Comparative Antimicrobial Spectrum and Activity of BMS284756 (T-3811; A Desfluoroquinolone) Against *H. influenzae* and *M. catarrhalis*, Including *In Vitro* Test Development Comparisons

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ABSTRACT

Purpose: To determine BMS284756 activity against recent clinical isolates of *H. influenzae* and *M. catarrhalis*.

Method: *H. influenzae* (n=1,871) and *M. catarrhalis* (n=810) from the SENTRY Antimicrobial Surveillance Program were selected to reflect geographically diverse samples with contemporary β-lactamase prevalence (30-35% *H. influenzae*; >90% *M. catarrhalis*). Isolates were tested by reference broth microdilution Etest, and disk methodologies against BMS284756 and selected agents. HTM (*H. influenzae*) or Mueller-Hintonbroth (*M. catarrhalis*) were used.

Results: Among the *M. catarrhalis* isolates, BMS284756 was very active against all strains (MIC_{90} ≤0.03) regardless of β-lactamase production. BMS284756 activity was comparable to trovafloxacin and superior to levofloxacin (MIC_{90}, 0.06) or ciprofloxacin (MIC_{90} 0.25). *H. influenzae* (MIC_{90}, ≤0.03) were also very susceptible to BMS284756 and other newer quinolones. For *H. influenzae* MIC comparisons between reference and Etests showed generally elevated Etest results. The disk (5-µg) diffusion results showed acceptable categorical correlation to the broth microdilution test.

Conclusions: The activity of BMS284756 shows a potency greater or equivalent to other recently developed quinolones against fastidious respiratory pathogens. Documented minimal toxicity and other pharmacokinetic profiles (≤4µg/ml breakpoint) suggest BMS284756 is a potential therapeutic option against common Gram-negative respiratory pathogens.

BMS284756, *H. influenzae*, *M. catarrhalis*, SENTRY

INTRODUCTION

The activity of fluoroquinolones against common respiratory tract pathogens including those most often associated with community-acquired pneumonia, otitis media, sinusitis and acute exacerbation of chronic bronchitis has been well documented. Fluoroquinolone resistance among, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Haemophilis influenzae*, has been a rare finding. Confirmed case reports of fluoroquinolone-resistant *M. catarrhalis* and *H. influenzae* strains have been linked to at-risk patients who have had extensive prior therapeutic exposure to drugs in this class, and some patients were receiving suboptimal dosing regimens. In addition, documented high-level fluoroquinolone resistances (ciprofloxacin MICs at >32 i g/ml) associated with multiple point mutations in *gyr A* and *par C* of the quinolone resistance determining region (QRDR), indicates that both *H. influenzae* and *M. catarrhalis* can evolve to a resistance level that renders all currently available fluoroquinolones clinically useless.

Surveillance studies such as the SENTRY Antimicrobial Surveillance Program (1997-present) show that resistance to older and newer generation quinolone agents among fastidious respiratory tract infection pathogens is indeed rare. Initial European results found no fluoroquinolone-resistant *H. influenzae* and only one *M. catarrhalis* isolate that was not susceptible to sparfloxacin only. Results in North America indicated that the highest level of reduced quinolone susceptibility was 0.25 i g/ml. A more recent study (1999 SENTRY Program) from Latin America also reported no confirmed

INTRODUCTION – Continued

fluoroquinolone-resistant isolates of either pathogen. World-wide prevalence of fluoroquinolone resistance including the Asia Pacific area (1997-99), showed resistance among *H. influenzae* and *M. catarrhalis* accounted for ≤0.01% of each species. The 2000 SENTRY Program reported no *M. catarrhalis* isolate resistant to tested fluoroquinolones, and only two *H. influenzae* isolates with MICs to ciprofloxacin of ≥0.5 i g/ml among over 3,000 strains (0.06%) tested from Europe, Latin and North America.

BMS284756 (Figure 1) is an investigational desfluoro-quinolone with a broad spectrum of activity against both Gram-positive and -negative pathogens, including fastidious strains that commonly cause community-acquired respiratory tract infections. The pharmacokinetic profile including toxicology results suggest that the side chain substitutions of the BMS284756 molecule may enhance the activity of this compound against Gram-positive cocci, some anaerobes and fastidious pathogens, without serious acute toxicity.

