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Correlations of Broth Microdilution MIC and Disk Diffusion Results for an Investigational Agent, BC-3205 Among Potentially Indicated Species

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Abstract

Objectives: To determine the correlation between the CLSI reference broth microdilution (BMD) MIC (mg/l) and the zone diameter diffusion (mm) results obtained for BC-3205 when tested against targeted Gram-positive pathogens. BC-3205 is semi-synthetic pleuromutilin derivative that interferes with bacterial protein synthesis. Cross resistance with other antimicrobial classes has not been observed with BC-3205 and it is being developed for the treatment of skin and skin structure infections (SSSI) including multidrug-resistant (R) species.

Methods: Recent (2006-2009) clinical isolates of *S. aureus* (214), coagulase-negative staphylococci (102), *E. faecium* (EFM; 112), beta-haemolytic streptococci (202) and viridans group streptococci (100) were tested by CLSI BMD and disk diffusion (20-µg) using reference methods and appropriate media (M07-A8, 2009; M02-A10, 2009). Staphylococcal strains with non-wildtype (non-WT; ≥2 mg/l) MIC values for other pleuromutilin compounds (carrying *vga*[A] or *cfr*) were included in the study to determine tentative epidemiologic cutoff values (ECV) for MIC and disk diffusion tests with BC-3205. Comparator agents included azithromycin (AZ), linezolid (LZ) and clindamycin (CL).

Results: Using tentative ECV breakpoints (≤1 mg/l and ≥20 mm) BC-3205 produced rare intermethod errors (see Figure). Excellent discrimination by BMD between WT and non-WT populations was evident among staphylococci, regardless of methicillin susceptibility. A single non-WT MRSA strain (MIC, 1 mg/l) fell below the proposed ECV (≤1 mg/l) introducing a major interpretive error (0.1 %). The WT EFM population contained MIC values at ≤1 mg/l and zone diameters ≥20 mm with no zones between 16-19 mm, enabling excellent separation between WT and non-WT EFM (MICs, ≥2 mg/l). BC-3205 scattergrams for the streptococci demonstrated dominant susceptibility (MICs, ≤0.5 mg/l and zone diameters at ≥21 mm). Intermethod interpretive agreement for the control agents ranged from 98.6 (AZ, CL) to 100.0 % (LZ).

Conclusions: Correlations of 20-µg BC-3205 zone diameters with the CLSI reference MIC values were excellent and extremely rare intermethod error (0.1 %) was noted when using a susceptible or ECV breakpoints of ≤1 mg/l and ≥20 mm. These tentative criteria should be considered for use in the early clinical trials. Significant cross-R or -susceptibility with other agents (macrolides, oxazolidinones, lincosamides) was not observed.

Introduction

The pleuromutilin class of antimicrobial agents have a novel mode of action involving the inhibitition of protein synthesis by binding to the peptidyl transferase component of the 50S subunit of ribosomes. BC-3205, a member of this class of agents, is highly active against Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), and Gram-negative pathogens associated with community-acquired bacterial pneumonia.

A disk content study was performed to determine the appropriate disk concentration to be applied for BC-3205 against target pathogens. Data derived from the preliminary investigation established that the 20-µg disk should be evaluated more thoroughly as the most appropriate concentration for providing correlation with MIC values and to differentiate the wildtype susceptible isolates from the resistant organism population.

This current evaluation of the 20-µg disk concentration utilized a large collection to determine the correlation between the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution MIC values and disk diffusion zone diameter results for BC-3205 and targeted pathogens including, staphylococci, streptococci, and *Enterococcus faecium*.

