaxone resistance. U-100592 and U-100766 MIC ranges for strains classified as susceptible (MIC ≤ 0.5 μg/ml), intermediate (MIC = 1 μg/ml), or resistant (MIC ≥ 2.0 μg/ml) were similar. Imipenem MICs ranged from 0.06 to 2.0 μg/ml for ceftriaxone-susceptible and -intermediate strains and from 0.125 to 2.0 μg/ml for ceftriaxone-resistant strains. All strains were susceptible to rifampin and vancomycin regardless of ceftriaxone susceptibility.

Two of 66 blood isolates, 3 of 92 middle-ear isolates, and 2 of 44 upper respiratory tract isolates required a maximal concentration of U-100592 of 1.0 μg/ml for inhibition. In contrast, 82 isolates from the same anatomic sites required 1.0 μg of U-100766 per ml for inhibition. The MIC of U-100766 for one blood isolate was 2.0 μg/ml, and that for one upper respiratory isolate was 4 μg/ml.

Reproducible MICs that varied by only 1 dilution were recorded for both investigational compounds by using the *S. pneumoniae* (ATCC 49619) quality control strain. The median MIC of U-100592 was 0.5 μg/ml (coefficient of variation = 0.12; range, 0.25 to 0.5 μg/ml), and the median value for U-100766 was 1 μg/ml (coefficient of variation = 0.24; range, 0.5 to 1 μg/ml).

The exact clinical significance of penicillin-resistant pneumococci causing infections outside the central nervous system remains unknown (13). Freidland has recently reported that beta-lactam therapy is effective in the treatment of nonmeningeal infections in children caused by intermediately resistant *S. pneumoniae* (4). This study does not address the issue of systemic infections caused by resistant pneumococci (MIC > 1.0 μg/ml) or infections in the middle ear caused by either intermediate or resistant strains. Therapy for meningitis and otitis media caused by strains resistant to the extended-spectrum cephalosporins or multiply resistant to other antibiotics does, however, present a significant problem (10). Until an effective protein-conjugated multivalent pneumococcal vaccine is available, development of new antibiotics remains the only alternative in dealing with these infections.

The oxazolidinones are a new class of antibiotics with activity against aerobic and anaerobic gram-positive bacteria (2). Antibiotic activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci has been demonstrated (14). Our study demonstrates that, in vitro, the oxazolidinones U-100592 and U-100766 at MICs of ≤4.0 μg/ml are active against strains of pneumococci that are resistant to penicillin and ceftriaxone. Intravenous infusion of 10 mg/kg of body weight in animals (rats and dogs) resulted in peak serum drug levels of 20.78 and 25.06 μg/ml (9). Pharmacokinetic parameters for a limited number of human subjects indicate that peak U-100592 concentrations in serum following oral doses of 1.5 and 2.0 g were 7.02 and 9.78 μg/ml respectively (12). The usefulness of either agent will depend on further determination of the achievable concentrations in serum under therapeutic conditions, followed by clinical assessment of efficacy in treating pneumococcal infections.

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