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

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ABSTRACT/ AMENDED

Purpose: To determine the *in vitro* activity and MIC/disk test comparison of BMS284756 against isolates of staphylococci and enterococci from the SENTRY Program, 2000.

Methods: Isolates were tested by reference broth and disk diffusion methods.

Results: 300 staphylococci (208 *S. aureus*, 92 coagulase-negative) and 102 enterococci were tested against BMS284756. The activity of BMS284756 was highly correlated with oxacillin resistance among staphylococci. Oxacillin susceptible staphylococci (n=201) were all inhibited by BMS284756 at ≤0.5 µg/ml whereas oxacillin resistant strains (n=99) required ≥1 µg/ml. Excellent correlation was observed between the MIC and 5-µg disk zone diameters for staphylococci (r=0.91). Among enterococci, 47% of *E. faecalis*, 4% of *E. faecium*, and 68% of *Enterococcus* spp. were inhibited by BMS284756 at ≤1 µg/ml. All vancomycin-resistant enterococci (VRE, 11 *E. faecalis* and 15 *E. faecium*) were inhibited by ≥ 2 µg/ml. Among the non-VRE, non-*faecium* isolates (n=61), 64% were inhibited by ≤1 µg/ml. Comparison of MICs and zone diameters revealed excellent correlation (r=0.93) through breakpoints of ≤4 µg/ml.

Conclusions: BMS284756 showed excellent activity against oxacillin susceptible staphylococci and moderate activity against enterococci other than VRE and *E. faecium*. Excellent correlations were observed between MIC and disk tests for staphylococci and enterococci

INTRODUCTION

- Infections caused by Gram-positive cocci, especially staphylococci and enterococci are a major problem world-wide. Quinolones are used widely for both empiric and directed therapy due to their excellent spectrum and potency enhanced by favorable pharmacokinetics. Newer advanced generation fluoroquinolone derivatives (gatifloxacin, gemifloxacin, moxifloxacin) with expanded activity against Gram-positive pathogens are being introduced in an effort to meet the ever increasing challenge of resistance.
- BMS284756 is a novel des-fluoro (6)-quinolone. These des-fluoro compounds have been shown to have lower cerebral toxicity in mice. Previous studies have demonstrated comparable *in vitro* activity to that of gatifloxacin and trovafloxacin, even against anaerobic species.
- As the newer, more potent quinolones become available for therapy, it is important to develop *in vitro* susceptibility testing criteria for staphylococci and enterococci. The present study was designed to evaluate the potency of BMS284756 against staphylococci and enterococci and to provide MIC and disk test methods comparison data.

MATERIALS AND METHODS

Organisms tested. A total of 300 strains of staphylococci (208 *S. aureus* and 92 coagulase-negative staphylococci [CoNS]) and 102 enterococci (47 *E. faecalis*, 27 *E. faecium*, 28 *Enterococcus* spp.) from the SENTRY 2000 organism collection were tested using reference broth microdilution and standardized disk diffusion methods. The staphylococci included 187 oxacillin-susceptible strains (148 *S. aureus*, 39 CoNS) and 113 oxacillin-resistant strains (60 *S. aureus*, 53 CoNS). Among the enterococci, 76 were vancomycin-susceptible (36 *E. faecalis*, 12 *E. faecium*, 28 *Enterococcus* spp.) and 26 were vancomycin-resistant (11 *E. faecalis*, 15 *E. faecium*). Isolates were selected to represent strains from North America, Latin America, and Europe.

MATERIALS AND METHODS (continued)

Antimicrobials tested. BMS284756 was provided by Bristol-Myers Squibb (Princeton, NJ, USA). BD Microbiological Systems (Cockeysville, MD, USA) provided the 5-µg BMS284756 disks.

Susceptibility methods. All the tests were performed in accordance with NCCLS [2000a and b] standards. The tests were validated using the following quality control strains: *Staphylococcus aureus* ATCC 25923 and 29213, and *Enterococcus faecalis* ATCC 29212. MIC results and disk inhibitory zone diameters were analyzed by regression statistics to determine inter-method correlation and a breakpoint suggested by Fung-Tomcet al. (2000) was applied to propose breakpoint criteria (≤ 4 µg/ml).

