Comparative Antimicrobial Spectrum and Activity of BMS284756 (T-3811; A Desfluoroquinolone) Against Three Groups of Non-fermentative Gram-negative Bacilli, Including *In Vitro* Test Comparisons And Development

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ABSTRACT

Purpose: To compare the in vitro activity of BMS284756 (formerly T-3811), a desfluoroquinolone, with four other fluoroquinolones when tested against 129 *Pseudomonas aeruginosa*, 97 *Stenotrophomonas/Burkholderia* gr. and 43 *Acinetobacter* spp. Also in vitro test methods were compared for accuracy and reproducibility.

Methods: The BMS284756 MICs were determined using the broth microdilution method of the NCCLS and Etest (AB BIODISK, Solna, Sweden). These methods were compared for test accuracy and 5- µg disk zone diameters were compared for interpretative accuracy using the proposed susceptible breakpoint of ≤4 µg/ml (Fung-Tomc et al. AAC 44:3351-3356, 2000). All organisms tested were from the 1999-2000 SENTRY Antimicrobial Surveillance Program collection derived from medical centers Europe and the Americas.

Results: Comparative fluoroquinolone potency against *P. aeruginosa* in the studied collection was as follows: ciprofloxacin (CIP; MIC_{50}, 0.25 µg/ml) > gemifloxacin (GEMI; MIC_{50}, 0.5 µg/ml) > levofloxacin (LEVO; MIC_{50}, 1 µg/ml) = gatifloxacin (GATI; MIC_{50}, 1 µg/ml) > BMS284756 (MIC_{50}, 4 µg/ml). The BMS284756 MIC_{50} values for the *Stenotrophomonas/Burkholderia* gr. was 2 µg/ml. This potency was similar and spectrum superior to those of the other fluoroquinolones reported previously (Biedenbach et al. Eur J Clin Microbiol Infect Dis 18:428-431, 1999). The MIC_{50} results for the *Acinetobacter* spp. are as follows: CIP (>2 µg/ml), LEVO (4 µg/ml), GATI (4 µg/ml), GEMI (4 µg/ml), and BMS284756 (>4 µg/ml). The Etest results compared favorably to the reference dilution test results for *P. aeruginosa* (100% ± two log_2 dilutions) and for *Acinetobacter* spp. (100% ± one log_2 dilution). Etest results were slightly lower when testing *Stenotrophomonas/Burkholderia* gr. (r=0.81), but the essential agreement remained acceptable at 94.2% ± two log_2 dilutions. The disk diffusion method correlated well for *P. aeruginosa* (r=0.94) without serious false-susceptible errors. The *Stenotrophomonas/Burkholderia* gr. strains also showed a maximum potential for major errors of only 0-3%. The disk diffusion test compared to the reference method with *Acinetobacter* spp. showed a bi-modal susceptibility test result population (MICs , ≥4 versus ≤0.12 µg/ml) with minimal possible interpretive errors.

Conclusions: BMS284756 was generally less active than other comparison fluoroquinolones versus non-fermentative Gram-negative bacilli. However, its spectrum remains more equivalent if elevated dosing schedules substantiates a susceptible breakpoint of ≤4 µg/ml with acceptable safety. In vitro tests appear accurate.

INTRODUCTION

Among the clinically important non-fermenting Gram-negative bacilli, the most commonly isolated species are *Pseudomonas aeruginosa* , *Stenotrophomonas maltophilia/Burkholderia* and *Acinetobacter spp.* These opportunistic pathogens are usually found in the environment, but can become problematic as nosocomial pathogens in the generally at risk often ICU patient populations. Because these non-fermenters tend to be resistant to many antimicrobial agents, a search for newer agents to use for therapy is imperative.

BMS284756 (formerly T-3811) is a novel so-called "desfluoroquinolone". It has been shown to be among the most active quinolones against Gram-positive bacteria and anaerobic bacterial strains. This study compares the activity of this new desfluoroquinolone and other fluorinated quinolones against the three most commonly isolated groups of non-fermenters from hospitalized patient infections. The results obtained from disk diffusion and Etest (AB BIODISK, Solna, Sweden) methods were also compared to those produced by the reference broth microdilution method described by the National Committee for Clinical Laboratory Standards (NCCLS).

