Commercial Broth Microdilution Panel Validation and Reproducibility Trials for Garenoxacin (BMS-284756), a Novel Desfluoroquinolone

Kelley A. Gordon,^{1*} Paul R. Rhomberg,¹ and Ronald N. Jones^{1,2}

The JONES Group/JMI Laboratories, North Liberty, Iowa,¹ and Tufts University School of Medicine, Boston, Massachusetts²

Received 13 February 2003/Returned for modification 14 May 2003/Accepted 24 May 2003

Results from garenoxacin dry-form broth microdilution MIC panels prepared commercially (Sensititre, TREK Diagnostics) were compared to reference frozen-form MICs to ensure the validity of the longer-shelf-life product. A total of 1,078 organisms from seven major organism groups were used in this trial. All commercial MIC results were within $\pm$ one log₂ dilution of reference garenoxacin values, and reproducibility trials produced identical MIC results for 90.5 to 92.1% of garenoxacin MIC comparisons. Control quinolones (ciprofloxacin and gatifloxacin) also performed at a similarly high level of accuracy.

Because of a combination of several characteristics, including favorable pharmacokinetics, the ability to be given orally or parenterally, and spectrum of activity, the quinolones as a class have been extensively used to treat a wide range of bacterial infections. Garenoxacin (formerly BMS-284756) is a novel desfluoro(6) quinolone. Relative to other fluoroquinolones, nearly complete bioavailability and lower toxicity in animal models have been documented for garenoxacin (9; M. Takahata, J. Mitsuyama, Y. Yamashiro, H. Araki, H. Yamada, H. Hayakawa, Y. Todo, S. Minami, Y. Watanabe, and H. Narita, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. T-3811, 1997). Garenoxacin has also demonstrated potent in vitro activity against a wide variety of bacterial pathogens, including many gram-positive bacterial species (2, 3, 8, 9). Similarly, other reports have shown the potential utility of garenoxacin against multidrug-resistant gram-positive pathogens, including many methicillin-resistant *Staphylococcus aureus* or *Staphylococcus epidermidis* organisms and penicillin-resistant *Streptococcus pneumoniae* (2, 9). Garenoxacin potency is comparable to that of other newer quinolones against gram-negative organisms and has proven to be active against *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, and *Chlamydia trachomatis* (9).

Appropriate disk diffusion and MIC quality control (QC) ranges for garenoxacin have been previously established (1). These QC ranges were determined for seven commonly used American Type Culture Collection (ATCC) control strains, which were tested over a 10-day period by more than seven laboratories using reference methods (5). With QC ranges established for garenoxacin, and as this compound nears completion of Phase III clinical trials and advances into clinical use, routine susceptibility testing using commercially prepared MIC products will be necessary. To validate the garenoxacin MIC results derived from commercially prepared products with extended shelf lives, a comparison (validation) study conforming to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines was performed (6).

In accordance with NCCLS M7-A6 methods (5) and established precedent (4), a minimum of 100 isolates per organism group listed in the M100-S13 document (7) tables for which garenoxacin has proven activity were tested. The spectrum of garenoxacin activity was determined for the following seven organism groups: *Staphylococcus* spp. (153 strains), *Streptococcus* spp. (141 strains; viridans group and β -hemolytic streptococcal species), *S. pneumoniae* (167 strains), *Enterococcus* spp. (102 strains), *Haemophilus influenzae* (308 strains), *Enterobacteriaceae* (105 strains), and other gram-negative bacilli (102 strains). Garenoxacin susceptibility was determined by using both commercial dry-form (Sensititre/TREK Diagnostics, Cleveland, Ohio) and frozen broth microdilution panels. All frozen-form reference panels were processed using the manufacturer's package insert procedures (also produced by TREK Diagnostics) and were maintained at -70°C or below until used. All panels were inoculated with a final concentration of 5×10^5 CFU/ml. The panels were incubated in ambient air at 35°C and were interpreted at 16 to 20 h for gram-negative rapid-growing species or 20 to 24 h for gram-positive and fastidious organisms (5). Gatifloxacin was tested concurrently as an internal QC quinolone agent for this MIC validation study. The tested range for garenoxacin on both the reference and commercial dry-form panels was 0.008 to 16 $\mu\text{g/ml}$. The tested gatifloxacin concentration range on commercial dry-form panels was 0.03 to 4 $\mu\text{g/ml}$, and it was 0.004 to 8 $\mu\text{g/ml}$ for frozen-form panels.

