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Antimicrobial Activity of BP-102, A Novel Carbacephem, Tested Against *H. influenzae*, *M. catarrhalis* and Various Gram-Positive Cocci

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ABSTRACT

Background: BP-102 is a parenteral, bactericidal carbacephem having low protein binding and potency against some resistant (R) Gram-positive species (MRSA and multidrug-R [MDR] pneumococci [SPN]). A highly selected collection of recent clinical isolates representing MDR phenotypes was tested against BP-102 (125 strains) using reference CLSI methods.
Methods: *S. aureus* (SA; 32, all different MDR patterns), CoNS (17; 14 MDR patterns), enterococci (29; 7 species, 12 ampicillin [AMP]-R, 10 VRE, 1 linezolid-R, 7 chloramphenicol-R), SPN (18; 13 penicillin [PEN]-non-S), vir. gr. streptococci (6; 1 PEN-R), *M. catarrhalis* (6), *H. influenzae* (HI; 12, 5 AMP-R) and 5 QC strains were tested by broth microdilution methods. Results with ATCC QC strains were: SA 25923 (≤ 0.12 µg/ml) and 29213 (0.25); *E. faecalis* 29212 (1); *E. coli* 25922 (≤ 0.12) and *P. aeruginosa* 27853 (> 16).
Results: BP-102 MIC results for isolates R to ß-lactams (oxacillin, ampicillin, penicillin) were usually higher than S strains: SA (MIC_{90}, 2 vs 0.25 µg/ml), CoNS (0.25 vs ≤ 0.12), HI (1 vs 0.06) and SPN (0.25 vs 0.008); potency effected by enzyme hydrolysis and/or PBP alterations. Among 32 different MDR-MR staphylococci the highest BP-102 MIC was only 4 µg/ml (MIC_{50}, 1 µg/ml). 93% of enterococci were inhibited by BP-102 at ≤ 8 µg/ml.

	BP-102 MIC (µg/ml)			
Organism (no. tested)	Range	50%	90%	% ≤ 8 µg/ml
SA (32)	≤0.12-4	1	4	100
CoNS (17)	≤0.12-2	0.25	2	100
E. faecium (12)	0.25-16	2	8	92
HI (12)	≤0.004-2	0.06	1	100
All streptococci (24)	≤0.004-4	0.06	0.25	100

R to glycopeptides, oxazolidinones and other non-ß-lactam agents did not adversely effect BP-102 activity.
Conclusions: BP-102 is a promising anti-MRSA carbacephem with potent activity against other MDR Gram-positive pathogens. HI and *M. catarrhalis* were also inhibited (MIC_{90}, ≤ 1 µg/ml). Further development alone or with enzyme inhibitors appears warranted.

INTRODUCTION

As resistances among clinically important Gram-positive cocci continue to develop, few newer agents have highly active coverage of pathogens among the Enterobacteriaceae or key organisms associated with community-acquired respiratory tract infections (*Haemophilus influenzae* or *Moraxella catarrhalis*). Furthermore, methicillin- (oxacillin-) resistant *Staphylococcus aureus* (MRSA) has emerged as a significant cause of morbidity and mortality in the community setting, leading to the need for a paradigm shift to combination agents or parenteral antimicrobials in that clinical setting. Some ß-lactams have recently been advanced into human clinical trials that possess activity against MRSA, but these have been strictly parenteral and require prodrug formulations to enhance solubility (ceftobiprole, RWJ-5442 and TAK-599).

