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Bactericidal Action and Synergy Studies of BAL9141, A Novel Pyrrolidinone-3-ylidenemethyl Cephem, Tested Against Enterococci and Methicillin-Resistant Staphylococci (MRSA)

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ICAAC 2002

AMENDED ABSTRACT

Background: BAL9141 has been reported to have inhibitory activity against MRSA, many enterococci and streptococci having various resistant patterns. Its potency against MRSA (MIC_{90}, 4 µg/ml) and enterococci (MIC, 4 - 8 µg/ml) was assessed by time kill-curves when used alone or with subinhibitory concentrations of gentamicin.

Methods: Initially four *S. aureus* (two MRSA), four coagulase-negative staphylococci (two MR CoNS), three *S. pneumoniae* (two PENR), three viridans group streptococci, one *E. faecalis* and four *E. faecium* (two ampicillin resistant) were tested over 24 hours by kill-curve. For synergy studies, 10 strains of enterococci (two *E. faecalis* and eight *E. faecium*) each having a BAL9141 MIC of 1 µg/ml and gentamicin MIC of 4 µg/ml were tested at a concentration 8x and MIC/4, respectively. Three strains of MRSA and seven CoNS (BAL9141 MIC 1 µg/ml, gentamicin susceptible, MIC 0.016 - 1 µg/ml) were also tested in the same manner. Synergy was defined as ≥ 1 (at 4 to 8 hours) and ≥ 2 $\log_{10}$ (at 24 hours) CFU/ml greater killing for the combination when compared to BAL9141 alone. Clinical isolates were from bacteremic patients in the USA, Canada, Europe and Latin America referred to the SENTRY Program (2001).

Results: BAL9141 exhibited bactericidal activity against all the streptococci and oxacillin resistant staphylococci. BAL9141 demonstrated bactericidal action versus both *E. faecalis* and four *E. faecium* (-4.8 to -6.0 $\log_{10}$ CFU/ml), but static effects for other four *E. faecium* (-1.0 to -1.4 $\log_{10}$ CFU/ml). Activity of BAL9141 and gentamicin was slightly enhanced (not synergy) or indifferent over the 24 hour interval against staphylococci. Drug interactions with gentamicin documented early synergy (4 to 8 hours) for all enterococci and indifference (5) or synergy (5) at 24 hours. No antagonism was detected.

Conclusions: BAL9141 showed promising bactericidal activity alone and synergy with gentamicin against the vancomycin resistant enterococci tested using modest concentrations (8 µg/ml). BAL9141 was bactericidal against oxacillin-resistant *S. aureus* and CoNS, indifferent in combination. Clinical trials for BAL9141 appear warranted against these species that cause serious nosocomial infections.

INTRODUCTION

BAL9141 is an anti-MRSA cephalosporin with additional antimicrobial qualities similar to that of a "fourth-generation" cephalosporin. It also exhibits good in vitro activity against other Gram-positive cocci. Anti-MRSA activity of BAL9141 originates from its high affinity for and efficient inhibition of PBP2'. The PBP2' functions as a transpeptidase and is not efficiently inhibited by ß-lactams such as cephalosporins. Since BAL9141 showed all the properties of an advanced spectrum cephalosporin in addition to the anti-MRSA activity, we investigated cidal action and the possibility of classical ß-lactam-aminoglycoside combination synergy.

MATERIALS AND METHODS

The isolates were recent clinical isolates from bacteremic patients (SENTRY Antimicrobial Surveillance Program, 2001), standard quality control strains or well characterized resistant isolates. The isolates were from different countries and continents (USA, Canada, Europe and Latin America) forming a diverse geographic sample.

Bactericidal activity as well as synergy was determined by timed kill-curve methodology described in the ASM Clinical Microbiology Procedures' Handbook (1992). Cidal activity was measured against *S. pneumoniae* (three strains), viridans group streptococci (three strains), *Staphylococcus aureus* (four strains), coagulase-negative staphylococci (four strains), *Enterococcus faecalis* (one strain), and *E. faecium* (four strains). Appropriate resistant phenotypes were included to challenge the spectrum of BAL9141 (Table 1). BAL9141 activity alone was tested at 4, 8, and 16 µg/ml.

Synergistic interaction tests between BAL9141 and gentamicin were determined against staphylococci (10 strains) and enterococci (10 strains) with BAL9141 MICs of 1 µg/ml and susceptibility to gentamicin. All staphylococci were resistant to oxacillin and the enterococci showed a varying degree of resistance to vancomycin (Table 2). BAL9141 was tested at 8 µg/ml (8x MIC) and gentamicin at MIC/4. Synergy was defined as ≥ 1 (at 4 to 8 hours) and ≥ 2 $\log_{10}$ (at 24 hours) CFU/ml greater killing for the combination when compared to BAL9141 tested alone.