Materials and Methods

Bacterial isolates: A total of 730 recent (2008-2009) clinical isolates were tested from patients hospitalized in North America (USA; 52.2 %), Europe (39.2 %) and smaller numbers in the Asia-Pacific Region and Latin America. Isolates included methicillin-susceptible *S. aureus* (MSSA; 102), methicillin-resistant *S. aureus* (MRSA; 112 strains, including various SCC*mec*A types and USA community-acquired clones). Coagulase-negative *Staphylococcus* spp. (CoNS) included equal numbers of methicillin-susceptible (51) and methicillin-resistant (51) strains. The non-wildtype sub-population (eight strains, MIC values of 2 to >16 mg/l) was selected based on elevated MIC values for other pleuromutilin compounds (retapamulin and/or tiamulin) and was included in the study to facilitate the evaluation of the epidemiologic MIC and disk diffusion categorical breakpoints for BC-3205. *E. faecium* isolates (112 strains) included vancomycin-susceptible (78 strains) and vancomycin-resistant strains (34 strains; VanA and VanB phenotypes). Streptococci (302 strains) included β-haemolytic streptococci (groups A, B, C, F and G; 202 strains) and viridans group streptococci (≥6 species including *S. bovis*-group; 100 strains).

Susceptibility testing: MIC values for pathogens were determined using the reference CLSI broth microdilution method as described in M07-A8 (2009). Disk diffusion per the CLSI M02-A10 (2009) method using commercially prepared (Remel, Lenexa, Kansas, USA) 150 mm agar plates containing, Mueller-Hinton agar or Mueller-Hinton agar with 5 % sheep blood for streptococci. Frozen-form assay panels were produced by JMI Laboratories (North Liberty, Iowa, USA) consisting of two media types, cation-adjusted Mueller-Hinton broth and cation-adjusted Mueller-Hinton broth with 2-5 % lysed horse blood (for testing of streptococci). BC-3205 disks (20-µg) were provided by MAST Group (Merseyside, United Kingdom). Comparison susceptibility disks were provided by Becton-Dickinson (Sparks, Maryland, USA) and included linezolid, azithromycin and clindamycin. Direct broth suspensions of isolated colonies were diluted to achieve a final concentration of approximately 5 x 10^5 CFU/ml.

Quality control (QC) ranges and interpretive criteria for both MIC and zone diameters for comparator compounds were as published in the CLSI M100-S20 (2010) document. Tested QC strains included *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, and *Streptococcus pneumoniae* ATCC 49619. The MIC and zone diameter results were compared using analysis found in CLSI M23-A3 (2008).

Results

- The highest BC-3205 MIC value among the wildtype *S. aureus* and CoNS population was 0.25 mg/l and 1 mg/l, respectively (Table 1). The non-wildtype sub-population included eight strains of *S. aureus* with MIC values of 1 to >16 mg/l and three strains of CoNS with BC-3205 MIC value of ≥16 mg/l which were included to facilitate the evaluation of the epidemiologic cutoff values (ECV) for MIC and disk diffusion categorical breakpoints for BC-3205.
- Against *E. faecium*, BC-3205 demonstrated a wide range of activity (MIC range, 0.03 to >16 mg/l) with $MIC_{50/90}$ values of 0.12 and 16 mg/l (Table 1). Streptococcal strains were highly susceptible to BC-3205 ($MIC_{50/90}$, 0.06/0.06-0.12 mg/l).
- Figure 1 provides a graphic representation of the MIC distribution of all tested isolates with a tentative breakpoint criteria established at ≤1 mg/l for the susceptible wildtype population. The MIC range of the susceptible wildtype strains (≤0.008-1 mg/l) is clearly discernable from the non-wildtype organisms with MIC results at ≥2 mg/l.
- As observed in the scattergram analyses (Figure 2), excellent discrimination between wildtype and non-wildtype populations was evident among all staphylococci, regardless of methicillin susceptibility phenotypes.
- A single non-wildtype MRSA strain (MIC, 1 mg/l) fell below the proposed breakpoint introducing a major interpretation error event (susceptible by MIC and resistant by disk diffusion results [16 mm]) as shown in Figure 3.