MATERIALS AND METHODS

The bacterial strains tested in this study consisted of *P. aeruginosa* (129 strains), *Stenotrophomonas/Burkholderia gr* (97 strains) and *Acinetobacter* spp. (43 strains). All organisms were obtained from the SENTRY Antimicrobial Surveillance Program collection isolated in 1999-2000. These organisms were isolated in medical centers in Europe and the Americas.

All organisms were tested by reference broth microdilution method and the standardized disk diffusion test. The broth microdilution trays were produced by TREK Diagnostics, Inc. (Westlake , OH, USA) and were validated to be equivalent to NCCLS tests. The BMS284756 5- µg disks were made by BD Microbiology Systems (Cockeysville , MD, USA). Etests (AB BIODISK) were performed as described in the manufacturer product package insert on Mueller-Hinton agar plates.

The reference broth microdilution results were compared to the Etest results. Inter-method essential agreement was defined as the Etest result (MIC) being within ± two log2 dilution of the reference result for ≥ 90% of strains. The disk diffusion tests were compared using the proposed susceptibility breakpoint of ≤ 4 µg/ml. Linear regression statistics and the determination of potential interpretive errors were used to assess diagnostic accuracy applying M23-A2 criteria.

RESULTS

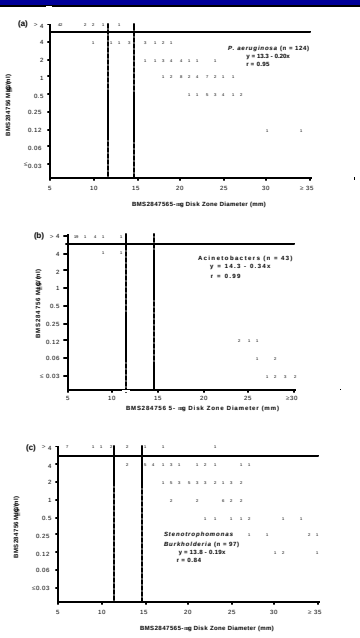
- As shown in Table 1, it was found that the potency of BMS284756 was reduced when compared to other testedquinolones. However, the BMS284756 spectrum of activity as demonstrated by the percent susceptibility was in general higher or more similar to the other agents. Specifically, *Acinetobacter* spp. isolates were shown to have the least susceptibility to the quinolones tested with percentage rates of only 34.9 to 39.5%.
- Among the *Pseudomonas aeruginosa* tested, BMS284756 had a two- to four-fold higher MIC_{50} compared to ciprofloxacin or levofloxacin.
- The *Stenotrophomonas/Burkholderia* group had equivalent MIC_{50} results (2 µg/ml) to BMS284756 and ciprofloxacin, but a narrower spectrum of activity for ciprofloxacin (43.3% versus 83.5%).
- Table 2 and Figure 1a show the intermethod comparison results for the BMS284756 Etest and disk diffusion methods using *P. aeruginosa* strains. Etest results showed a slightly lower value, but 128 of 129 results were within ± one log2 dilution of the reference broth microdilution MIC. Similarly the disk diffusion zone diameters around a 5-µg BMS284756 disk correlated well (r=0.95) and a proposed breakpoint MIC of ≤ 4 µg/ml (correlate zone of ≥ 15 mm) would produce no false-susceptible (very major) errors and only 0.8% false-resistant (major) discrepancies.
- Also in Table 2 and Figure 1b, these highly resistant *Acinetobacter* spp. strains demonstrated a clear bi-modal MIC population distribution for BMS284756 (≤ 0.12 and ≥ 4 µg/ml). Etest MIC results were all within ± one log2 dilution and the correlation coefficient for the disk diffusion test (r=0.99) was nearly perfect. Applying the interpretive criteria for susceptibility listed above, no very major errors were encountered and 4.7% major errors.
- The comparisons of Etest and disk diffusion results to reference test MICs using 97 strains of *S. maltophilia* and *Burkholderia* spp. (Table 2 and Figure 1C) shows Etest results that were clearly skewed toward a lower MIC value (65.9% ± one log2 dilution). The correlation coefficient (r) for the disk diffusion test was also decreased to 0.84, but still acceptable. Application of the ≤ 4 µg/ml and ≥ 15 mm susceptible breakpoints produced modest potential very major and minor types of errors (≤ 5.2% combined).
- Overall, the Etest results for the so-called "non-fermenters" (269 strains) had an essential agreement ± one log_2 dilution of nearly 90% and 97.0% ± two log2 dilutions. The use of a lower, alternative breakpoint MIC for BMS284756 (susceptible at ≤ 2 µg/ml and ≥17 mm) would have combined error rates for the disk diffusion test of: very major = 0.7%, major = 0.0%, and minor errors = 8.9%.