BP-102 is a novel carbacephem (Figure 1) developed by Blanca Pharmaceuticals, Inc. (Mountain View, CA) as a derivative of loracarbef. Preliminary pharmacokinetic studies in animals suggest a low serum protein binding, relatively slow free-drug clearance and a high volume of distribution. Several investigations by the sponsor and by independent laboratories suggest the following MIC_{90} data: methicillin- (oxacillin-) susceptible *S. aureus* (MSSA; 0.25 - 0.5 µg/ml), MRSA (1 - 4 µg/ml), MS-CoNS (2 µg/ml), MR-CoNS (0.5 - 4 µg/ml), glycopeptide-intermediate staphylococci (GISA, 2 µg/ml), penicillin-resistant pneumococci (0.5 µg/ml), ß-haemolytic streptococci (≤ 0.004 µg/ml), viridans group streptococci (1 µg/ml) and *E. faecium* ampicillin-susceptible/-resistant (2/8 µg/ml). BP-102 was also observed to be bactericidal at concentrations of ≤ four-fold greater than the MIC for MSSA, MRSA, many enterococci and various streptococcal species including penicillin-resistant *S. pneumoniae*. Inoculum affects were minimal over the range of 10^4 to 10^7 CFU/ml (Blanca, data on file) and vancomycin-resistant *E. faecalis* were more likely to be killed than *E. faecium*. PAE was determined at 80 - 180 minutes. *H. influenzae* (MIC_{90}, 0.12 µg/ml; BLNAR, 1 µg/ml) and *M. catarrhalis* (MIC_{90}, 2 µg/ml) were inhibited by BP-102, as were many *E. coli*, but not *Pseudomonas aeruginosa*.

To challenge this novel agent with potential for oral, parenteral or even combination applications, a highly selected organism set was collected that featured numerous resistance groups among Gram-positive pathogens (total strains, 125) tested by reference methods of the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS).

MATERIALS AND METHODS

Organisms. The resistant strains were selected to represent as many contemporary patterns as possible by searching an international collection of pathogens at the Jones Microbiology Institute (North Liberty, IA). A total of 32 *S. aureus* were chosen (21 MRSA), each having a unique multidrug-resistance (MDR) pattern (see example in Table 1) from among seven other drug classes. Two strains with a seven-drug resistant pattern were tested to represent the most worrisome clinical isolates.

Similarly, MR- and MS-CoNS were selected to represent 11 and six co-resistance patterns, respectively. Equal numbers of ampicillin-susceptible (six strains) and -resistant *E. faecalis* and *E. faecium* were processed. Five other *Enterococcus* spp. (ampicillin-susceptible) were tested, each a different species. Among the enterococci tested, other resistances to vancomycin (*vanA, vanB, vanC*$_{2\text{-}3}$), linezolid and chloramphenicol were present (see Table 2 footnotes for details). *S. pneumoniae* (18 strains) were equally distributed between penicillin-susceptible, -intermediate and -resistant isolates, and one of the six viridans group streptococci was highly resistant to penicillin (MIC, 32 µg/ml).

The 12 *H. influenzae* (five ß-lactamase positive) included one isolate that was ß-lactamase-negative and ampicillin-resistant (ATCC 49247), and the four *M. catarrhalis* produced either BRO-1 or -2 enzymes.

Susceptibility tests. All testing was performed using reference CLSI methods in broth microdilution trays containing Mueller-Hinton broth (staphylococci, enterococci and *M. catarrhalis*) and supplemented for some species (2-5% lysed horse blood for streptococci; HTM for *H. influenzae*). A number of replicates of quality control (QC) strains were determined including: *E. coli* (modal MIC, ≤ 0.12 µg/ml) and *P. aeruginosa* (modal MIC, > 16 µg/ml), for BP-102 results. Comparison agents included oxacillin, ampicillin, penicillin, chloramphenicol, gentamicin, linezolid, quinupristin/dalfopristin, glycopeptides (vancomycin and teicoplanin), amoxicillin/clavulanate, cefepime, ceftriaxone, clindamycin, erythromycin, levofloxacin, ciprofloxacin, rifampin, tetracycline, and trimethoprim/sulfamethoxazole. These antimicrobials were used to classify the resistance subsets. All QC strains (six organisms) had MIC results within CLSI published ranges.