Figure 1. MIC distribution of BC-3205 comparing wildtype and non-wildtype results for all strains (730)

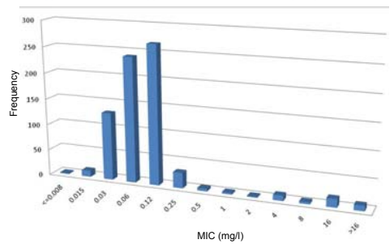
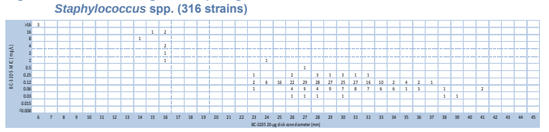


Figure 2. BC-3205 scattergram comparing MIC and zone diameter results for all *Staphylococcus* spp. (316 strains)



Includes: *Staphylococcus aureus* (214) and coagulase-negative staphylococci (102).

Figure 3. BC-3205 scattergram comparing MIC and zone diameter results for all MRSA (112 strains)

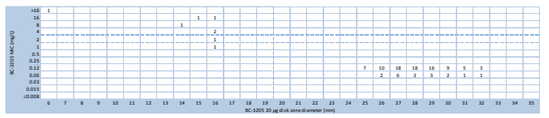
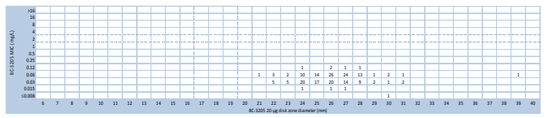
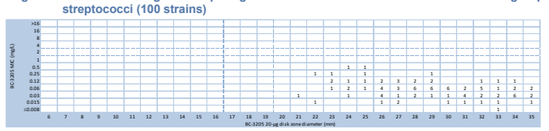


Figure 4. BC-3205 scattergram comparing MIC and zone diameter results for all β-haemolytic streptococci (202 strains)



Includes: Group A *Streptococcus* (105 strains), Group B *Streptococcus* (67 strains), Group C *Streptococcus* (nine strains), Group F *Streptococcus* (two strains), and Group G *Streptococcus* (19 strains).

Figure 5. BC-3205 scattergram comparing MIC and zone diameter results for all viridans group streptococci (100 strains)



Includes: *Streptococcus anginosus* (11 strains), *S. bovis* (10 strains), *S. constellatus* (five strains), *S. gordonii* (two strains), *S. intermedius* (one strain), *S. mitis* (27 strains), *S. oralis* (six strains), *S. parasanguinis* (nine strains), *S. salivarius* (19 strains),*S. sanguinis* (10 strains), and *S. vestibularis* (one strain).

Table 1. MIC frequency distributions of the investigational Nabriva agent BC-3205 tested against 730 Gram-positive cocci

	Percentage of strains inhibited at each MIC [mg/l] number tested (%)											
Organism (no. tested)	≤ 0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥ 16
S. aureus (214)	2 (0.9)	32 (15.0)	167 (78.0)	5 (2.3)	-			1 (0.5) [a]	1 (0.5)	2 (0.9)	1 (0.5)	3 (1.4)
Oxacillin-susceptible (102)	2 (2.0)	14 (13.7)	81 (79.4)	5 (4.9)								
Oxacillin-resistant (112)		18 (16.1)	86 (76.8)	-	-			1 (0.9) [a]	1 (0.9)	2 (1.8)	1 (0.9)	3 (2.7)
Coagulase-negative staphylococci [b] (102)	4 (3.9)	36 (35.3)	50 (49.0)	7 (6.9)	1 (1.0)	1 (1.0)		1 (1.0)[a]	-	-	-	3 (3.0)
Oxacillin-susceptible (51)	1 (2.0)	25 (49.0)	21 (41.2)	1 (2.0)	1 (2.0)	1 (2.0)[a]			-	-	-	1 (2.0)
Oxacillin-resistant (51)	3 (5.9)	11 (21.6)	29 (56.9)	6 (11.8)	-	-[a]		-	-	-	-	-
E. faecium (112)	2 (1.8)	33 (29.5)	28 (25.0)	14 (12.5)	2 (1.8)	1 (0.9)		1 (0.9)	1 (0.9)	8 (7.1)	3 (2.7)	20 (18.9)
Vancomycin-susceptible (78)	1 (1.3)	20 (25.6)	18 (23.1)	9 (11.5)	1 (1.3)	1 (1.3)		1 (1.3)	1 (1.3)	6 (7.7)	3 (3.9)	18 (23.1)
Vancomycin-resistant (34)	1 (2.9)	13 (38.2)	10 (29.4)	5 (14.7)	1 (2.9)	-		-	-	2 (5.9)	-	2 (5.8)
β-hemolytic streptococci [c] (202)	1 (0.5)	3 (1.5)	95 (47.0)	98 (48.5)	5 (2.5)							
Viridans streptococci group [d] (100)	1 (1.0)	9 (9.0)	27 (27.0)	41 (41.0)	16 (16.0)	4 (4.0)	2 (2.0)					

