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Antipneumococcal Activity of BAY 12-8039, a New Quinolone, Compared with Activities of Three Other Quinolones and Four Oral β-Lactams

MELISSA A. VISALLI,[1] MICHAEL R. JACOBS,[2] AND PETER C. APPELBAUM[1]*

Department of Pathology (Clinical Microbiology), Hershey Medical Center, Hershey, Pennsylvania 17033,[1] and Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106[2]

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Activities of BAY 12-8039 against 205 pneumococci were tested by agar dilution. MICs (in micrograms per milliliter) at which 50 and 90% of the isolates are inhibited (MIC_{50}s and MIC_{90}s, respectively) were 0.125 and 0.25 (BAY 12-8039), 2.0 and 4.0 (ciprofloxacin and ofloxacin), and 0.25 and 0.5 (sparfloxacin). β-Lactam MIC_{50}s and MIC_{90}s for penicillin-susceptible, -intermediate, and -resistant strains, in that order, were 0.016 and 0.03, 0.25 and 2.0, and 2.0 and 4.0 (amoxicillin); 0.03 and 0.06, 0.25 and 4.0, and 4.0 and 8.0 (ampicillin); 0.03 and 0.06, 0.5 and 4.0, and 4.0 and 8.0 (cefuroxime); and 0.03 and 0.125, 0.25 and 2.0, and 4.0 and 8.0 (cefpodoxime). At two times their MICs after 24 h, BAY 12-8039, ciprofloxacin, ampicillin, and cefuroxime were uniformly bactericidal (99.9% killing) against 12 strains; other compounds were bactericidal at four times their MICs.

The incidence of pneumococci resistant to penicillin G and other β-lactam and non-β-lactam compounds has increased at an alarming rate (1). The problem is exacerbated by the tendency of these organisms to spread from country to country and from continent to continent (14, 15). In the United States a recent survey has shown an increase in penicillin resistance from <5% before 1989 (<0.02% of isolates for which MICs are ≥2.0 μg/ml) to 6.6% in 1991 to 1992 (1.3% of isolates for which MICs are ≥2.0 μg/ml) (3). In another recent survey, 23.6% of pneumococci were not susceptible to penicillin (5). High rates of isolation of penicillin-intermediate and -resistant pneumococci occur in middle ear fluids from patients with refractory otitis media (2).

There is a need of oral compounds for treatment of otitis media, sinusitis, bronchitis, and community-acquired pneumonia caused by penicillin-intermediate and -resistant pneumococci (7, 8, 11, 12). Quinolones such as ciprofloxacin and ofloxacin yield moderate in vitro activities against pneumococci (11, 12, 17, 18, 21, 22).

BAY 12-8039 is a new experimental broad-spectrum 8-methoxyquinolone with activities against gram-positive and -negative aerobes, anaerobes, chlamydiae, *Mycoplasma* spp., and *Legionella* spp. (4, 6, 9, 10, 13, 25). This study employs standard agar dilution MIC methodology to test the activities of BAY 12-8039, ciprofloxacin, ofloxacin, sparfloxacin, ampicillin, amoxicillin, cefuroxime, and cefpodoxime against 205 penicillin-susceptible and -resistant pneumococci. Additionally, the activities of these compounds against 12 penicillin-susceptible and -resistant pneumococci were investigated by broth dilution (MIC) and time-kill analyses.

To obtain MICs by agar dilution, we used 53 penicillin-susceptible (MICs, ≤0.06 μg/ml), 76 penicillin-intermediate (MICs, 0.125 to 1.0 μg/ml), and 76 penicillin-resistant (MICs, 2.0 to 16.0 μg/ml) pneumococcal strains. For time-kill studies, four penicillin-susceptible, four penicillin-intermediate, and four penicillin-resistant strains were tested. Agar dilution was performed as described previously (11). Broth dilutions to obtain MICs for 12 strains were performed according to National Committee for Clinical Laboratory Standards recommendations (16). Standard quality-control strains were included in each run of agar and broth dilutions (16).