This study was conducted to determine the activity of BMS284756 compared to other selected quinolones against recent clinical isolates of *H. influenzae* and *M. catarrhalis* using National Committee for Clinical Laboratory Standards (NCCLS) reference broth microdilution tests. Etest (AB BIODISK, Solna Sweden) and disk diffusion methodologies were also tested on a subset of *H. influenzae* isolates to determine inter-method correlation with the reference test results.

MATERIALS AND METHODS

The collection included strains from Europe, Canada, and the US (44 medical centers) that were forwarded for reference MIC testing during the SENTRY Program (2000). The 2,681 isolates tested includes *H. influenzae* (1,871 strains) and *M. catarrhalis* (810 strains). Identifications were confirmed and β-lactamase production determined. Greater than 95% of *M. catarrhalis* strains and around one-third of *H. influenzae* produced β-lactamase enzymes. A selected subset of 292 *H. influenzae* strains were also tested using the Etest and disk diffusion (5-i g) methods for inter-method comparison purposes.

All strains were tested using NCCLS methods in validated, dry form panels (TREK Diagnostics, Inc., Westlake , OH) including BMS284756 (Bristol-Myers Squibb, Princeton, NJ) and four other quinolones including ciprofloxacin, gatifloxacin levofloxacin, and moxifloxacin. Purified subcultures of each strain were suspended into cation-adjusted Mueller-Hinton broth to a density of a 0.5 McFarland turbidity standard, diluted into HTM (*H. influenzae*) or cation-adjusted MuellerHinton broth (*M. catarrhalis*) and delivered by autoinoculator into the panels targeting a final concentration of 5 x 10⁵ CFU/ml per well. Weekly colony counts and ATCC quality control strain tests ensured valid results. Concurrently, from the same 0.5 McFarland inoculum , the selected 292 isolates of *H. influenzae* were applied to the surface of 150 mm HTM plates with a cotton swab and allowed to dry. To each plate were then applied a BMS284756 Etest strip and a 5-i g disk. All tests were incubated in atmospheres and for time frames recommended for each species by the NCCLS. Interpretations of susceptibility utilized the current NCCLS [2001] tables with ≤4 i g/ml as susceptible employed for BMS284756.

FIGURE 1: Chemical Structure of BMS284756

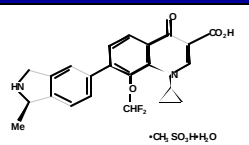

FIGURE 2: The evaluation of the 5-i g BMS284756 disk zone diameters compared to broth microdilution results

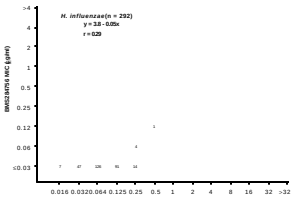

FIGURE 3: Etest versus broth microdilution MIC results for the same panel of H. influenzae isolates tested by disk diffusion

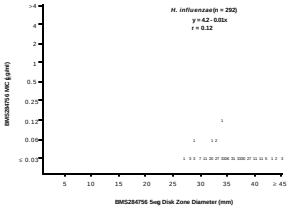

Table 1. Antimicrobial activity of BMS284756 and four other fluoroquinolones tested against 1,871 *H. influenzae* strains isolated in SENTRY Antimicrobial Surveillance Program (2000) medical centers in Europe, Canada and the United States.