RESULTS

- The rank order of quinolone potency versus the staphylococci (blood stream isolates; 3,789 strains) was: BMS284756 (MIC_{50}, ≤ 0.03 - 0.12 µg/ml) > gatifloxacin > levofloxacin > ciprofloxacin (Table 1). BMS284756 had a 20-30% wider spectrum than comparison drugs, levofloxacin and ciprofloxacin.
- BMS284756 was superior or equal in potency versus enterococci (Table 1) and using proposed breakpoints inhibited 76.2% of strains tested (> 20% more than comparators).
- The activity of BMS284756 was highly correlated with oxacillin resistance among staphylococci (Table 2). Oxacillin-susceptible staphylococci (n=187) were all inhibited by BMS284756 at ≤ 0.5 µg/ml, whereas oxacillin-resistant strains (n=113) required ≥ 1 µg/ml.
- Overall, 97.6% of staphylococci were susceptible to BMS284756 at the ≤ 4 µg/ml breakpoint concentration proposed by the manufacturer.
- Among the tested enterococci, 47% of *E. faecalis*, 4% of *E. faecium*, and 64% of *Enterococcus* spp. were inhibited by BMS284756 at ≤ 0.5 µg/ml (Table 3).
- All vancomycin-resistant enterococci (VRE; 11 *E. faecalis* and 15 *E. faecium*) were inhibited by concentrations at ≥ 2 µg/ml.
- Among the non-VRE, non-*E. faecium* (n=64), 62% of strains were inhibited by ≤0.5 µg/ml and 84% were inhibited by ≤ 4 µg/ml. Only one-half of non-VRE *E. faecium* isolates were inhibited by ≤ 4 µg/ml of BMS284756.
- Overall, 75.5% of enterococci were inhibited by ≤ 4 µg/ml of BMS284756.
- Figure 1 illustrates the comparison of BMS284756 MIC and disk (5-µg) inhibitory zone diameter results for staphylococci. The diverse population of strains produced an excellent linear correlation between tests (y=14.6 - 0.33x; r=0.91). Oxacillin-resistant strains were clearly positioned at the upper limits of the BMS284756 MIC distribution.
- The susceptibility breakpoint of ≤ 4 µg/ml suggested by Fung-Tomcet al. (2000) and the accompanying zone diameter of ≥ 12 mm (solid lines) classifies most of the oxacillin-resistant strains as susceptible to BMS284756 (Figure 1). A more conservative breakpoint of ≤ 0.5 µg/ml (≥ 22 mm; dotted lines in Figure 1) clearly separates the oxacillin-susceptible and -resistant populations.
- Figure 2 illustrates the comparison of BMS284756 MIC and inhibitory zone diameter results for enterococci. Again an excellent linear correlation was observed between tests (y = 14.2 - 0.29x; r=0.93) through the proposed breakpoint of ≤ 4 µg/ml. Again, a more conservative breakpoints of ≤ 0.5 µg/ml (≥ 20 mm) separates two distinct populations of isolates (broken lines).

RESULTS (continued)

FIGURE 1

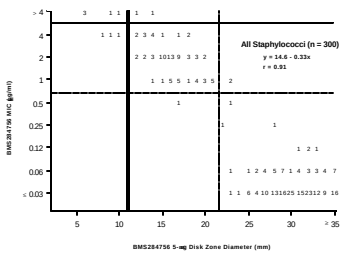

Figure 1: Scattergram comparing the reference broth dilution BMS284756 MICs and zone diameters around 5-µg disks for staphylococci (300 strains; r=0.91). Suggested breakpoints are shown as solid vertical and horizontal lines. More conservative criteria are noted as broken lines.

FIGURE 2

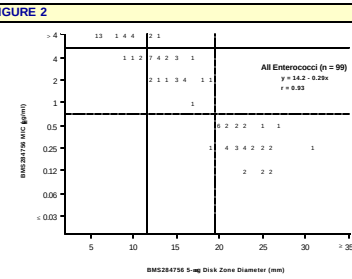

Figure 2: Scattergram comparing the reference broth dilution BMS284756 MICs and zone diameters around 5-µg disks for enterococci (102 strains; r=0.93). Suggested breakpoints are shown as solid vertical and horizontal lines. More conservative criteria are noted as broken lines.

RESULTS (continued)

TABLE 1: Comparative antimicrobial activity of BMS284756 tested against staphylococci (3,789 strains) and enterococci (877 strains) isolated from blood stream infections (SENTRY Antimicrobial Surveillance Program, 2000).