RESULTS

- Table 1 illustrates the extreme nature of the resistance subsets utilized to test the potency of BP-102. The *S. aureus* strains alone had 30 different antibiograms among 32 challenge isolates including strains resistant to all seven, non-oxacillin compounds tested as representative of other drug classes.
- BP-102 MRSA strain MIC values ranged from 0.25 to 4 µg/ml (MIC_{90}, 4 µg/ml), generally eight-fold higher than MSSA strains (MIC_{90}, 0.25 µg/ml). MS- and MR-CoNS BP-102 MICs varied from ≤ 0.12 to 2 µg/ml (Table 2).
- BP-102 was also active against *E. faecalis* (MIC_{50}, 4 µg/ml) and ampicillin-susceptible *E. faecium* (MIC_{90}, 1 µg/ml); but ampicillin-resistant *E. faecium* MIC results for BP-102 were elevated by approximately eight-fold compared to ampicillin-susceptible strains (Table 2).
- *M. catarrhalis* (Table 2) were all inhibited by ≤ 1 µg/ml of BP-102, regardless of ß-lactamase activity.

Table 1. Resistance patterns included in the *S. aureus* collection (32 strains) used to challenge BP-102 activity.

	Co-resistance pattern for:						
S. aureus oxacillin pattern (no. patterns)	Chloramphenicol	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Tetracycline	Trim/Sulfa
Oxacillin-resistant (20)							
	S	S	S	S	S	S	S
	S	S	S	S	R	S	S
	S	S	S	R	S	S	S
	S	R	S	S	S	S	S
	S	R	S	R	S	S	S
	S	R	S	S	S	R	S
	S	R	S	S	R	S	S
	S	R	R	R	S	S	S
	S	R	S	S	R	R	R
	S	R	R	R	R	S	S
	S	R	R	R	R	R	R
	S	R	R	R	R	S	R
	S	R	R	R	S	R	R
	R[a]	R[a]	R[a]	R[a]	R[a]	R[a]	R[a]
	R	R	R	R	R	S	S
	R	R	R	R	R	S	S
	R	R	R	R	S	S	S
	R	R	R	R	R	S	R
	R	R	S	R	R	S	S
Oxacillin-susceptible (10)							
	S	S	S	S	S	S	S
	S	S	S	S	S	S	R
	S	S	S	S	S	R	S
	S	S	S	R	S	S	S
	S	R	S	S	S	S	S
	S	S	S	R	S	R	S
	S	S	R	R	S	S	S
	S	R	R	R	S	S	S
	R	S	S	S	S	S	S
	R[a]	R[a]	R[a]	R[a]	R[a]	R[a]	R[a]

a. Two strains were tested of this resistance phenotype.

Table 2. In vitro activity of BP-102, a novel carbacephem, tested against *S. aureus*, coagulase-negative staphylococci (CoNS), *E. faecalis*, *E. faecium*, other enterococcal species, *M. catarrhalis* and selected QC strains (89 total strains).

		Occurrences at MIC (µg/ml):								
Organism (no. tested)		≤0.12	0.25	0.5	1	2	4	8	16	>16
S. aureus,	oxacillin-resistant (21)[a]	-	1	3	5	6	6	-	-	-
	oxacillin-susceptible (11)[b]	3	8	-	-	-	-	-	-	-
CoNS,	oxacillin-resistant (11)[c]	3	3	4	1	-	-	-	-	-
	oxacillin-susceptible (6)[d]	3	-	-	-	3	-	-	-	-
E. faecalis,	ampicillin-resistant (6)[e]	-	-	-	1	1	1	2	-	1
	ampicillin-susceptible (6)[f]	-	-	-	2	-	3	1	-	-
E. faecium,	ampicillin-resistant (6)[g]	-	-	-	-	-	-	5	1	-
	ampicillin-susceptible (6)[h]	-	1	1	3	1	-	-	-	-
Other enterococci (5)[i]		2	1	2	-	-	-	-	-	-
M. catarrhalis (6)		4	-	1	1	-	-	-	-	-
QC strains (7 replicates)										
ATCC 25923 (*S. aureus*)		1	-	-	-	-	-	-	-	-
ATCC 29213 (*S. aureus*)		-	3	-	-	-	-	-	-	-
ATCC 29212 (*E. faecalis*)		-	-	-	1	-	-	-	-	-
ATCC 25922 (*E. coli*)		1	-	-	-	-	-	-	-	-
ATCC 27853 (*P. aeruginosa*)		-	-	-	-	-	-	-	-	1

