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Antimicrobial Activity of the Investigational Pleuromutilin Compound BC-3781 against Gram-positive Organisms Commonly Associated with Cutaneous Infections

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Amended Abstract

Objectives: To determine the antimicrobial activity of BC-3781 against recent clinical isolates of Gram-positive cocci. BC-3781 is an investigational semi-synthetic pleuromutilin derivative, which inhibits ribosomal protein synthesis. BC-3781 binds to 50S ribosomal subunit and cross resistance with other antimicrobial classes is very uncommon.

Methods: Antimicrobial activity of BC-3718 and comparator agents was determined against 827 non-duplicate Gram-positive organisms, including staphylococci (413), streptococci (302) and enterococci (112). Susceptibility (S) testing used the CLSI reference broth microdilution method (M07-A8, 2009) for MIC determinations with BC-3781 and comparator drugs (azithromycin [AZ], clindamycin [CL] and linezolid [LZ]). Interpretation of MIC values was based on CLSI (M100-S20, 2010) and EUCAST (2009) S criteria.

Results: Staphylococcal isolates were very S to BC-3781 with highest MIC value being 0.25 and 1 mg/l for *S. aureus* (SA) and coagulase-negative staphylococci (CoNS), respectively. BC-3781 was eight- to 16-fold more potent than LZ and also showed greater potency compared to AZ and CL when tested against staphylococci (Table). Methicillin-S and -resistant (R) staphylococci showed similar BC-3781 MIC distributions. Highest BC-3781 MIC value among beta-haemolytic streptococci (BHS) was 0.12 mg/l. BC-3781 was slightly more active than CL and eight- to 16-fold more active than LZ when tested against BHS. Viridans group streptococci (VGS) were also BC-3781-S, but MIC values were slightly elevated compared to BHS. BC-3781 showed potent activity against vancomycin-S and -R *E. faecium* (EFM), and it was significantly more active than comparators against EFM. However, 28.6 % of EFM exhibited higher (≥2 mg/l) BC-3781 MIC values (mechanism unknown).

Conclusions: BC-3781 was very active against a contemporary (2008-2009) collection of staphylococci, streptococci and enterococci, organisms commonly associated with cutaneous infections. BC-3781 activity was not adversely influenced by R to methicillin among staphylococci or vancomycin among enterococci. Further studies are warrant to determine the role of this novel pleuromutilin for the treatment skin and skin structure infections.

Table: Activity of BC-3781 and comparator agents tested against selected Gram-positive cocci

Organism (no. tested)	BC-3781		Azithromycin		Clindamycin		Linezolid	
	$MIC_{50/90}$	%S	$MIC_{50/90}$	%S[a]	$MIC_{50/90}$	%S[a]	$MIC_{50/90}$	%S[a]
SA (314)	0.12/0.12	-	16/>16	34.1	0.12/>16	77.1	2/2	100.0
CoNS (99)	0.06/0.12	-	>16/>16	47.5	0.12/>16	76.8	1/1	99.0
BHS (202)	0.03/0.06	-	0.12/8	84.7	0.06/0.12	94.1	1/1	100.0
VGS (100)	0.12/0.5	-	0.25/8	-[a]	0.03/0.12	91.0	1/1	-[a]
EFM (112)	0.12/16	-	>16/>16	-[a]	>16/>16	-[a]	2/2	97.3

[a] Breakpoint criteria published by EUCAST; and - = no breakpoint has been established.

Introduction

BC-3781 is an investigational semi-synthetic pleuromutilin derivative. Pleuromutilin antimicrobial agents inhibit protein synthesis through interaction with the 50S ribosomal subunit and cross resistance with other antimicrobial classes is uncommon. BC-3781 has demonstrated potent antimicrobial activity against Gram-positive cocci relevant for skin and skin structure infections and Gram-negative pathogens associated with community-acquired bacterial pneumonia.

Furthermore, its antimicrobial spectrum includes methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

BC-3781 is currently under clinical development for treatment of acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP) with intravenous and oral dosing formulations. In the present study, we determined the *in vitro* antimicrobial activity of BC-3781 tested against recent clinical isolates of Gram-positive cocci collected worldwide (2008-2009).

