Poster F1-844

Activity of ACHN-490 in Combination with Daptomycin, Ceftobiprole, or Linezolid against 47 Methicillin-resistant *Staphylococcus aureus* by Synergy Time-kill

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Revised Abstract

Background. A combination of new drugs with existing agents represents a promising avenue for treatment of multidrug-resistant *Staphylococcus aureus*. ACHN-490 is a neoglycoside, a next-generation aminoglycoside, currently in clinical development. ACHN-490 was tested by the synergy time-kill method alone and in combination with daptomycin (DAP), ceftobiprole (BPR; synthesized by Achaogen Inc., South San Francisco, CA), or linezolid (LZD) against methicillin-resistant *S. aureus* (MRSA) by the synergy time-kill method.

Methods. The 47 isolates tested included 2 vancomycin-hetero-intermediate (hVISA), 20 vancomycin-intermediate (VISA), 5 vancomycin-resistant (VRSA), 10 hospital-acquired and 10 community-acquired MRSA strains. Minimum inhibitory concentrations (MICs) were determined by broth macrodilution. Potency of each of the 4 drugs alone was determined via time-kill methodology. For synergy time-kills, the concentration of each drug was chosen from the MIC result so that 1 drug demonstrated minimal activity while the other was more active. Synergy was defined as ≥2 $\log_{10}$ decrease in colony-forming units/mL between the combination and its more active component at 3, 6, 12, and 24 h.

Results. MICs (µg/mL) of drugs alone were: ACHN-490, 0.5–8; DAP, 0.25–4; BPR, 0.5–4; and LZD, 1–4. Synergy results were:

	3 h		6 h		12 h		24 h	
	Syn[a]	Range[b]	Syn[a]	Range[b]	Syn[a]	Range[b]	Syn[a]	Range[b]
ACHN-490/ DAP	23	0.125-2/ 0.06-1	28	0.25-2/ 0.125-2	37	0.125-2/ 0.125-2	43	0.25-2/ 0.125-2
ACHN-490/ BPR	2	1/ 0.125-0.25	7	0.5-4/ 0.25-1	12	0.25-2/ 0.25-1	17	0.5-2/ 0.25-2
ACHN-490/ LZD	0	—	0	—	1	0.125/1	6	0.125-2/ 0.5-1

[a]No. strains showing synergy out of 47 strains tested
[b]Range (µg/mL) where synergy was observed

Combination of ACHN-490 + DAP yielded the highest rate of synergy at the various time points at sub-MIC levels, with 23 strains showing synergy at 3 h, 28 strains at 6 h, 37 strains at 12 h, and 43 strains at 24 h. ACHN-490 + BPR yielded synergy in 2 strains at 3 h, 7 strains at 6 h, 12 strains at 12 h, and 17 strains at 24 h. ACHN-490 + LZD yielded 1 strain with synergy at 12 h and 6 strains at 24 h.

Conclusions. Combination of subinhibitory concentrations of ACHN-490 + DAP yielded significant synergy even in 10 VISA strains which were initially DAP non-susceptible.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are not susceptible to conventional ß-lactams, and most are resistant to all available quinolones. In addition, vancomycin-hetero-intermediate (hVISA), vancomycin-intermediate (VISA), and vancomycin-resistant (VRSA) *S. aureus* strains have emerged.[1,2] While most multidrug-resistant *S. aureus* strains are hospital-acquired, there has been a worrisome increase in the incidence of community-acquired MRSA.[3] Such strains are currently more susceptible to most other antibiotics, but typically are more virulent than hospital strains. An increase in community-acquired MRSA infections may potentially lead to an increase in the selective pressure for vancomycin resistance. Thus an alternative to vancomycin is urgently needed. ACHN-490 (see Poster F1-840 for chemical structure) is a neoglycoside, a next-generation aminoglycoside, currently in clinical development. The combination of new drugs, such as ACHN-490, with existing agents represents a promising avenue for the treatment of multidrug-resistant *S. aureus*. In the present study, we examine the synergistic activity of ACHN-490 with daptomycin (DAP), ceftobiprole (BPR), or linezolid (LZD) against 47 MRSA strains with various resistotypes.

