Comparative Antimicrobial Spectrum and Activity of BMS284756 (T-3811; A Desfluoroquinolone) Against *Neisseria gonorrhoeae*, Including *In Vitro* Test Comparisons and Development

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ABSTRACT

Purpose: To determine the potency of BMS284756, a novel des-F(6)-quinolone, tested against clinical isolates of *Neisseria gonorrhoeae* including strains observed to be resistant to ciprofloxacin and other newer quinolones.

Methods: A total of 137 strains of *N. gonorrhoeae* were tested using NCCLS methods (agar dilution, disk diffusion) and Etest (AB BIODISK, Solna, Sweden). Only three strains were susceptible to penicillin (PEN; ≤0.06 µg/ml); and 50 strains had ciprofloxacin (CIP) MIC values of ≥0.12 µg/ml, seven with MICs of ≥1 µg/ml (resistant). CIP-non-susceptible strains were collected from patients in Europe, Japan and the United States. Comparisons between methods were determined by regression analysis, dilution step variations, and errors at a breakpoint of ≤2 µg/ml.

Summary of Results: BMS284756 potency versus *N. gonorrhoeae* was generally 2- to 4-fold greater than CIP. PEN resistance in the absence of CIP resistance did not effect BMS284756 activity. However, elevated CIP MICs were associated with higher BMS284756 MICs as follows (BMS284756 MIC_{50}/MIC range in µg/ml): CIP-susceptible strains (0.016 or 0.03/0.004-0.06), CIP-intermediate strains (0.06 or 0.12/0.008-0.25) and CIP-resistant strains (0.12 or 0.5/0.12-1). Etest MICs were routinely 4-fold lower than those produced by the agar dilution method, but the correlation coefficient (r) was 0.87. Similarly acceptable correlation was achieved with 5-µg disk zone diameters (r=0.78) where all zones were ≥28 mm (MIC, 1 µg/ml).

Conclusions: BMS284756 was very active against *N. gonorrhoeae* (MIC_{50}, 0.03 µg/ml overall) including CIP-resistant strains. All BMS284756 MICs were ≤1 µg/ml and single-dose therapy could be effective for >95% of strains if clinical dosing and breakpoints of peer drugs (trovafloxacin; ≤0.25 µg/ml) were utilized. These results confirm those of Fung-Tomc et al. (AAC 44:3351, 2000).

INTRODUCTION

- Infections caused by multi-drug resistant *Neisseria gonorrhoeae* are an expanding global problem. Quinolones have become a very attractive alternative for therapy of uncomplicated gonorrhea because of their excellent penetration into infected genitourinary tissue and favorable single-dose pharmacokinetics. Newer advanced generation fluoroquinolone derivatives are being introduced, as older drugs of this class become less effective because of emerging resistance.
- BMS284756 is a novel des-fluoroquinolone (lacks a fluorine at the C-6 position). The des-F(6)-quinolones have been shown to have lower cerebral toxicity in mice. In a previous study, *in vitro* activity as well as *in vivo* model efficacy of BMS 284756 was comparable to peer drugs such as ciprofloxacin, levofloxacin and trovafloxacin.
- As the newer, more potent quinolone compounds become available for therapy, it is important to develop *in vitro* susceptibility testing criteria for *N. gonorrhoeae*. Agar dilution is considered the standard for susceptibility testing, but it is cumbersome and time consuming. Disk diffusion and Etest (AB BIODISK, Solna, Sweden) are more practical for determining antimicrobial susceptibility in routine clinical laboratory settings. The present study was designed to evaluate potency of BMS284756 against *N. gonorrhoeae* and to develop *in vitro* susceptibility testing criteria.

MATERIALS AND METHODS

Organisms tested. A total of 137 strains of *N. gonorrhoeae* were tested against BMS284756 using agar dilution, Etest (AB BIODISK) and disk diffusion methods. Based on the penicillin MIC values, these strains were distributed as follows: three penicillin-susceptible, 33 penicillin-intermediate and 101 penicillin-resistant (44 penicillinase producing *N. gonorrhoeae*; PPNG). Fifty of the 137 isolates had elevated ciprofloxacin MIC values (≥ 0.12 µg/ml) and were collected from Japan, The Netherlands and the United States (USA), many of which have documented *gyr A* and/or *par C* mutations.

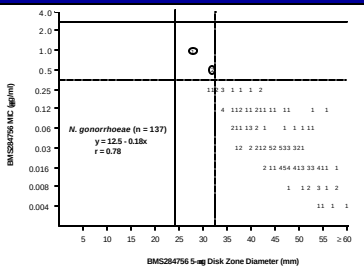
Antimicrobials tested. BMS284756 was provided by Bristol-Myers Squibb (Princeton, NJ, USA). AB BIODISK (Solna, Sweden) manufactured the BMS284756 Etest strips. BD Microbiological Systems (Cockeysville, MD, USA) made 5-µg disks for both quinolones, and IsoVitalex GC agar supplement.