Materials and Methods

Susceptibility Testing: MIC values for pathogens were determined using 96-well frozen-form assay panels were produced by JMI Laboratories (North Liberty, Iowa, USA) 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). Quality control (QC) ranges and interpretive criteria for comparator compounds were as published in the CLSI M100-S20 (2010) document, and the EUCAST (2009) breakpoints were also applied. Tested QC strains included: *S. aureus* ATCC 29213 (MIC QC range for BC-3781, 0.06 - 0.25 mg/l) and *Streptococcus pneumoniae* ATCC 49619 (MIC QC range, 0.06 - 0.5 mg/l). These ranges were determined in an earlier JMI Laboratories report (ECCMID 2010, Poster # P912).

Organisms: A total of 827 contemporary (2008-2009) clinical isolates were tested from samples distributed by species or genus groups as follows: *S. aureus* (314 strains; 67.5 % MRSA), coagulase-negative *Staphylococcus* spp. (CoNS; 99 strains; 49.5 % oxacillin-resistant), *E. faecium* (112 strains; 30.4 % VRE); β-haemolytic streptococci (202 strains) and viridans group streptococci (100 strains).

All strains were isolated from patients with documented bacteremia in the following geographic regions: North America (USA; 51.0 %), Europe (40.0 %), Asia-Pacific Region (8.7 %) and Latin America (0.3 %).

Results

- BC-3781 was very active against *S. aureus* (314 isolates; Tables 1 and 2) and showed similar activity against MSSA and MRSA isolates. The highest BC-3781 MIC value among *S. aureus* was only 0.25 mg/l (Table 1). Furthermore, BC-3781 activity was greater than that of the comparator agents, especially against MRSA (Table 2).
- BC-3781 was also very active against CoNS (Tables 1 and 2) and showed very similar MIC distributions for oxacillin-susceptible and -resistant strains ($MIC_{50/90}$, 0.06/0.12 mg/l for both groups). Furthermore, BC-3781 activity against CoNS was greater than those documented for the comparators azithromycin, clindamycin and linezolid (Table 2).
- Against *E. faecium*, BC-3781 showed potent activity ($MIC_{50/90}$, 0.12/16 mg/l) and was at least eight- and 16-fold more active than the nearest comparator antimicrobial agents, vancomycin (MIC_{50}, 1 mg/l) and linezolid (MIC_{50}, 2 mg/l), respectively (Table 2).
- Linezolid demonstrated good activity against *E. faecium* ($MIC_{50/90}$, 2/2 mg/l) with 96.4 and 97.3 % of strains being susceptible according to CLSI and EUCAST breakpoints, respectively. In contrast, vancomycin ($MIC_{50/90}$, 1/>16 mg/l) was active against only 69.6 % of *E. faecium* strains at the CLSI and EUCAST breakpoints of ≤4 mg/l (Table 2). BC-3781 inhibited 71.4 % of *E. faecium* at ≤1 mg/l (Table 1).
- BC-3781 was active against vancomycin-susceptible ($MIC_{50/90}$, 0.12/>16 mg/l) and -resistant *E. faecium* ($MIC_{50/90}$, 0.12/8 mg/l). Interestingly, a higher proportion of vancomycin-susceptible strains (27 of 78; 34.6 %) exhibited elevated BC-3781 MIC values when compared to VRE strains (4 of 34; 11.8 %; Table 1).

Table 1. MIC frequency distributions of the investigational Nabriva agent BC-3781 tested against all strains (n = 827)

Organism (no. tested)	no. (%) of strains inhibited at each MIC (mg/l):												
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
S. aureus (314)			9 (2.9)	84 (26.8)	200 (63.7)	21 (6.7)	-	-					
Oxacillin-susceptible (102)			2 (2.0)	10 (9.8)	79 (77.5)	11 (10.8)							
Oxacillin-resistant (212)			7 (3.3)	74 (34.9)	121 (55.0)	10 (4.7)	-	-					
CoNS (99)		1 (1.0)	12 (12.1)	69 (69.7)	12 (12.1)	2 (2.0)	2 (2.0)	1 (1.0)	-	-	-	-	
Oxacillin-susceptible (50)			7 (14.0)	37 (64.0)	4 (8)	-	1 (2.0)	1 (2.0)	-	-	-	-	
Oxacillin-resistant (49)		1 (2.0)	5 (10.2)	32 (65.3)	8 (16.3)	2 (4.1)	1 (2.0)	-	-	-	-	-	
E. faecium (112)			2 (1.8)	16 (14.3)	46 (41.1)	10 (8.9)	5 (4.5)	1 (0.9)	1 (0.9)	-	6 (5.4)	14 (12.5)	11 (9.8)
Vancomycin-susceptible (78)			1 (1.3)	9 (11.5)	30 (38.5)	7 (9.0)	2 (2.6)	1 (1.3)	1 (1.3)	-	5 (6.4)	13 (16.7)	9 (11.5)
Vancomycin-resistant (34)			1 (2.9)	7 (20.6)	16 (47.1)	3 (8.8)	3 (8.8)	-	-	-	1 (2.9)	1 (2.9)	2 (5.9)
β-hemolytic streptococci (202)	1 (0.5)	21 (10.4)	140 (69.3)	37 (18.3)	3 (1.5)								
Viridans group streptococci (100)	3 (3.0)	4 (4.0)	11 (11.0)	20 (20.0)	27 (27.0)	24 (24.0)	7 (7.0)	2 (2.0)	2 (2.0)				

