Lysin CF-301 (exebacase) Demonstrates Potent *in vitro* Activity Against a Range of *Staphylococcus* and *Streptococcus* Species Associated with Complicated Bacteremia and Infective Endocarditis (IE) in Humans

Sunday AAR LB5

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Abstract

Background: CF-301 (exebacase) is a novel, recombinantly-produced bacteriophage-derived lysin (cell wall hydrolase) and is the first agent of this class to enter clinical development in the US for the treatment of bacteremia including endocarditis due to *S. aureus*. In the current study, we evaluated the antimicrobial activity of CF-301 against a wide range of staphylococcal and streptococcal species known to be the primary causative agents of infective endocarditis (IE) in humans.

Methods: MICs of CF-301 were determined against 6 species of staphylococci using an AST medium for broth microdilution endorsed by the CLSI for use with CF-301 comprised of cation-adjusted MHB supplemented with 25% horse serum and 0.5 mM DTT (caMHB-HSD). The determination of MICs against 13 species of streptococci was performed in caMHB-HSD that was supplemented with 2.5% laked horse blood (LHB). Daptomycin (DAP) and vancomycin (VAN) were used as comparator antibiotics and were tested in the manner defined by the CLSI (M07-A10). Bacterial isolates were obtained from the following sources: ATCC, BEI Resources, ARS (NRRL Collection), CCUG, BCCM/LMG, DSMZ, CDC, Nakano Lab (Osaka University), and Weill Cornell Medical Center (Clinical Microbiology Lab).

Results: *Staphylococcus* spp. tested with the lowest MICs of CF-301 with a range of 0.12 – 2 µg/mL, whereas CF-301 exhibited more variable activity against *Streptococcus* spp. with *S. pyogenes* (Group A) and *S. agalactiae* (Group B) which resulted in the lowest MIC values ranging from 0.25 – 4 µg/mL.

Conclusions: The targeted activity of CF-301 against a wide range of *Staphylococcus* spp. and Groups A and B streptococci suggests that CF-301 may provide therapeutic benefit in the treatment of bacteremia including endocarditis caused by these pathogens.

Approach

Objective: While the potent activity of CF-301 (exebacase) against *S. aureus* is well described (1,2), limited activity data exists for other common pathogens causing infective endocarditis. Our objective here was to examine the CF-301-susceptibility of a broad range staphylococcal and streptococcal species known to cause clinical infective endocarditis to-inform our clinical development program. For benchmarking purposes, DAP and VAN MICs were also determined for all isolates in this study.

Design:

- Literature search to identify top pathogens causing IE
- Obtain multiple strains of each pathogen
- Determine MIC values
  - CF-301: caMHB/HSD (with 2.5% LHB for streptococci)
  - DAP: caMHB + $CaCl_2$ (with 2.5% LHB for streptococci)
  - VAN: caMHB (with 2.5% LHB for streptococci)

References

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Major Pathogens Causing IE

Organism	Primary Causative Agents by Study (%)					
	(1)*	(2)	(3)	(4)	(5)	(6)
S. aureus	50.9	17	7	40.3	10	26.6
S. epidermidis	1.8	5	4.5			
S. lugdunensis	1.8					
Other CoNS (*S. capitis*, *S. warneri*)	1.8			16.7	12.4	9.7
Streptococcus viridians group**	3.6		25.6	12.3	58.1	
S. agalactiae	3.6					
S. sanguis	1.8	22				
S. gallolyticus		5	2.2	6.4		12.5
S. oralis		5				
S. mitis		5				
S. pneumoniae		5				
"oral" streptococci***						18.7
Streptococcus group G		5				
Abiotrophia adiacens		5				
Enterococcus faecalis	6.6	5			4.8	
Enterococcus spp.			2.2	12.7		

*Study reference number (see references below)
** The most common viridans streptococci causing IE are: *S. mitis, S. sanguis, S. mutans, S. salivarius, Abiotrophia spp., S. intermedius*, and *S. anginosus* (7)
*** This reference (8) did not speciate the streptococci, however, the "oral streptococci" are: (i) Mutans group (prominent members are *S. mutans* and *S. sobrinus*), (ii) Salivarius group (*S. salivarius*), (iii) Anginosus group (*S. anginosus* and *S. intermedius*, (iv) Sanguinis group (*S. sanguinis* and *S. gordonii*), and (v) Mitis group (*S. mitis* and *S. oralis*).

