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Activity of BC-3205 When Tested against a Collection of Gram-positive Organisms Responsible for Skin and Skin Structure Infections

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Abstract

Objectives: To assess the *in vitro* activity for BC-3205, a novel semi-synthetic pleuromutilin, against a contemporary collection of Gram-positive cocci. Pleuromutilins inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. BC-3205 is in early stage clinical development for oral treatment of multidrug-resistant (R) skin and skin structure infections (SSSI).

Methods: Over 800 unique contemporary clinical isolates of Gram-positive organisms tested for susceptibility (S) by broth microdilution methods (CLSI, M07-A8) to BC-3205 and comparator agents, including azithromycin (AZ), clindamycin (CL) and linezolid (LZ). The organisms tested were *S. aureus* (SA; 314), coagulase-negative staphylococci (CoNS; 99), *E. faecium* (EFM; 112), beta-haemolytic streptococci (BHS; 202) and viridans group streptococci (VGS; 100). Interpretation of results was guided by CLSI (M100-S19, 2009) and EUCAST criteria.

Results: BC-3205 was very active against SA ($MIC_{50/90}$, 0.12/0.12 mg/l) and showed similar potency against methicillin-S (MSSA) and -R (MRSA) strains. Against MRSA, BC-3205 ($MIC_{50/90}$, 0.06/0.12 mg/l) showed significantly greater activity than CL ($MIC_{50/90}$, 0.12/>16 mg/l), LZ ($MIC_{50/90}$, 2/2 mg/l) and AZ ($MIC_{50/90}$, >16/>16 mg/l). Methicillin-S (MSCoNS) and -R CoNS (MRCoNS) were also very S to BC-3205 (see Table). LZ ($MIC_{50/90}$, 1/1 mg/l) was also very potent against CoNS, but eight- to 16-fold less active than BC-3205. CL and AZ showed more limited activity against CoNS, especially MRCoNS strains ($MIC_{50/90}$, 0.12/>16 and >16/>16 mg/l, respectively). BC-3205 was highly active against vancomycin-S (VS) and vancomycin-R (VR) EFM (MIC_{50}, 0.12 mg/l for both). BHS strains were very S to BC-3205 ($MIC_{50/90}$, 0.06/0.06 mg/l). BC-3205 ($MIC_{50/90}$, 0.06/0.12 mg/l) was four- and 16-fold more active than AZ ($MIC_{50/90}$, 0.25/8 mg/l) and LZ ($MIC_{50/90}$, 1/1 mg/l); but slightly less potent than CL ($MIC_{50/90}$, 0.03/0.12 mg/l), when tested against VGS.

Conclusions: BC-3205 was very active against a representative collection of contemporary pathogens associated with SSSI. Pending further pharmacokinetic/pharmacodynamic and early clinical trial studies, BC-3205 appears to be a promising treatment for cutaneous infections caused by R Gram-positive organisms.

Organism (no. tested)	No. (cumulative percentage) of strains inhibited at BC-3205 MIC [mg/l] of: ≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
MSSA (102)	2 (2.0)	14 (15.7)	81 (95.1)	5 (100.0)						
MRSA (212)	18 (8.5)	106 (58.5)	88 (100.0)							
MSCoNS (50)	1 (2.0)	25 (52.0)	21 (94.0)	1 (96.0)	1 (98.0)	1 (100.0)				
MRCoNS (49)	3 (5.9)	11 (28.6)	29 (87.8)	6 (100.0)						
VS-EFM (78)	1 (1.3)	20 (26.9)	18 (50.0)	9 (61.5)	1 (62.8)	1 (64.1)	1 (65.4)	6 (73.1)	3 (76.9)	18 (100.0)
VR-EFM (34)	1 (2.9)	13 (41.2)	10 (70.6)	5 (85.3)	1 (88.2)	1 (88.2)	1 (88.2)	2 (94.1)	2 (94.1)	2 (100.0)
BHS (202)	99 (49.0)	98 (97.5)	5 (100.0)							
VGS (100)	37 (37.0)	41 (78.0)	16 (94.0)	4 (98.0)	2 (100.0)					

Introduction

Pleuromutilins are natural antibiotics that were first isolated in 1951 from basidiomycetes *Pleurotus* and *Pleurotus passeckerianus*. Further studies have shown that this class of antibiotics selectively inhibit bacterial protein synthesis by specifically targeting the 50S subunit of the bacterial ribosome.