For time-kill testing, tubes containing 5 ml of cation-adjusted Mueller-Hinton broth plus 5% lysed horse blood with doubling antibiotic concentrations were inoculated with 5×10^5 to 5×10^6 CFU/ml and incubated at 35°C in a shaking water bath (17, 20). Tubes were vortexed, and the contents were plated for viability counts. The original inoculum was determined by using the untreated growth control (17, 20). Viability counts of antibiotic-containing suspensions were performed at 0, 3, 6, 12, and 24 h, by plating dilutions onto 5% sheep blood agar plates. The lower limit of sensitivity of colony counts was 300 CFU/ml (17, 20).

Time-kill results were analyzed by determining the number of strains which yielded a change in $\log_{10}$ CFU per milliliter of −1, −2, or −3 at 0, 3, 6, 12, and 24 h, compared to counts at time zero. Antimicrobials were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥3 $\log_{10}$ CFU/ml (99.9%) at each of the time periods, and they were considered bacteriostatic if the inoculum was reduced by 0 to 3 $\log_{10}$ CFU/ml. With the sensitivity threshold and inocula used in these studies, no problems were encountered in delineating 99.9% killing, when this was present. Bacterial carryover was addressed as described previously (20).

Results of MIC testing are presented in Table 1. MICs of quinolones were independent of those of penicillin G. BAY 12-8039 yielded the lowest MICs of the quinolones tested (its MICs at which 50 and 90% of isolates are inhibited [MIC_{50} and MIC_{90}, respectively] were 0.125 and 0.25 μg/ml), with all strains being inhibited by 0.5 μg/ml. Sparfloxacin was the next-most-active quinolone, with a MIC_{50} and a MIC_{90} of 0.25 and 0.5 μg/ml, respectively, followed by ciprofloxacin and ofloxacin (both of which had a MIC_{50} and a MIC_{90} of 2.0 and 4.0 μg/ml, respectively). MICs of β-lactams rose with those of penicillin

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelba@psuhmc.hmc.psu.edu.

TABLE 1. MICs obtained by agar dilution of individual agents for 205 pneumococcal strains

Compound	Type of strain[a]	MIC (μg/ml)[b]		
		Range	50%	90%
Penicillin G	S	0.016–0.06	0.03	0.06
	I	0.125–1.0	0.5	1.0
	R	2.0–16.0	4.0	8.0
BAY 12-8039	S	0.06–0.5	0.125	0.25
	I	0.06–0.5	0.125	0.25
	R	0.06–0.5	0.125	0.25
Ciprofloxacin	S	0.5–8.0	2.0	4.0
	I	0.5–8.0	2.0	8.0
	R	0.5–8.0	2.0	4.0
Ofloxacin	S	1.0–8.0	2.0	4.0
	I	1.0–8.0	2.0	4.0
	R	1.0–8.0	2.0	4.0
Sparfloxacin	S	0.06–2.0	0.25	0.5
	I	0.125–1.0	0.25	0.5
	R	0.125–1.0	0.5	0.5
Amoxicillin	S	0.008–0.125	0.016	0.03
	I	0.008–4.0	0.25	2.0
	R	0.125–8.0	2.0	4.0
Ampicillin	S	0.03–0.125	0.03	0.06
	I	0.03–4.0	0.25	4.0
	R	0.5–16.0	4.0	8.0
Cefuroxime	S	0.008–0.25	0.03	0.06
	I	0.03–8.0	0.5	4.0
	R	0.5–16.0	4.0	8.0
Cefpodoxime	S	0.03–0.25	0.03	0.125
	I	0.03–4.0	0.25	2.0
	R	0.5–32.0	4.0	8.0

[a] S, penicillin susceptible; I, penicillin intermediate; R, penicillin resistant.
[b] 50% and 90%, MIC_{50}s and MIC_{90}s, respectively.

G. Amoxicillin was the most active β-lactam, followed by ampicillin, cefuroxime, and cefpodoxime. MIC_{50} and MIC_{90} values (in micrograms per milliliter) of β-lactams for penicillin-susceptible, -intermediate, and -resistant strains (in that order) were 0.016 and 0.03, 0.25 and 2.0, and 2.0 and 4.0 (amoxicillin); 0.03 and 0.06, 0.25 and 4.0, and 4.0 and 8.0 (ampicillin); 0.03 and 0.06, 0.5 and 4.0, and 4.0 and 8.0 (cefuroxime); and 0.03 and 0.125, 0.25 and 2.0, and 4.0 and 8.0 (cefpodoxime).