Commercial MIC panel reproducibility was assessed for garenoxacin by testing 14 strains. These strains included 10 commonly used ATCC strains: *Escherichia coli* ATCC 25922 and ATCC 35218, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and ATCC 25923, *Enterococcus faecalis* ATCC 29212, *H. influenzae* ATCC 49247 and ATCC 49766, and *S. pneumoniae* ATCC 49619, as well as four clinical isolates. Susceptibility testing was performed three times daily for 3 days, generating a total of 126 determinations. Ciprofloxacin was utilized as the control antimicrobial agent for this portion of the study. The acceptable agreement definition for susceptibility test reproducibility was that $\geq 95\%$ of MIC results be within ± 1 log₂ dilution step.

* Corresponding author. Mailing address: The JONES Group/JMI Laboratories, Inc., 345 Beaver Creek Centre, Suite A, North Liberty, IA 52317. Phone: (319) 665-3370. Fax: (319) 665-3371. E-mail: kelley-gordon @jmilabs.com.

TABLE 1. Variations of garenoxacin MIC results performed on commercially prepared dry-form panels versus reference frozen-form broth microdilution panels

Organism collection and species or type (no. tested)	No. (%) ^c of organisms with MIC variation (log ₂ dilutions) of (<i>n</i> = 1,078) ^a :					No. of organisms with MIC variation (log ₂ dilutions) of (<i>n</i> = 497) ^b :				
	-2	-1	0	+1	+2	-2	-1	0	+1	+2
Gram-positive cocci										
<i>Enterococcus</i> spp. (102)	0	6	60	36	0	0	6	48	30	0
<i>Staphylococcus</i> spp. (153)	0	3	129	21	0	0	3	55	16	0
<i>S. pneumoniae</i> (167)	0	0	71	96	0	0	0	4	96	0
<i>Streptococcus</i> spp. (141)	0	2	102	37	0	0	1	63	37	0
Subtotal (563)	0	11	362	190	0	0	10	170	179	0
Gram-negative bacilli										
<i>H. influenzae</i> (308)	0	3	291	14	0	0	2	9	2	0
<i>Enterobacteriaceae</i> (105)	0	4	64	37	0	0	3	30	34	0
Nonfermentative bacilli (102)	0	2	73	27	0	0	2	31	25	0
Subtotal (515)	0	9	428	78	0	0	7	70	61	0
All strains (1,078)	0 (0, <1)	20 (2, 3)	790 (73, 73)	268 (25, 23)	0 (0, <1)	0 (0, <1)	17 (3, 4)	240 (49, 53)	240 (48, 42)	0 (0, <1)

^a *n*, all strains tested.^b *n*, MIC values only with on-scale results.^c Data in parentheses are percentages of organisms with the indicated variation in garenoxacin and gatifloxacin MICs respectively. Gatifloxacin was utilized as a control drug in the same class.

All MIC results for QC strains were within published NCCLS M100-S13 ranges (7).

Table 1 summarizes the validation phase results for testing garenoxacin and gatifloxacin to compare reference NCCLS frozen panels (5) to commercial dry-form panels. Garenoxacin MIC results were slightly less variable than gatifloxacin test comparisons. All dry-form MIC results for garenoxacin were within ± 1 log₂ dilution. The gatifloxacin MICs for six strains

were 2 log₂ dilutions higher or lower than the reference method MIC.

Table 1 also includes a subset analysis for MIC data pairs that had on-scale values. A trend toward a higher (0.5-log₂ dilution) MIC was observed for garenoxacin. With the on-scale results, 48% of MIC determinations were at a twofold-higher value. Likewise, gatifloxacin commercial-panel MICs trended higher, with 42% being twofold greater but <1% being four-

TABLE 2. Garenoxacin dry-form MIC panel reproducibility results testing three replicates daily for 3 days (126 total results)