Susceptibility methods. All the tests were performed in accordance with National Committee for Clinical Laboratory Standards. The tests were validated using the following quality control strains: *N. gonorrhoeae* (ATCC 49226), *Enterococcus faecalis* (ATCC 29212) and *Pseudomonas aeruginosa* (ATCC 27853). Results of agar dilution MICs were compared to Etest to establish essential agreement between the results of the two quantitative methods. MIC results and disk zone diameters were also analyzed by regression statistics to determine inter-method correlation and a breakpoint suggested by Fung-Tomc et al. (2000) was applied to propose breakpoint criteria.

RESULTS

- BMS284756 MICs correlated with ciprofloxacin (CIPRO) susceptibility (Table 1). MIC50s for BMS 284756 of CIPRO susceptible (cipro-S) strains were in the range of 0.016 - 0.03 µg/ml, - intermediate (cipro-I) were in the range 0.06 - 0.12 µg/ml and -resistant (cipro-R) strains were in the range 0.12 - 0.5 µg/ml.
- Penicillin MICs did not correlate with BMS284756 for the cipro-S and cipro-I strains, but all the cipro-R strains were also non-susceptible to penicillin.
- Overall BMS284756 showed activity comparable or two-fold superior to CIPRO which confirms the results of Fung-Tomc et al. (2000).
- Etest values were two- to eight-fold lower than agar dilution MIC with almost half of the (49.7%) isolates clustered at a reference MIC/ Etest MIC ratio of 0.25.
- Figure 1 illustrates comparison of BMS284756 MIC and disk zone diameter results (5-µg). The diverse population of strains with regards to their fluoroquinolone resistance produced an acceptable linear correlation between tests (y=12.5 - 0.18x; r=0.78). CIPRO-resistant strains were clearly positioned at the upper limits of the BMS284756 MIC distribution.
- The susceptibility breakpoint of ≤ 2 µg/ml suggested by Fung-Tomc et al. (2000) classifies many strains with mutations in QRDR as BMS 284756-susceptible (Figure 1). A more conservative breakpoint comparable to that of trovafloxacin or gemifloxacin (showed in dotted line in Figure 1) classifies strains with ≥ 3 QRDR mutations as non-susceptible to BMS284756.
- Although BMS284756 shows activity only slightly greater than currently available quinolones, its low toxicity may subsequently support administration of higher doses (> 400 mg) for treatment of *N. gonorrhoeae* infections. In that situation it may be more reasonable to use the higher breakpoint of ≤ 2 µg/ml suggested by the manufacturer.

FIGURE 1: Scattergram comparing the reference agar dilution BMS284756 MICs and zone diameters around 5-µg disks (137 strains; r=0.78).



Suggested breakpoint concentrations and zone diameters are shown as solid vertical and horizontal lines. More conservative criteria are noted as broken lines (see text).

TABLE 1: Antigonococcal activity of BMS284756 against strains indexed by their susceptibility to ciprofloxacin and penicillin.[a]

Susceptibility category for:		*No. tested*	*BMS284756 MIC (mg/ml)*		
Ciprofloxacin	*Penicillin*		*50%*	*90%*	*Range*
Susceptible	Susceptible	1	0.016	-	0.016
	Intermediate	27	0.016	0.03	0.004-0.06
	Resistant	59[b]	0.03	0.03	0.004-0.06
Intermediate	Susceptible	2	0.12	-	0.12
	Intermediate	4	0.06	-	0.06-0.12
	Resistant	37[c]	0.12	0.25	0.008-0.25
Resistant	Susceptible	0	-	-	-
	Intermediate	2	0.12	-	0.12
	Resistant	5[d]	0.5	-	0.25-1

a. Ciprofloxacin susceptibility was defined as ≤ 0.06 µg/ml and resistance as ≥ 1 µg/ml. Penicillin-susceptible was defined as ≤0.06 µg/ml and resistant at ≥ 2 µg/ml [9].
b. Includes 33 penicillinase -producing (PPNG) strains.
c. Includes nine PPNG strains.
d. Includes two PPNG strains.

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CONCLUSIONS

- BMS284756 showed activity comparable or two-fold superior to CIPRO that confirms earlier studies.
- The Etest MIC values were generally four-fold lower compared to the agar dilution MIC values.
- Disk diffusion versus MIC results led to an acceptably good correlation indicating the possibility of using disk diffusion as a routine susceptibility test method for gonococci against BMS284756.
- The conservative susceptible disk diffusion breakpoint of ≥ 32 mm zone diameter was able to discriminate *N. gonorrhoeae* strains with reduced susceptibility to BMS284756 (MICs, 0.5 or 1 µg/ml).