Table 2. Antimicrobial activity of BC-3781 and comparator antimicrobial agents when tested against 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
S. aureus (314)					
BC-3781	0.12	0.12	0.03 – 0.25	- / -	- / -
Azithromycin	16	>16	0.25 – >16	34.1 / 65.6	32.2 / 65.9
Clindamycin	0.12	>16	0.06 – >16	77.1 / 22.9	76.1 / 22.9
Linezolid	2	2	0.5 – >16	100.0 / 0.0	100.0 / 0.0
Oxacillin	>2	>2	≤0.25 – >2	32.5 / 67.5	32.5 / 67.5
MSSA (102)					
BC-3781	0.12	0.25	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 / 0.0	100.0 / 0.0
MRSA (212)					
BC-3781	0.12	0.25	0.06 – 0.25	- / -	- / -
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)					
BC-3781	0.06	0.12	0.015 – 1	- / -	- / -
Azithromycin	>16	>16	0.12 – >16	46.1 / 53.9	46.1 / 53.9
Clindamycin	0.12	>16	0.06 – >16	75.5 / 22.5	74.5 / 24.5
Linezolid	1	1	0.25 – >16	100.0 / 0.0	100.0 / 0.0
Oxacillin	≤0.25	>2	≤0.25 – >2	50.5 / 49.5	50.5 / 49.5
MSCoNS (50)					
BC-3781	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 / 0.0	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
E. faecium (112)					
BC-3781	0.12	16	0.03 – >16	- / -	- / -
Azithromycin	>16	>16	0.12 – >16	- / -	- / -
Clindamycin	>16	>16	0.12 – >16	- / -	- / -
Linezolid	2	2	1 – 8	96.4 / 2.7	97.3 / 2.7
Vancomycin	1	>16	0.5 – >16	69.6 / 30.4	69.6 / 30.4
Vancomycin-susceptible (78)					
BC-3781	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 (34)					
BC-3781	0.12	8	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-3781	0.03	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-3781	0.12	0.5	≤0.008 – 4	- / -	- / -
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]

- β-haemolytic streptococcal strains were highly susceptible to BC-3781 ($MIC_{50/90}$, 0.03/0.06 mg/l). The highest BC-3781 MIC value observed was 0.12 mg/l (1.5 % of strains; Table 1). BC-3781 was slightly more active (two-fold) than clindamycin ($MIC_{50/90}$, 0.06/0.12 mg/l), four-fold more active than azithromycin ($MIC_{50/90}$, 0.12/8 mg/l) and 32-fold more active than linezolid ($MIC_{50/90}$, 1/1 mg/l) when tested against β-haemolytic streptococci (Table 2).
- BC-3781 showed potent activity against viridans group streptococci ($MIC_{50/90}$, 0.12/0.5 mg/l) with an activity two- and eight-fold greater 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 contrast, BC-3781 was slightly less active than clindamycin ($MIC_{50/90}$, 0.03/0.12 mg/l; 91.0 % susceptible) against these organisms (Table 2).

Conclusions

- BC-3781 was very active against a contemporary (2008-2009) worldwide collection of staphylococci, streptococci and enterococci, organisms commonly associated with cutaneous infections.
- BC-3781 activity was not adversely influenced by resistance to methicillin among staphylococci or vancomycin among enterococci.
- Pending appropriate toxicological, pharmacokinetic/pharmacodynamic studies and clinical trial results, BC-3781 remains a promising adjunct for management of acute bacterial SSSI.

References

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