Organism (no. tested)	*Quinolone*	*MIC (µg/ml) 50%*	*MIC (µg/ml) 90%*	*% inhibited at MIC (µg/ml) ≤1*	*% inhibited at MIC (µg/ml) 2*	*% inhibited at MIC (µg/ml) 4*
S. aureus (2,555)	BMS284756	≤0.03	4	77.6	89.3	(97.1) [a]
	Ciprofloxacin	0.5	>2	(67.5)	68.4	-
	Gatifloxacin	0.12	4	69.7	(78.8)	91.0
	Levofloxacin	0.25	>4	68.5	(69.7)	78.5
CoNS (1,234) [c]	BMS284756	0.12	4	68.4	86.1	(96.0)
	Ciprofloxacin	1	>2	(50.7)	52.0	-
	Gatifloxacin	0.25	2	58.2	(90.1)	96.2
	Levofloxacin	0.5	>4	51.9	(57.6)	72.0
Enterococci (877)	BMS284756	1	>4	51.3	62.0	(76.2)
	Ciprofloxacin	>2	>2	(39.9)	50.6	-
	Gatifloxacin	1	>4	50.5	(54.0)	56.3
	Levofloxacin	2	>4	43.2	(53.1)	54.4

a. Percentage in parenthesis indicates the susceptible rate [NCCLS, 2001]. The breakpoint of ≤ 4 µg/ml was used for BMS284756 [Fung-Tomcet al., 2000].
b. - = untested concentration.
c. CoNS = coagulase-negative staphylococci

TABLE 2: In vitro susceptibility of 300 clinical isolates of oxacillin-susceptible and -resistant staphylococci to BMS284756[a]

Species	*Oxacillin susceptibility (no. tested)[b]*	*BMS284756 MIC (µg/ml) Range*	*BMS284756 MIC (µg/ml) 50%*	*BMS284756 MIC (µg/ml) 90%*	*Cum. % susceptible at (µg/ml) ≤0.25*	*0.5*	*1*	*0.5*	*0.5*
S. aureus	Susceptible (148)	≤0.03-0.5	≤0.03	0.06	99	100	100	100	100
	Resistant (60)	1->4	2	>4	0	0	22	73	88
Coag.-neg. staphylococci	Susceptible (39)	≤0.03-0.5	0.06	0.06	98	100	100	100	100
	Resistant (53)	1->4	2	4	0	0	36	77	95

a. Isolates from the SENTRY Antimicrobial Surveillance Program, 2000.
b. Oxacillin susceptibility categories according to NCCLS (2001) guidelines.

TABLE 3: In vitro susceptibility of 102 clinical isolates of vancomycin-susceptible and -resistant enterococci to BMS284756[a]

Species	*Vancomycin susceptibility (no. tested)[b]*	*BMS284756 MIC (µg/ml) Range*	*BMS284756 MIC (µg/ml) 50%*	*BMS284756 MIC (µg/ml) 90%*	*Cum. % susceptible at (µg/ml) ≤0.25*	*0.5*	*1*	*2*	*4*
E. faecalis	Susceptible (36)	0.12->4	0.25	>4	44	61	61	67	83
	Resistant (11)	2-4	2	4	0	0	0	64	100
E. faecium	Susceptible (12)	0.5->4	4	>4	0	8	8	25	50
	Resistant (15)	4->4	>4	>4	0	0	0	0	40
Enterococcus spp.[c]	Susceptible (28)	0.12->4	0.5	>4	36	64	68	76	84

a. Isolates from the SENTRY Antimicrobial Surveillance Program, 2000.
b. Vancomycin susceptibility criteria according to NCCLS [2001] guidelines.
c. Includes *E. avium* (one isolate), *E. casseliflavus* (seven isolates), *E. durans* (two isolates), *E. gallinarum* (four isolates), *E. raffinosus* (two isolates) and *Enterococcus* spp. (12 isolates).

SELECTED REFERENCES

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CONCLUSIONS

- BMS284756 showed excellent activity against oxacillin-susceptible staphylococci and more moderate activity against enterococci other than VRE and *E. faecium*
- Excellent correlations were observed between MIC and disk test results for staphylococci and enterococci indicating the possibility of additionally using disk diffusion as a routine susceptibility test method for these organisms against BMS284756.
- The lower toxicity of BMS284756 may support administration of higher doses (> 400 mg) for treatment of Gram-positive infections. In that situation, it may be reasonable to use the higher breakpoint of ≤ 4 µg/ml and correlate zone diameters.