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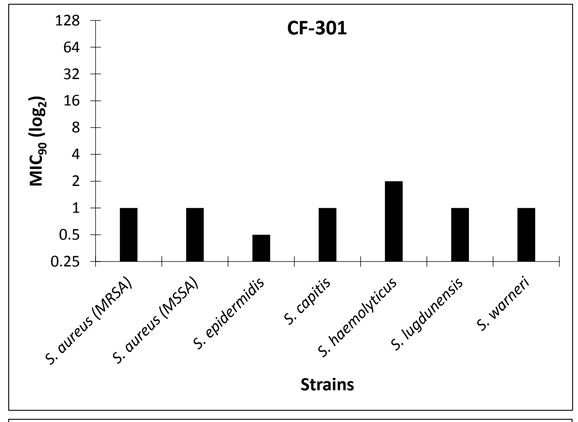
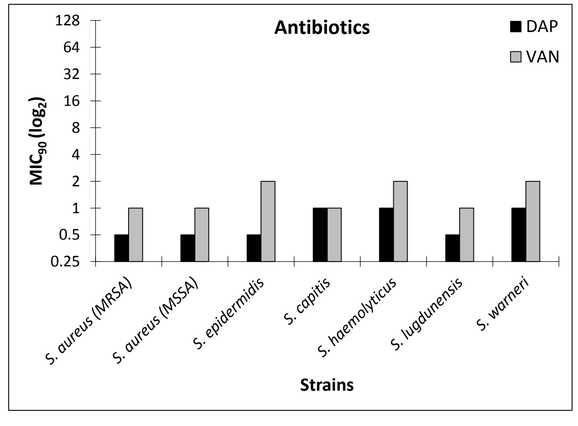
Results: MICs for *Staphylococcus* spp.

Organism	N	CF-301 MIC (µg/mL)			DAP MIC (µg/mL)			VAN MIC (µg/mL)		
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
S. aureus (MRSA)	315	0.5	1	0.12-1	0.5	0.5	0.06-1	1	1	0.5-2
S. aureus (MSSA)	310	0.5	1	0.25-1	0.5	0.5	0.25-1	1	1	0.5-2
S. epidermidis	54	0.5	0.5	0.12-2	0.5	0.5	0.12-1	1	2	1-2
S. capitis	5	0.25	1	0.25-4	0.5	1	0.25-1	0.5	1	0.5-1
S. haemolyticus	22	1	2	0.25-2	0.5	1	0.25-2	2	2	0.5-4
S. lugdunensis	23	0.5	1	0.25-2	0.5	0.5	0.25-0.5	1	1	0.5-1
S. warneri	19	0.5	1	0.06-1	0.5	1	0.25-2	1	2	0.25-2