	Europe (n=677) MIC (i g/ml)			Canada (n=261) MIC (i g/ml)			United States (n=933) MIC (i g/ml)		
Antimicrobialagent	50%	90%	% Susc.[a]	50%	90%	% Susc.[a]	50%	90%	% Susc.[a]
BMS284756	≤0.03	≤0.03	(100.0)[b]	≤0.03	≤0.03	(100.0)[b]	≤0.03	≤0.03	(100.0)[b]
Ciprofloxacin	≤0.016	≤0.016	100.0	≤0.016	≤0.016	99.6[c]	≤0.016	≤0.016	100.0
Gatifloxacin	≤0.03	≤0.03	100.0	≤0.03	≤0.03	99.6[c]	≤0.03	≤0.03	100.0
Levofloxacin	≤0.03	≤0.03	100.0	≤0.03	≤0.03	100.0[c]	≤0.03	≤0.03	100.0
Moxifloxacin	≤0.03	≤0.03	100.0	≤0.03	≤0.03	99.6[c]	≤0.03	≤0.03	100.0

a. Interpretive criteria as published by the NCCLS (2001).
b. Percentage of tested strains inhibited at proposed susceptible breakpoint of ≤4 i g/ml [Fung-Tomc et al., 2000].
c. Two strains with elevated fluoroquinolone MICs were detected during the monitored interval (levofloxacin MICs, 0.5 and 2 i g/ml).

Table 2. Antimicrobial activity of BMS284756 and four other fluoroquinolones tested against 810 *M. catarrhalis* strains isolated in SENTRY Antimicrobial Surveillance Program (2000) medical centers in Europe, Canada and the United States.

	Europe (n=286) MIC (i g/ml)			Canada (n=77) MIC (i g/ml)			United States (n=447) MIC (i g/ml)		
Antimicrobialagent	50%	90%	% Susc.[a]	50%	90%	% Susc.[a]	50%	90%	% Susc.[a]
BMS284756	≤0.03	≤0.03	100.0(≤4)	≤0.03	≤0.03	100.0(≤4)	≤0.03	≤0.03	100.0(≤4)
Ciprofloxacin	≤0.016	≤0.016	100.0(≤1)	0.03	0.03	100.0(≤1)	0.03	0.03	100.0(≤1)
Gatifloxacin	≤0.03	≤0.03	100.0(≤0.5)	≤0.03	≤0.03	100.0(≤0.5)	≤0.03	≤0.03	100.0(≤0.5)
Levofloxacin	≤0.03	≤0.03	100.0(≤2)	≤0.03	≤0.03	100.0(≤2)	≤0.03	≤0.03	100.0(≤2)
Moxifloxacin	≤0.03	≤0.03	100.0(≤1)	≤0.03	≤0.03	100.0(≤1)	≤0.03	≤0.03	100.0(≤1)

a. Susceptibility criteria are listed in parenthesis derived from *H. influenzae* tables of the NCCLS [2001] standard. A projected breakpoint of ≤ 4ug/ml was used for BMS284756 [Fung-Tomc et. al., 2000].

RESULTS AND CONCLUSIONS

- With the exception of two genetically and epidemiologically different isolates from Canada, the 1,871 *H. influenzae* strains were highly susceptible to allquinolones and no significant geographic variability could be evaluated.
- Among the two isolates with elevated MICs, the levofloxacin and moxifloxacin MIC values were 0.5 and 2 i g/ml, respectively. BMS284756 and gatifloxacin were slightly more potent (0.25 and 1 i g/ml) and ciprofloxacin was slightly less potent (0.5 and>2 i g/ml).
- The quinolone results for the tested *M. catarrhalis* isolates (Table 2), showed a highly susceptible population with MIC_{90}s generally at the lowest tested concentrations (0.03 mg/ml), independent of geographic region.
- The evaluation of the 5-i g BMS284756 disk zone diameters compared to broth microdilution results (Figure 2) suggested that only a susceptible breakpoint was necessary when testing *H. influenzae*.
- Figure 3 shows the Etest versus broth microdilution MIC results for the same panel of *H. influenzae* isolates tested by disk diffusion. All five strains with comparative on-scale values had Etest values that were four-fold elevated and only 14 isolates (4.8%) had Etest results >2 log_2-dilutions higher than broth microdilution (> 95% quantitative and 100% categorical agreement).
- This study documents the excellent comparative potency of BMS284756 against recent clinical and geographically diverse strains of *H. influenzae* and *M. catarrhalis*. The use of surveillance studies is necessary to determine if increased use of quinolones that alters "selective pressure" may increase the frequency of single- or multiple-step mutations in the QRDR of these common species.

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