FIGURE LEGENDS

Figure 1.

Scattergrams comparing the BMS284756 MIC with the zone of inhibition around a 5-µg disk when testing: (a) P. aeruginosa, (129 strains); (b) Acinetobacter spp., 43 strains; and (c) Stenotrophomonas/Burkholderia spp., 97 strains. Vertical broken lines indicate preliminary zone diameter breakpoint criteria correlating to the proposed ≤ 4 µg/ml MIC indicating susceptibility.

RESULTS (continued)



RESULTS (continued)

Table 1.
Activity of BMS284756 and four comparison quinolones tested against 279 strains of P. aeruginosa, Stenotrophomonas/ Burkholderia gr. and Acinetobacter spp.

Organism/antimicrobial agent (no. tested)	MIC (µg/ml) MIC_{50}	MIC_{90}	Range	% susceptible (breakpoint)[a]
***Acinetobacter* spp. (43)**				
BMS284756	>4	>4	0.03->4	39.5 (≤4)
Ciprofloxacin	>2	>2	0.25->2	37.2 (≤1)
Gatifloxacin	4	>4	0.03->4	34.9 (≤2)
Gemifloxacin	4	>4	0.03->4	34.9 (≤0.25)
Levofloxacin	4	>4	0.03->4	34.9 (≤2)
***P. aeruginosa* (129)**				
BMS284756	4	>4	0.12->4	60.5 (≤4)
Ciprofloxacin	0.25	>2	0.25->2	62.8 (≤1)
Gatifloxacin	1	>4	1->4	58.9 (≤2)
Gemifloxacin	0.5	>4	0.03->4	45.0 (≤0.25)
Levofloxacin	1	>4	0.06->4	62.0 (≤2)
***Stenotrophomonas/ Burkholderia gr* (97)**				
BMS284756	2	>4	0.12->4	83.5 (≤4)
Ciprofloxacin	2	>2	0.25->2	43.3 (≤1)
Gatifloxacin	1	2	0.06->4	91.8 (≤2)
Gemifloxacin	0.5	2	0.03->4	26.8 (≤0.25)
Levofloxacin	1	2	0.12->4	81.4 (≤2)

a. Interpretive breakpoint criteria are those published by the NCCLS or suggested in cited references.

RESULTS (continued)

TABLE 2:
In vitro susceptibility of 4,105 Gram-negative blood stream infection isolates from North America, Latin America, and Europe to BMS284756, gatifloxacin , ciprofloxacin, and levofloxacin: SENTRY, 2000.

Organism (no. tested)	Etest MIC/reference MIC ≥8	4	2	1	0.5	0.25	≤0.12
Acinetobacter (43)	0	0	5	4	0	0	0
P. aeruginosa (129)	0	0	6	38	33	1	0
Stenotrophomonas/Burkholderia spp. (97)	0	0	4	31	29	12	5
TOTAL (269)	0	0	15[b]	73[b]	62[b]	13	5

a. Only on-scale results for both tests were used to assess essential agreement (168 organism tests).
b. Essential agreement ± one log2 dilution was 89.3% (97.0% ± two log2 dilutions steps).

CONCLUSIONS

- The Etest appears to be an excellent and practical alternative to the use of the reference broth microdilution method for BMS284756, a finding supported by similar success with this method for other quinolones. The disk diffusion method also provided results that appear acceptable for the proposed breakpoint for BMS284756 susceptibility (≤ 4 µg/ml) or a lower alternative ≤ 2 µg/ml.
- BMS284756, a novel desfluoroquinolone, has demonstrated a wide spectrum of activity versus Gram-positive and -negative aerobic pathogens, as well as, *Bacteroides fragilis* (MIC_{90}, 0.5 - 0.78 µg/ml). Modest activity for BMS284756 has been documented here, for non-fermentative Gram-negative bacilli. Continued in vivo investigations are encouraged.

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