Over the past years, a few pleuromutilin analogues have been developed for use in veterinary and human medicine. Tiamulin and valnemulin were successfully developed as veterinary medicines to treat serious infections in swine and poultry. More recently, another pleuromutilin analogue with excellent activity *in vitro*, retapamulin, was the first approved for human use as a topical antimicrobial agent to treat skin infections.

BC-3205 is a novel semi-synthetic pleuromutilin which is being developed for systemic treatment of human infections. BC-3205 is in early stage clinical development for oral treatment of skin and skin structure infections (SSSI), including those caused by multidrug-resistant Gram-positive cocci. The objective of this study was to assess the *in vitro* activity for BC-3205, against a contemporary collection of Gram-positive cocci.

Methods

Susceptibility testing:

A. MIC values for pathogens were determined using the reference CLSI broth microdilution method as described in M07-A8 (2009).

B. 96-well frozen-form assay panels were produced by JMI Laboratories and consisted 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). Inocula were prepared by making direct broth suspensions of isolated colonies selected from an 18- to 24-hour agar plate. These inoculum preparations (0.5 McFarland standard or approximately 1 to 2 x 10^8 CFU/ml) were diluted to achieve a final concentration of approximately 5 x 10^5 CFU/ml and used to inoculate the wells of the MIC panels.

C. Quality control (QC) ranges and interpretive criteria for both MIC and zone diameters for comparator compounds were as published in the CLSI M100-S19 (2009) document; tested QC strains included *Staphylococcus aureus* ATCC 29213 (MIC QC range for BC-3205 was 0.03–0.25 mg/l), and *Streptococcus pneumoniae* ATCC 49619 (MIC QC range was 0.06–0.25 mg/l). These ranges were determined in an earlier JMI Laboratories study (ECCMID 2010, Poster # P912).

Antimicrobial agents:

A. BC-3205: MIC concentrations of 16-0.008 mg/l.

B. Controls: azithromycin, linezolid and clindamycin.

Organisms:

A. A total of 827 recent (2008-2009) clinical isolates were tested from samples distributed by species or genus groups as follows: North America (USA; 51.0 %), Europe (40.0 %), Asia-Pacific Region (8.7 %) and Latin America (0.3 %).

B. Species/genus groups

1. *S. aureus* (314 strains)
   a. Methicillin-susceptible *S. aureus* (MSSA; 102)
   b. Methicillin-resistant *S. aureus* (MRSA; 212 strains). Representing strains from various SCC*mec*A types and USA typed clones including USA300 community-acquired MRSA.
2. Coagulase-negative *Staphylococcus* spp. (CoNS; 99 strains)
   a. Methicillin-susceptible CoNS (50 strains)
   b. Methicillin-resistant CoNS (49 strains).
3. *E. faecium* (112 strains)
   a. Vancomycin-susceptible (78 strains)
   b. Vancomycin-resistant (34 strains; VanA and VanB phenotypes).
4. Streptococci (302 strains)
   a. β-haemolytic streptococci (BHS; groups A, B, C, F and G; 202 strains).
   b. Viridans group streptococci (VGS; ≥6 species including *S. bovis*-group; 100 strains). All isolated from documented bacteremias.