MICs obtained by broth dilution for the 12 strains studied by time-kill analysis are listed in Table 2. MICs of individual strains were within one dilution of those obtained by agar dilution. Time-kill analysis (Table 3) showed that, at two times the MIC after 24 h, BAY 12-8039, ciprofloxacin, ampicillin, and cefuroxime were bactericidal (99.9% killing) against all strains; other compounds were bactericidal at four times the MIC. After 12 h, BAY 12-8039 showed 90% killing of all strains at the MIC and 99% killing at two times the MIC; after 6 h, it showed 99% killing of all strains at two times the MIC; and after 3 h, it showed 90% killing of all strains at two times the MIC. Similar results relative to the MICs were obtained for ciprofloxacin, ofloxacin, and sparfloxacin. After 12 h, amoxicillin showed 90% killing of all strains at two times the MIC and 99% killing at four times the MIC; after 6 h, it showed 90% killing of all strains at four times the MIC; and after 3 h, it showed 90% killing of all strains at eight times the MIC. Kill patterns of all β-lactams were similar, relative to their respective MICs. However, quinolones killed strains slightly more rapidly than β-lactams after 3 and 6 h.

TABLE 2. MICs obtained by broth dilution of agents for 12 pneumococcal strains

Drug	MIC or MIC range for four pneumococcal strains of type[a]:		
	S	I	R
Penicillin G	0.03	0.125–0.5	2.0–4.0
BAY 12-8039	0.125–0.25	0.25	0.125–0.25
Ciprofloxacin	1.0–2.0	2.0–4.0	1.0–4.0
Ofloxacin	2.0–4.0	2.0–4.0	2.0–4.0
Sparfloxacin	0.25–1.0	0.5–1.0	0.25–0.5
Amoxicillin	0.016–0.03	0.03–0.5	2.0
Ampicillin	0.03	0.06–1.0	2.0–4.0
Cefuroxime	0.03	0.125–1.0	2.0–4.0
Cefpodoxime	0.03	0.125–2.0	2.0–4.0

[a] S, penicillin susceptible; I, penicillin intermediate; R, penicillin resistant.

BAY 12-8039 is a new broad-spectrum methoxyquinolone (4, 6, 9, 10, 13, 25). Other fluoroquinolones with expanded gram-positive coverage which are currently being developed or are in use are levofloxacin, sparfloxacin, grepafloxacin, gatifloxacin, clinafloxacin, trovafloxacin, and DU-6859a (12, 17, 18, 20–24). MICs of levofloxacin range between 0.5 and 2 μg/ml, while those of grepafloxacin, sparfloxacin, and gatifloxacin are similar (0.125 to 0.5 μg/ml) for these strains (18, 21–23). MICs of clinafloxacin and DU-6859a are a few dilutions lower than those of the last two compounds (18, 24). MICs of trovafloxacin are similar to those of BAY 12-8039 (18). The superior antipneumococcal activity of amoxicillin compared to those of other oral β-lactam agents has been described before (19).

MIC and time-kill data of BAY 12-8039 for pneumococci are similar to those published previously from studies of smaller numbers of strains (4, 25). With an oral dosage regimen of 400 mg daily, a maximum concentration of the drug in serum of 3.2 μg/ml has been obtained in humans; the time to maximum concentration of the agent in serum is 1 to 2 h, and its half-life is approximately 12 h (13).

Dalhoff et al. have shown BAY 12-8039 to have concentration-dependent killing throughout all concentrations studied, whereas the bactericidal effects of ciprofloxacin, ofloxacin, and sparfloxacin were concentration dependent only up to 0.25 to 1 times the MIC. Although MICs of BAY 12-8039 and sparfloxacin differed by a factor of 2, killing rates were qualitatively and quantitatively different. Resistance to BAY 12-8039 developed less frequently than resistance to ciprofloxacin in pneumococci (4). Protein binding of BAY 12-8039 (<50%) is similar to that of many quinolones but less than that of trovafloxacin (85%) (25).