Methods

- The strains studied were recent isolates and comprised: 2 hVISA (Hershey Medical Center isolates, screened by the Etest® macromethod and confirmed by population analyses); 20 VISA (4 Hershey and 16 from the Network on Antimicrobial Resistance in *S. aureus*); 5 VRSA (3 Michigan, 1 Hershey, and 1 from New York City); 10 community-acquired MRSA (9 Panton-Valentine Leukocidin [PVL]-positive and 1 PVL-negative) and 10 hospital-acquired MRSA (including *S. aureus* ATCC 33591) with differing resistance phenotypes.
- Minimum inhibitory concentrations (MICs) for ACHN-490, BPR, DAP, and LZD were determined by macrobroth dilution after incubation for 24 h.
- Kill kinetics of each drug alone were determined by incubating an initial inoculum of 5 x 10^5 to 5 x 10^6 colony-forming units (CFU)/mL with drug concentrations at the MIC, 2 dilutions above (2x and 4x MIC), and 3 dilutions below the MIC (1/2x, 1/4x, and 1/8x MIC).[4]
- Viability counts were performed at 0, 3, 6, 12, and 24 h incubation by plating undiluted and 10-fold serial diluted samples.[4]
- ACHN-490 was combined with either BPR, DAP, or LZD and tested at 1 and 2 dilutions below the MIC of each drug.

Results

***Definitions*:**

Synergy: ≥2 $\log_{10}$ decrease in CFU/mL between the combination and its most active constituent after 3, 6, 12, and 24 h, with the number of surviving organisms in the presence of the combination ≥2 $\log_{10}$ CFU/mL below the starting inoculum.

Indifference: between a 2 $\log_{10}$ CFU/mL decrease and a 2 $\log_{10}$ CFU/mL increase in counts between the combination and its most active constituent.

Antagonism: ≥2 $\log_{10}$ CFU/mL increase in counts between the combination and its most active constituent.

Table 1: *In Vitro* Antimicrobial Activities of ACHN-490 and Other Agents Used

Strain Phenotype	MIC (µg/mL)[a]			
	ACHN-490	DAP	BPR	LZD
All (n=47)	0.5-8	0.25-4	0.5-4	1-4
VSSA (n=20)	2-8	0.25-1	0.5-2	2-4
VISA (n=20)	1-4	0.5-4	0.5-4	1-4
hVISA (n=2)	1	0.5-1	2	2-4
VRSA (n=5)	0.5-2	0.25-0.5	0.5-2	2-4

[a]Determined by broth macrodilution at 24 h
VSSA = vancomycin-susceptible *S. aureus*

Table 2: Results of *In Vitro* Antimicrobial Combinations

	ACHN-490 + DAP				ACHN-490 + BPR				ACHN-490 + LZD			
	3 h	6 h	12 h	24 h	3 h	6 h	12 h	24 h	3 h	6 h	12 h	24 h
Synergy[a]	49	60	79	92	4	15	26	36	0	0	2	13
Indifference[a]	51	40	21	8	96	81	72	64	72	43	51	83
Antagonism[a]	0	0	0	0	0	4	2	0	28	57	47	4

[a]Percent of strains (47 total strains tested)

Figure 1. Synergy with Subinhibitory Concentrations of ACHN-490

Figure 1a. ACHN-490 + DAP Synergy vs VISA Strain SA 1008

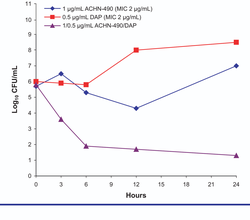


Figure 1b. ACHN-490 + DAP Synergy vs VRSA Strain SA 510

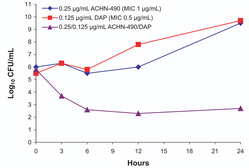


Figure 1c. ACHN-490 + DAP Synergy vs hVISA Strain SA 873

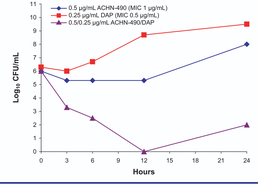


Figure 1d. ACHN-490 + DAP Synergy vs Hospital-acquired MRSA Strain SA 1808

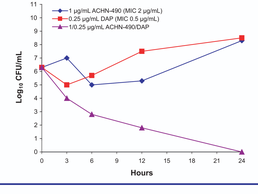
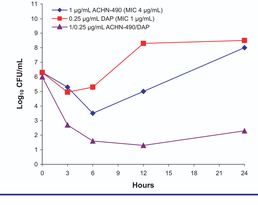


Figure 1e. ACHN-490 + DAP Synergy vs Community-acquired MRSA Strain SA 1468



Conclusions

- The combination of ACHN-490 and DAP provided the most strains showing synergy at 24 h, followed by ACHN-490 and BPR; ACHN-490 and LZD had the least number of strains showing synergy at 24 h.
- Subinhibitory concentrations of ACHN-490 and DAP showed very clear synergy in 43 of the 47 strains tested, irrespective of the resistance phenotype.
- In view of the fact that DAP has been described to select for resistant strains during therapy for systemic infections,[5,6] combinations with ACHN-490 may greatly improve the spectrum and therapeutic efficacy of DAP. Clinical studies are required to test the validity of this hypothesis.

Acknowledgment

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