Results

- All QC results for data generated, based on CLSI M23-A3 (2008) and included in this report, were within the ranges as specified by the CLSI (M100-S19, 2009) or determined in an earlier JMI Laboratories report (ECCMID 2010, Poster # P912).
- BC-3205 demonstrated potent activity against a large collection of contemporary Gram-positive organisms collected within the SENTRY Program platform (719 strains; $MIC_{50/90}$, 0.06/0.25 mg/l) and exhibited superior activity when compared to the other tested antimicrobial agents azithromycin, clindamycin and linezolid (Tables 1 and 2).
- *S. aureus* (314 isolates) was very susceptible to BC-3205 and this compound showed similar antimicrobial activity against MSSA ($MIC_{50/90}$, 0.12/0.12 mg/l) and MRSA isolates ($MIC_{50/90}$, 0.06/0.12 mg/l for both groups). The highest BC-3205 MIC value was 0.25 mg/l; Tables 1 and 2).
- BC-3205 activity against CoNS was similar to that against *S. aureus* (MIC_{90}, 0.12 mg/l for both organisms). CoNS susceptibility to BC-3205 was minimally influenced by oxacillin resistance and BC-3205 activity against CoNS was greater than those shown by the comparators azithromycin, clindamycin and linezolid (Tables 1 and 2).
- BC-3205 demonstrated a wide range of activity against *E. faecium* (MIC range, 0.03->16 mg/l), with MIC_{50} and MIC_{90} values of 0.12 and 16 mg/l, respectively (Table 1). BC-3205 was eight- and 16-fold more active than vancomycin (MIC_{50}, 1 mg/l; data not shown) and linezolid (MIC_{50}, 2 mg/l), respectively, when tested against *E. faecium* (Table 2).
- Vancomycin-resistant *E. faecium* ($MIC_{50/90}$, 0.12/4 mg/l) strains exhibited BC-3205 MIC values slightly lower than vancomycin-susceptible strains ($MIC_{50/90}$, 0.12/16 mg/l; Tables 1 and 2).
- BC-3205 showed potent activity against β-haemolytic streptococcal strains ($MIC_{50/90}$, 0.06/0.06 mg/l). The highest BC-3205 MIC value observed was 0.12 mg/l (2.5 % of strains; Tables 1 and 2).
- Viridans group streptococcal strains were also very susceptible to BC-3205 ($MIC_{50/90}$, 0.06/0.12 mg/l). BC-3205 was four- and 16-fold more active than azithromycin ($MIC_{50/90}$, 0.25/8 mg/l; 51.0 % susceptible) and linezolid ($MIC_{50/90}$, 1/1 mg/l; 100 % susceptible), respectively. In addition, BC-3205 and clindamycin ($MIC_{50/90}$, 0.03/0.12 mg/l; 91.0 % susceptible) showed similar activity when tested against these organisms.
- Cross-susceptibility analysis of BC-3205 compared to azithromycin, clindamycin and linezolid indicate no significant correlation between BC-3205 MIC values and those of these comparator agents (data not shown).

Table 1. MIC frequency distributions of the investigational Nabriva agent BC-3205 tested against 827 Gram-positive cocci

Organism (no. tested)	Percentage of strains inhibited at each MIC [mg/l] number tested (%) ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
S. aureus (314)											
Oxacillin-susceptible (102)		2 (2.0)	14 (13.7)	81 (79.4)	5 (4.9)						
Oxacillin-resistant (212)			18 (8.5)	106 (50.0)	88 (41.5)						
CoNS (99)		1 (2.0)	25 (50.0)	21 (42.0)	1 (2.0)	1 (2.0)	1 (2.0)				
Oxacillin-susceptible (50)											
Oxacillin-resistant (49)		3 (6.1)	11 (22.4)	29 (59.2)	6 (12.2)						
E. faecium (112)		1 (1.3)	20 (25.6)	18 (23.1)	9 (11.5)	1 (1.3)	1 (1.3)	1 (1.3)	6 (7.7)	3 (3.9)	18 (23.1)
Vancomycin-susceptible (78)											
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-haemolytic streptococci (202)	4 (1.5)	95 (47.0)	98 (48.5)	5 (2.5)							
Viridans streptococci group (100)	10 (9.0)	27 (27.0)	41 (41.0)	16 (16.0)	4 (4.0)	2 (2.0)					

Table 2. Antimicrobial activity of BC-3205 and comparator antimicrobial agents when tested against 719 clinical isolates of Gram-positive cocci