a. Includes 20 patterns of co-resistance (see Table 1).
b. Includes 10 patterns of co-resistance (see Table 1).
c. Includes 11 patterns of co-resistance.
d. Includes six patterns of co-resistance.
e. Includes two VRE (*vanA*) and three strains resistant to chloramphenicol.
f. Includes three VRE (two *vanA* and one *vanB*), three strains resistant to chloramphenicol and one linezolid resistant isolate.
g. Includes five VRE (four *vanA* and one *vanB*), and one chloramphenicol-resistant strains.
h. Includes one VRE (one *vanA*).
i. Includes *E. casseliflavus* (one strain), *E. durans* (one strain), *E. avium* (one strain), *E. gallinarum* (one strain) and *Enterococcus* spp., not *E. faecalis* or *E. faecium* (one strain).

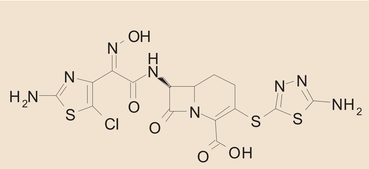
- Table 3 shows that BP-102 activity versus *S. pneumoniae* was related to penicillin MIC values. In fact, penicillin-susceptible strains had BP-102 MIC values ranging from ≤ 0.004 - 0.008 µg/ml, penicillin-intermediate strains from 0.03 - 0.25 µg/ml and penicillin-resistant pneumococci had BP-102 MICs of 0.25 - 4 µg/ml. Only one *S. pneumoniae* isolate had a BP-102 MIC value > 0.25 µg/ml.
- BP-102 MIC values for viridans group streptococci corresponding to penicillin-susceptible, -intermediate, and -resistant strains were 0.008, 0.06 and 2 µg/ml, respectively.
- ß-lactamases produced by *H. influenzae* appear to hydrolyze BP-102 to some degree since enzyme-producing strains had MIC_{50} results eight-fold greater than ß-lactamase-negative isolates.

Table 3. Activity of BP-102 tested against streptococci (24 strains) and *H. influenzae* (12 strains; five ß-lactamase-positive).

	Occurrences at MIC (µg/ml):											
Organism (no. tested)	≤0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
S. pneumoniae (18)[a]	1	5	-	1	2	1	7	-	-	-	1	-
viridans group streptococci (6)	-	3	-	-	2	-	-	-	-	1	-	-
H. influenzae												
ß-lactamase-negative (7)	1	-	-	-	5	-	-	1[b]	-	-	-	-
ß-lactamase-positive (5)	-	-	-	-	-	-	2	2	-	1	-	-

a. Includes six penicillin-susceptible, six penicillin-intermediate and six strains resistant to penicillin.
b. ß-lactamase-negative ampicillin-resistant organism.

Figure 1. Chemical structure of BP-102.



CONCLUSIONS

- BP-102 appears to be a promising carbacephem with potencies against troublesome Gram-positive pathogens including:
  - MRSA (MIC_{90}, 4 µg/ml)
  - MR-CoNS (MIC_{90}, 0.5 µg/ml)
  - VRE, usually ampicillin-susceptible strains
  - Streptococci, penicillin-susceptible or -intermediate strains
- *H. influenzae* and *M. catarrhalis* were susceptible to BP-102, but modestly affected by ß-lactamase activity (TEM-1 and BRO-1).
- Numerous co-resistance patterns involving several non-ß-lactam drug classes did not significantly influence BP-102 activity, especially versus the staphylococci.
- Applications of BP-102 by parenteral or oral routes should be pursued as community-associated MRSA evolves.

SELECTED REFERENCES

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