RESULTS

Table 1. BAL9141 bactericidal activity tested by kill-curves against 18 strains of Gram-positive cocci at concentrations of 4, 8 and 16 µg/ml.

Organism/strain number	Susceptibility (MIC [µg/ml], category) phenotype[a]	Activity at concentration (µg/ml): 4	8	16
S. pneumoniae				
11-43B	Penicillin (0.12, I)	Bactericidal[b]	Bactericidal	Bactericidal
30-21B	Penicillin (4, R)	Bactericidal	Bactericidal	Bactericidal
42-4512B	Penicillin (4, R)	Bactericidal	Bactericidal	Bactericidal
viridans group streptococci				
14-2152A	Penicillin (≤ 0.015, S)	Bactericidal	Bactericidal	Bactericidal
31-7254A	Penicillin (0.25, I)	Bactericidal	Bactericidal	Bactericidal
63-962C	Penicillin (16, R)	Bactericidal	Bactericidal	Bactericidal
S. aureus				
ATCC 29213	Oxacillin (0.25, S)	Bactericidal	Bactericidal	Bactericidal
82-11A	Oxacillin (0.5, S)	Bactericidal	Bactericidal	Bactericidal
82-8A	Oxacillin (> 8, R)	Bactericidal	Bactericidal	Bactericidal
Mu50[c]	Oxacillin (> 8, R)	Bactericidal	Bactericidal	Bactericidal
CoNS[d]				
15-320A	Oxacillin (0.12, S)	Bactericidal	Bactericidal	Static[e]
82-9A	Oxacillin (0.12, S)	Bactericidal	Bactericidal	Bactericidal
48-388A	Oxacillin (> 8, R)	Bactericidal	Bactericidal	Bactericidal
63-294A	Oxacillin (> 8, R)	Bactericidal	Bactericidal	Bactericidal
E. faecalis				
ATCC 29212	Ampicillin (2, S)	Bactericidal	Bactericidal	Bactericidal
E. faecium				
69-2323A	Ampicillin (2, S)[f]	Bactericidal	Bactericidal	Bactericidal
15-2666A	Ampicillin (4, S)[f]	Static	Static	Static
15-4011A	Ampicillin (> 16, R)	[g]	[g]	[g]
30-7648A	Ampicillin (> 16, R)	[g]	[g]	[g]

a. S = susceptible, I = intermediate, and R = resistant.
b. Bactericidal activity defined by ≥ three $\log_{10}$ decrease in the initial inoculum (approximately 5 x 10^5 to 1 x 10^6 CFU/ml) at ≤ 24 hours incubation.
c. VISA MRSA from reference (Mu50).
d. CoNS = coagulase-negative staphylococci.
e. Bactericidal activity was noted at 8 hours, but regrowth occurred to 10^5 CFU/ml at 24 hours.
f. Strain was quinupristin/dalfopristin-resistant.
g. No inhibition was observed as the BAL9141 parallels the MIC of ampicillin.

- BAL9141 showed cidal action against all the streptococci (*S. pneumoniae* and viridans group streptococci) regardless of penicillin MICs. There were no differences in the rate of killing based on drug concentration at 2 and 6 hours of incubation. A modest concentration dependence in killing was seen at 24 hours with some strains (Figure 1).
- All the *S. aureus* isolates (oxacillin-susceptible and -resistant) including the vancomycin-intermediate strain (Mu50) were killed by BAL9141.
- BAL9141 exerted cidal activity against all CoNS at 4 or 8 µg/ml. One isolate (15-320A) showed cidal activity at 8 hours with 16 µg/ml BAL9141 and regrowth at 24 hours, hence categorized as a static effect. "Eagle-like effects" were observed in some strains (Figure 2).
- BAL9141 was cidal against ampicillin-susceptible enterococci while it remained static for strains having slightly elevated ampicillin MICs (15-2666A).
- Ampicillin resistance in *E. faecium* also reflected BAL9141 resistance, thus no inhibition was detected in the ampicillin-resistant isolates.
- Table 2 shows the synergistic interaction between BAL9141 and gentamicin. The combination was cidal against all the staphylococci and enterococci tested. For some of the enterococci it changed the static action of BAL9141 alone, to cidal activity in combination. Thus based on the ≥ 2 $\log_{10}$ CFU/ml superior killing criteria, the combination was categorized as either synergistic (Figure 3) or indifferent. No antagonism was observed.

Figure 1: Kill curve for *S. pneumoniae* 42-4512C (BAL9141 MIC, 0.016 µg/ml) using BAL9141 concentrations at 4, 8 and 16 µg/ml.