Organism ^c	No. of tests with the indicated garenoxacin MIC variation (no. with indicated ciprofloxacin MIC variation) for:					
	Replicates on same day ^a			Replicates between days ^b		
	-1	0	+1	-1	0	+1
<i>E. coli</i> ATCC 25922	0 (0)	8 (9)	1 (0)	0 (0)	8 (9)	1 (0)
<i>E. coli</i> ATCC 35218	1 (0)	8 (9)	0 (0)	1 (0)	8 (9)	0 (0)
<i>K. pneumoniae</i> ATCC 13883	2 (0)	7 (8)	0 (1)	2 (0)	7 (8)	0 (1)
<i>P. aeruginosa</i> 2-5668	0 (0)	8 (9)	1 (0)	0 (0)	8 (9)	1 (0)
<i>P. aeruginosa</i> ATCC 27853	0 (1)	9 (7)	0 (1)	0 (0)	9 (6)	0 (3)
<i>S. aureus</i> 9144	0 (2)	8 (7)	1 (0)	0 (2)	8 (7)	1 (0)
<i>S. aureus</i> ATCC 29213	0 (0)	8 (8)	1 (1)	0 (0)	8 (8)	1 (1)
<i>S. aureus</i> ATCC 25923	0 (0)	8 (9)	1 (0)	2 (0)	7 (9)	0 (0)
<i>Enterococcus</i> sp. strain 96-10852A	0 (0)	9 (9)	0 (0)	0 (0)	9 (9)	0 (0)
<i>E. faecalis</i> ATCC 29212	0 (2)	8 (6)	1 (1)	0 (4)	8 (5)	1 (0)
<i>S. pneumoniae</i> ATCC 49619	1 (1)	8 (7)	0 (1)	0 (1)	7 (7)	2 (1)
<i>S. pneumoniae</i> 2666B	0 (0)	9 (8)	0 (1)	0 (0)	9 (8)	0 (1)
<i>H. influenzae</i> ATCC 49247	0 (0)	9 (9)	0 (0)	0 (0)	9 (9)	0 (0)
<i>H. influenzae</i> ATCC 49766	0 (0)	9 (9)	0 (0)	0 (0)	9 (9)	0 (0)
Total	4 (6)	116 (114)	6 (6)	5 (7)	114 (112)	7 (7)

^a Exact replicate-to-replicate reproducibilities were 92.1 and 90.5% for garenoxacin and ciprofloxacin, respectively. All MIC results (100.0%) were ± 1 log₂ dilution step for both. Values are log₂ dilutions.^b The exact MIC was achieved between days in 90.5 and 88.9% for garenoxacin and ciprofloxacin, respectively. All MIC results (100.0%) were ± 1 log₂ dilution step for both. Values are log₂ dilutions.^c Note that garenoxacin MICs yielded on-scale results for 12 of 14 organisms, and ciprofloxacin MICs yielded on-scale values for 8 of 14 organisms.

fold higher. Slightly elevated dry-form garenoxacin and gatifloxacin MIC results were common for all species groups tested, but among *S. pneumoniae* strains, MICs were equal on both test panels for only 4% of strains. Gatifloxacin dry-form panel MIC results were also elevated for *S. pneumoniae* isolates (data not shown). On-scale garenoxacin MICs for the *Streptococcus* spp. demonstrated that 62.4% of the dry-form MIC results were equal to the matched frozen-form MIC results. Among the *Staphylococcus* spp. with on-scale values, garenoxacin MICs were identical for 74.3% of isolates (59% for gatifloxacin). With the *Enterococcus* sp. isolates, garenoxacin and gatifloxacin had identical on-scale MICs for 57.1 and 57.4% of isolates, respectively.

Garenoxacin MICs for the *Enterobacteriaceae* also demonstrated a slight shift towards a 1-log₂-higher dilution for on-scale dry-form values than with frozen-form panels. Identical MICs occurred in 44.8% of tests with *Enterobacteriaceae*, while 50.7% of MICs were twofold higher. Among the nonfermentative gram-negative bacilli, identical MICs occurred for 53.4% of isolates, and 43.1% of tests demonstrated a twofold-higher MIC. All garenoxacin MICs for *H. influenzae* were within ± 1 log₂ dilution, with no significant shift or trend toward either higher or lower values. When all MIC results were analyzed for gatifloxacin, MICs for only 1.3% of the isolates were observed to be beyond the ± 1 -log₂-dilution range.