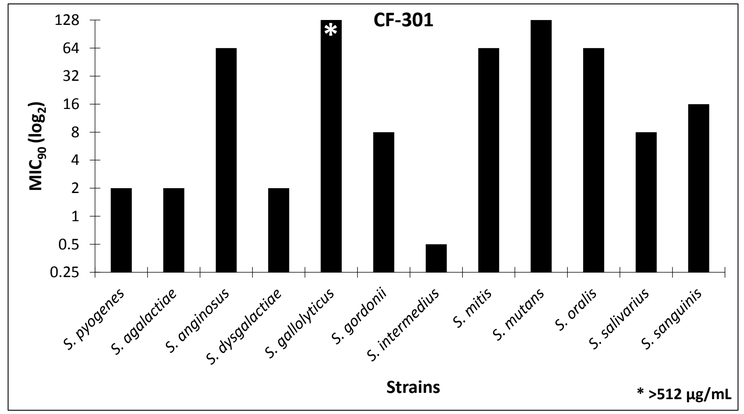


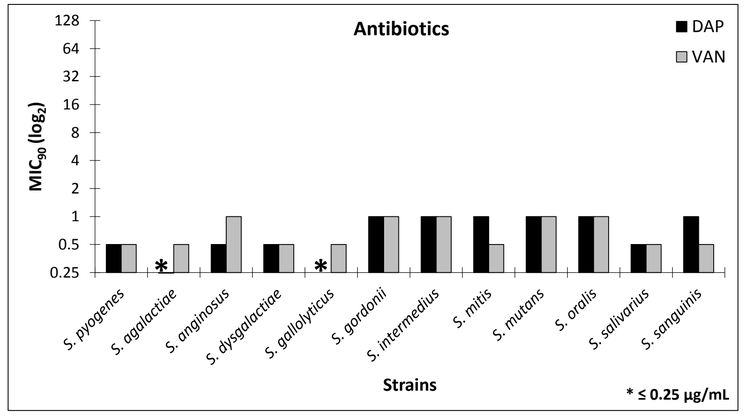
All *Staph.* spp. were highly susceptible to CF-301 with MIC_{90} values of 0.5 – 2 µg/mL

All isolates were highly susceptible to DAP and VAN, with MIC_{90} values of 0.5 – 2 µg/mL

Results: MICs for *Streptococcus* spp.

Organism	N	CF-301 MIC (µg/mL)			DAP MIC (µg/mL)			VAN MIC (µg/mL)		
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
S. pyogenes (Group A)	102	1	2	0.5-4	0.12	0.5	0.015-0.5	0.25	0.5	0.25-0.5
S. agalactiae (Group B)	101	1	2	0.25-4	0.09	0.12	0.015-0.25	0.5	0.5	0.5-2
S. anginosus	14	32	64	1-64	0.5	0.5	0.25-0.5	0.5	1	0.5-1
S. dysgalactiae	24	1	2	1-32	0.12	0.5	0.062-0.5	0.5	0.5	0.25-1
S. gallolyticus	23	64	>512	0.25->512	0.12	0.25	0.06-0.5	0.25	0.5	0.25-0.5
S. gordonii	12	4	8	0.5-8	0.5	1	0.25-1	0.5	1	0.5-1
S. intermedius	10	0.25	0.5	0.25-0.5	0.5	1	0.12-0.5	1	1	1
S. mitis	21	2	64	0.5-64	0.5	1	0.12-1	0.5	0.5	0.25-0.5
S. mutans	23	32	>64	1->64	0.5	1	0.25-8	1	1	0.25-1
S. oralis	15	4	64	0.5-64	0.5	1	0.5-1	0.5	1	0.5-1
S. salivarius	15	2	8	0.5-8	0.25	0.5	0.06-0.5	0.5	0.5	0.25-0.5
S. sanguinis	17	4	16	2-32	0.25	1	0.06-1	0.5	0.5	0.5





- ***S. pyogenes*** **and** ***S. agalactiae*** **were notably susceptible to CF-301 (MIC_{90} = 2 µg/mL);** ***S. intermedius*** **was the most susceptible with an MIC_{90} of 0.5 µg/mL**
- **Other *Streptococcus* spp. tested (with MICs from 8 – >512 µg/mL) are not expected to be susceptible to CF-301**
- **All isolates were susceptible to DAP and VAN (MIC_{90}= 0.12 – 1 µg/mL)**

Conclusions

CF-301 (exebacase) exhibits targeted, high level activity against all *Staphylococcus* spp. and Groups A and B streptococci and may have therapeutic benefit in treating bacteremia endocarditis caused by these pathogens.