a. Epidemiologic cutoff value (ECV) representing the highest MIC for the wildtype population. BC-3205 MIC results at ≥1 mg/l (*S. aureus* and CoNS) are non-wildtype values.

b. Includes: *Staphylococcus auricularis* (one strain), *S. capitis* (eight strains), *S. caprae* (one strain), *S. carnosus* (one strain), *S. chromogenes* (one strain), *S. epidermidis* (53 strains), *S. haemolyticus* (five strains), *S. hominis* (14 strains), *S. lugdunensis* (eight strains), *S. schleiferi* (one strain), *S. simulans* (three strains), *S. succinus* (one strain) and *S. warneriil* (four strains).

c. Includes: Group A *Streptococcus* (105 strains), Group B *Streptococcus* (67 strains), Group C *Streptococcus* (nine strains), Group F *Streptococcus* (two strains), and Group G *Streptococcus* (19 strains).

d. Includes: *Streptococcus anginosus* (11 strains), *S. bovis* (10 strains), *S. constellatus* (five strains), *S. gordonii* (two strains), *S. intermedius* (one strain), *S. mitis* (27 strains), *S. oralis* (six strains), *S. parasanguinis* (nine strains), *S. salivarius* (19 strains), *S. sanguinis* (10 strains), and *S. vestibularis* (one strain).

- *E. faecium* had a wildtype population that contains MIC values at ≤1 mg/l and zone diameters at ≥20 mm. No zones were generated by *E. faecium* strains at 16-19 mm, enabling excellent separation between the two populations of *E. faecium* isolates.
- The BC-3205 scattergram for the β-haemolytic and viridans streptococci (Figures 4 and 5) illustrate dominant susceptibility to BC-3205 with MIC values at ≤0.5 mg/l and zone diameters at ≥21 mm.
- If an intermediate category was defined at 2 mg/l and zones of 17-19 mm, the calculated error rates would be: very major error (false-susceptible by disk tests) = 0.0 %, major error (false-resistant by disk tests) = 1 strain (0.1 %), minor error (intermediate by one of the compared tests) = 2 occurrences (0.4 %).
- The *S. aureus* and CoNS isolates that exhibited a non-wildtype phenotype for BC-3205 harbored *cfr* or *vga*(A). Staphylococcal isolates recovered from human clinical species carrying these resistance determinants are extremely rare.

Conclusions

- All BC-3205 MIC versus zone diameter scattergrams indicate that the proposed/tentative ECV and susceptible breakpoints, regardless of species tested, provided a near perfect correlation (99.4 % absolute categorical agreement) between *in vitro* test results. Only a single strain would produce a potential serious major error using the proposed ECV/breakpoints from this study.
- Correlations of 20-µg BC-3205 disk zone diameters with the CLSI reference MIC values were excellent with extremely rare (0.4 %) intermethod error when using a susceptible (ECV) breakpoint of ≤1 mg/l (≥20 mm). These tentative criteria should be considered for early clinical trials.
- Cross-resistance with other agents (macrolides, oxazolidinones, lincosamides) was not demonstrated.
- BC-3205 shows promising activity against the most prevalent Gram-positive pathogens producing skin and skin structure infections. Pending appropriate clinical trial studies, BC-3205 remains a promising adjunct for management of cutaneous bacterial infections and has accurate MIC and disk diffusion test methods with tentative breakpoints.

Selected References

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