Antimicrobial agent (no. tested)	MIC_{50} [mg/l]	MIC_{90} [mg/l]	Range	CLSI[a] %S / %R	EUCAST[a] %S / %R
MSSA (102)					
BC-3205	0.12	0.12	0.03–0.25	- / -	- / -
Azithromycin	1	>16	0.25–>16	79.4 / 20.6	74.5 / 20.6
Clindamycin	0.12	0.25	0.06–>16	95.1 / 4.9	92.2 / 4.9
Linezolid	2	2	0.5–4	100.0 / -	100.0 / 0.0
MRSA (212)					
BC-3205	0.06	0.12	0.06–0.12	- / -	- / -
Azithromycin	>16	>16	0.5–>16	12.3 / 87.3	11.7 / 87.7
Clindamycin	0.12	>16	0.12–>16	68.3 / 31.7	68.3 / 31.7
Linezolid	2	2	1–>16	100.0 / 0.0	100.0 / 0.0
CoNS (99)					
MSCoNS (50)					
BC-3205	0.06	0.12	0.03–1	- / -	- / -
Azithromycin	0.5	>16	0.12–>16	66.0 / 34.0	66.0 / 34.0
Clindamycin	0.12	0.25	0.06–>16	94.0 / 6.0	94.0 / 6.0
Linezolid	1	1	0.25–2	100.0 / -	100.0 / 0.0
MRCoNS (49)					
BC-3781	0.06	0.12	0.015–0.5	- / -	- / -
Azithromycin	>16	>16	0.25–>16	27.5 / 72.5	27.5 / 72.5
Clindamycin	0.12	>16	0.06–>16	58.8 / 39.2	56.9 / 41.2
Linezolid	1	2	0.5–2	100.0 / 0.0	100.0 / 0.0
Vancomycin-susceptible *E. faecium (78)*					
BC-3202	0.12	>16	0.03–>16	- / -	- / -
Azithromycin	>16	>16	0.12–>16	- / -	- / -
Clindamycin	>16	>16	0.12–>16	- / -	- / -
Linezolid	2	2	1–8	97.4 / 1.3	98.7 / 1.3
Vancomycin-resistant *E. faecium (34)*					
BC-3205	0.12	4	0.03–>16	- / -	- / -
Azithromycin	>16	>16	4–>16	- / -	- / -
Clindamycin	>16	>16	0.12–>16	- / -	- / -
Linezolid	2	2	1–8	94.1 / 5.9	94.1 / 5.9
β-haemolytic streptococci (202)					
BC-3205	0.06	0.06	≤0.008–0.12	- / -	- / -
Azithromycin	0.12	8	≤0.008–>16	84.7 / 14.9	84.7 / 15.3
Clindamycin	0.06	0.12	0.015–>16	94.1 / 5.9	94.1 / 5.9
Linezolid	1	1	0.25–2	100.0 / -	100.0 / 0.0
Viridans group streptococci (100)					
BC-3205	0.06	0.12	≤0.008–0.5	- / -	- / -
Azithromycin	0.25	8	0.03–>16	51.0 / 47.0	- / -
Clindamycin	0.03	0.12	≤0.008–>16	91.0 / 9.0	91.0 / 9.0
Linezolid	1	1	≤0.008–2	100.0 / -	- / -

[a] Criteria as published by the CLSI [2010] and EUCAST [2009].
[B] Abbreviations: MSSA = methicillin- (oxacillin)-susceptible *Staphylococcus aureus*; MRSA = methicillin-(oxacillin)-resistant *S. aureus*; MSCoNS = methicillin- (oxacillin)-susceptible coagulase-negative staphylococci; MRCoNS = methicillin- (oxacillin)-resistant coagulase-negative staphylococci.

Conclusions

- BC-3205 was very active against *Staphylococcus* spp., including oxacillin-resistant strains. The highest BC-3205 MIC value among *S. aureus* was only 0.25 mg/l.
- BC-3205 inhibited all streptococci at ≤0.5 mg/l and *E. faecium* had a BC-3205 MIC_{50} at only 0.12 mg/l.
- This investigational agent (BC-3205) shows promising activity against the most prevalent Gram-positive pathogens producing skin and skin structure infections.

References

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