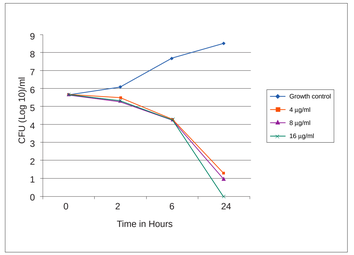

Figure 2: Kill curve for *S. aureus* 82-11A (BAL9141 MIC, 0.25 µg/ml) using BAL9141 concentrations at 4, 8 and 16 µg/ml. All concentrations achieved bactericidal action, but an Eagle-like effect was observed.

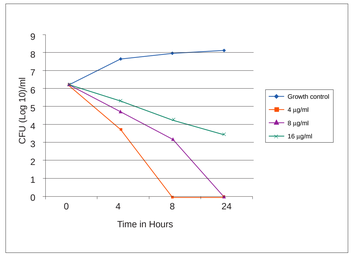

Figure 3: BAL9141 synergy experiment with gentamicin (MIC/4) using *E. faecium* 52-7395A (ampicillin- and vancomycin-susceptible).

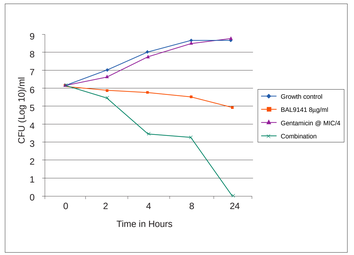

Table 2. Results of BAL9141 drug interaction (synergy) experiments using combinations with gentamicin (MIC/4) and the timed kill-curve method against various resistant and susceptible phenotype strains of staphylococci and enterococci.[a]

Organism/strain number	Susceptibility phenotype (MIC [µg/ml], category)	Strain origin	Activity for:[b] BAL9141 alone	BAL9141 + gentamicin	Interaction category[c]
S. aureus					
4-37C	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
4-111C	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
55-94A	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
CoNS[d]					
11-741A	Oxacillin (>8, R)	USA	Bactericidal	Bactericidal	Indifferent
23-1143A	Oxacillin (8, R)	USA	Bactericidal	Bactericidal	Indifferent
31-692A	Oxacillin (4, R)	Canada	Bactericidal	Bactericidal	Synergy
33-779A	Oxacillin (8, R)	Canada	Bactericidal	Bactericidal	Indifferent
48-933A	Oxacillin (>8, R)	Brazil	Bactericidal	Bactericidal	Indifferent
68-1852A	Oxacillin (>8, R)	Turkey	Bactericidal	Bactericidal	Indifferent
78-7893A	Oxacillin (>8, R)	France	Bactericidal	Bactericidal	Synergy
E. faecalis					
15-4006A	Vancomycin (1, S)[e]	USA	Bactericidal	Bactericidal	Indifferent
33-2134A	Vancomycin (1, S)[e]	Canada	Bactericidal	Bactericidal	Indifferent
E. faecium					
25-1368I	Vancomycin (0.5, S)[e]	USA	Bactericidal	Bactericidal	Synergy
52-7395A	Vancomycin (4, S)[e]	USA	Static	Bactericidal	Synergy
55-7032A	Vancomycin (8, I)[e]	USA	Static	Bactericidal	Synergy
33-3259A	Vancomycin (2, S)[e]	Canada	Bactericidal	Bactericidal	Synergy
31-3919A	Vancomycin (0.5, S)[e]	Canada	Bactericidal	Bactericidal	Indifferent
61-8000A	Vancomycin (4, S)[e]	France	Static	Bactericidal	Synergy
66-221A	Vancomycin (0.5, S)[e]	Spain	Static	Bactericidal	Indifferent
95-14745A	Vancomycin (0.5, S)[e]	Germany	Bactericidal	Bactericidal	Indifferent

a. All strains were tested at a BAL9141 concentration of 8 µg/L. Gentamicin was tested at MIC/4 alone and with BAL9141.
b. Bactericidal activity was defined as noted in Table 1, footnote b.
c. Interactive categories were defined as follows: Synergy if the combination exhibits ≥ two $\log_{10}$ superior killing compared to BAL9141 alone; antagonism if the combination exhibits ≥ two $\log_{10}$ inferior killing compared to BAL9141 alone; and Indifferent was used for all variations between the above defined extremes.
d. CoNS = coagulase-negative staphylococci.
e. These strains were also susceptible to ampicillin (MIC, ≤ 8 µg/ml).

CONCLUSIONS

- BAL9141 showed cidal action against streptococci, most staphylococci, and enterococci tested.
- Some *Staphylococcus* spp. strains exhibited an "Eagle-like effect".
- Gentamicin showed synergy with BAL9141, although indifference was observed in some cases.
- BAL9141 alone and in combination showed significant cidal activity against resistant Gram-positive pathogens.
- These results warrant further clinical evaluation of this compound alone and in selected combinations.

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