The results of our study indicate good in vitro antipneumococcal activity of BAY 12-8039, with MICs well within achievable levels in serum. If no significant toxicologic results are found, and pending favorable results of pharmacokinetic and in vivo efficacy studies, this compound will be a promising agent for outpatient treatment of pneumococcal respiratory tract infections in areas with a high incidence of penicillin-resistant strains.

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TABLE 3. Time-kill results

Drug	Dose	No. of strains with indicated change in $\log_{10}$ CFU/ml[a] at:											
		3 h			6 h			12 h			24 h		
		−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
BAY 12-8039	8 × MIC	12	9	6	12	12	9	12	12	11	12	12	12
	4 × MIC	12	8	4	12	12	8	12	12	11	12	12	12
	2 × MIC	12	4	0	12	12	6	12	12	11	12	12	12
	MIC	9	1	0	11	9	3	12	11	11	12	12	11
	0.5 × MIC	1	0	0	3	1	0	8	6	4	8	7	5
Ciprofloxacin	8 × MIC	12	8	4	12	12	9	12	12	11	12	12	12
	4 × MIC	12	8	4	12	12	9	12	12	11	12	12	12
	2 × MIC	12	8	1	12	12	8	12	12	11	12	12	12
	MIC	11	4	1	12	11	5	12	12	11	12	12	11
	0.5 × MIC	3	0	0	8	5	0	7	4	4	8	7	5
Ofloxacin	8 × MIC	12	8	6	12	12	9	12	12	11	12	12	12
	4 × MIC	12	8	5	12	12	8	12	12	11	12	12	12
	2 × MIC	11	8	4	12	12	8	12	12	11	12	12	11
	MIC	11	6	3	11	9	6	12	12	11	12	12	11
	0.5 × MIC	4	1	0	5	3	3	6	4	4	6	6	4
Sparfloxacin	8 × MIC	12	8	4	12	12	9	12	12	11	12	12	12
	4 × MIC	12	8	2	12	12	8	12	12	11	12	12	12
	2 × MIC	12	5	0	12	12	4	12	12	11	12	12	11
	MIC	8	1	0	11	7	2	12	11	10	11	11	11
	0.5 × MIC	1	0	0	5	0	0	6	6	5	8	7	4
Amoxicillin	8 × MIC	12	8	3	12	10	7	12	12	10	12	12	12
	4 × MIC	11	8	2	12	10	6	12	12	10	12	12	12
	2 × MIC	8	5	1	10	9	5	12	10	10	12	12	11
	MIC	7	2	0	9	7	4	10	9	8	11	11	10
	0.5 × MIC	3	1	0	4	2	0	5	5	4	5	4	4
Ampicillin	8 × MIC	12	7	1	12	12	8	12	12	11	12	12	12
	4 × MIC	11	7	1	12	12	8	12	12	11	12	12	12
	2 × MIC	11	6	1	12	11	6	12	12	11	12	12	12
	MIC	8	3	0	10	9	2	12	12	8	11	11	10
	0.5 × MIC	3	1	0	4	1	0	4	4	3	4	4	4
Cefuroxime	8 × MIC	12	7	2	12	12	7	12	12	11	12	12	12
	4 × MIC	11	6	2	12	12	7	12	12	11	12	12	12
	2 × MIC	11	6	1	12	11	6	11	11	10	12	12	12
	MIC	8	5	1	11	9	6	10	9	9	11	11	9
	0.5 × MIC	3	0	0	3	1	0	5	3	3	4	3	3
Cefpodoxime	8 × MIC	12	2	0	12	11	6	12	12	9	12	12	12
	4 × MIC	9	1	0	12	10	6	12	12	9	12	12	12
	2 × MIC	3	0	0	9	6	2	11	9	6	10	10	10
	MIC	1	0	0	3	1	0	6	5	4	7	7	6
	0.5 × MIC	0	0	0	0	0	0	0	0	0	0	0	0

[a] Negative numbers indicate the number of $\log_{10}$ CFU per milliliter below that at time zero.

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