Garenoxacin MIC results for the commercial dry-form panel reproducibility studies are listed in Table 2. Garenoxacin had a slightly greater same-day MIC reproducibility result, with 92.1% being identical versus 90.5% for the ciprofloxacin control. The MICs were identical for 90.5% of garenoxacin between-day replicates, compared to 88.9% for ciprofloxacin. There was no clear tendency for the MIC of either quinolone skewing towards higher or lower values or for unacceptable low levels of reproducibility. Ciprofloxacin MIC comparison results were on-scale for 8 of the 14 organisms tested, while garenoxacin demonstrated on-scale MIC results for 12 of the 14 strains tested. For both garenoxacin and ciprofloxacin, all replicate MICs were within ± 1 log₂ dilution for same-day and for between-day testing, meeting the definition of acceptable reproducibility.

The purpose of this study was to provide method and MIC endpoint validation for commercially prepared, dry-form panels containing garenoxacin when compared to the NCCLS frozen-form reference broth microdilution test (5). All MIC comparisons for garenoxacin between commercial and reference broth microdilution MIC results showed equality within ± 1 log₂ dilution. However, garenoxacin and the gatifloxacin con-

trol demonstrated a slight trend toward higher MICs on the dry-form panels, and this was most pronounced for *S. pneumoniae*. All reproducibility results obtained were within ± 1 log₂ dilution for the 14 organisms tested.

Garenoxacin, a novel des-fluoro(6) quinolone, has proven to be a potent antimicrobial agent in vitro, with a wide spectrum of activity, especially against gram-positive organisms and fastidious respiratory tract pathogens (2, 3, 8, 9; Takahata et al., 37th ICAAC). The study results reported here demonstrate that garenoxacin can be accurately and reproducibly tested following the Food and Drug Administration release of this product by using commercial dry-form (Sensititre, TREK Diagnostics) reagents and can be controlled by methods and QC ranges found in contemporary NCCLS tables (1, 5, 7).

We thank Bristol-Myers Squibb for the educational/research grant that made this project possible.

We also extend our thanks to K. Meyer, T. Anderegg, M. Beach, and D. Biedenbach for their technical support in the preparation of the manuscript and the execution of this comparative trial.

REFERENCES

1. Biedenbach, D. J., R. N. Jones, and The Quality Control Working Group. 2001. Assessment of BMS284756 MIC and 5- μ g disk diffusion quality control studies tested against seven American Type Culture Collection strains. *Diagn. Microbiol. Infect. Dis.* **41**:225–228.
2. Fung-Tomc, J. C., B. Minassian, B. Kolek, E. Huczko, L. Aleksunes, T. Stickle, T. Washo, E. Gradeliski, L. Valera, and D. P. Bonner. 2000. Antibacterial spectrum of a novel des-fluoro(6) quinolone, BMS284756. *Antimicrob. Agents Chemother.* **44**:3351–3356.
3. Gordon, K. A., M. A. Pfaller, R. N. Jones, and The SENTRY Participants Group. 2002. BMS284756 (formerly T-3811, a des-fluoroquinolone) potency and spectrum tested against over 10,000 bacterial bloodstream infection isolates from the SENTRY antimicrobial surveillance programme. *J. Antimicrob. Chemother.* **49**:851–855.
4. Jones, R. N., T. R. Anderegg, and D. J. Biedenbach. Validation of commercial dry-form broth microdilution panels for susceptibility testing of AZD2563, a new long-acting oxazolidinone. *Clin. Microbiol. Infect.*, in press.
5. National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7–A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
6. National Committee for Clinical Laboratory Standards. 2001. Development of in vitro susceptibility testing criteria and quality control parameters. Approved guideline M23–A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
7. National Committee for Clinical Laboratory Standards. 2003. Performance standards for antimicrobial susceptibility testing M100–S13. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. Pfaller, M. A., M. L. Beach, K. A. Gordon, and R. N. Jones. 2002. Comparative antimicrobial spectrum and activity of BMS284756 (T-3811; a desfluoroquinolone) tested against an international collection of staphylococci and enterococci, including in vitro test development and intermethod comparisons. *J. Chemother.* **14**:13–18.
9. Takahata, M., J. Mitsuyama, Y. Yamashiro, M. Yonezawa, H. Araki, Y. Todo, S. Minami, Y. Watanabe, and H. Narita. 1999. In vitro and in vivo antimicrobial activities of T-3811ME, a novel des-F(6)-quinolone. *Antimicrob. Agents Chemother.* **43**:1077–1084.