Lysin CF-301 (Exebacase) Resensitizes Methicillin-Resistant *Staphylococcus aureus* (MRSA) to Penicillin Derivatives and First Generation Cephalosporins

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Poster #99

Background

Although antimicrobial resistance (AMR) is a well-recognized global health threat, strategies to overcome resistance to β-lactam antibiotics (BLs) have been limited to the use of higher doses of BLs, combinations with β-lactamase inhibitors and development of new classes of antibiotics. Emerging resistance to drug classes used to treat MRSA (e.g. glycopeptides, cyclic lipopeptide, and oxazolidinones) represents a new threat. CF-301 (exebacase) is the first of a new class of recombinantly-produced, bacteriophage-derived lysins (cell wall hydrolases) to enter Phase 2 of development for the treatment of *S. aureus* infective endocarditis (IE) and bacteremia used in addition to standard-of-care (SOC) antibiotics. CF-301 demonstrates: 1) rapid and potent bacteriolytic effects against all *S. aureus* strains including MRSA and vancomycin-, daptomycin- and linezolid- resistant strains; 2) potent antibiofilm activity; 3) synergy with antistaphylococcal antibiotics ; 4) low propensity for resistance; and 5) the ability to suppress the emergence of resistance to antibiotics *in vitro* and *in vivo*. We now build on a previous studies (**1**) and examine CF-301 synergy with BLs which are inactive vs. MRSA (oxacillin (OXA), nafcillin (NAF), and cefazolin (CFZ)) to determine whether CF-301 can resensitize MRSA to these BLs, *in vitro* and *in vivo*.

Objective

To evaluate the potential for CF-301 treatment to resensitize MRSA to penicillin derivatives and first generation cephalosporins. The ability of CF-301 to resensitize antibiotic resistant strains (e.g. MRSA) to conventional antibiotics is expected to have important therapeutic implications

Methods

We used a stepwise approach to evaluate CF-301 as a resensitizing agent. First, broth microdilution checkerboard assays were used to determine fractional inhibitory concentration index (FICI) values for combinations of CF-301 + OXA, NAF, and CFZ against 9 MRSA strains. Reduction of BL MIC values to below established breakpoint values (**2**) are consistant with resensitization. We then performed 21-day *in vitro* serial passage resistance assays (**1**) to determine the impact of CF-301 (alone) on OXA MIC values and the potential for a "seesaw" effect similar to that previously shown, whereby exposures to daptomycin (DAP) or vancomycin (VAN) were accompanied by increased susceptibility (and the potential for resensitization) to OXA (**3,4**). Finally, we performed an ex vivo analysis on tissue samples recovered after CF-301 treatment in the standard rabbit model of MRSA IE (**5**). Four days after treatment with a single-dose of CF-301 (0.18 to 1.4 mg/kg) in the IE model, MIC values of MRSA isolates recovered from valvular vegetations were determined for both CF-301 and OXA. Isolates exhibiting resensitization phenotypes from both the serial passage assay and the rabbit IE study underwent whole genome sequencing (WGS) and additional genetic analyses to identify specific mutations-of-interest.

References

1. Schuch et al., 2014, J Infect Dis. 209(9):1469-78.
2. CLSI, 2018 (document M100, 28th edition).
3. Renzoni et al., 2017, Antimic Agents and Chemother. 61:e01634-16.
4. Werth et al., 2013, AAC, 57:2664-8.
5. Xiong et al., 2016, J Antimic Chemo. 71:2890-4.
6. Abdelhamed et al. 2015. Plasmid. 81:1-8

Synergy Between CF-301 and β-lactam Antibiotics

Data from checkerboard assays were generated to determine the interaction and potency of CF-301 with 3 BLs (OXA, NAF, and CFZ) in comparison to their individual activities. This comparison is represented as the fractional inhibitory concentration index (FICI) value, whereby values of ≤0.5 are consistent with synergy, values of >0.5-<1 are highly-additive, values of 1-≤2 are indifferent, and values >2 are antagonistic. Representative single agent MICs are also shown, determined for each agent alone (initial) and in combinations (final). The current CLSI breakpoints and interpretive categories for OXA (as well as NAF and CFZ) against *S. aureus* are ≤2 (sensitive) and ≥4 (resistant) (**2**).

TABLE 1.

Strain	MIC & FICI	Antimicrobial Agents: CF-301	OXA	CF-301	NAF	CF-301	CFZ
NRS 100	$MIC_{initial}$	1	256	1	64	1	256
	MIC_{final}	0.25	2	0.25	1	0.25	1
	FICI	0.258		0.266		0.254	
ATCC 43300	$MIC_{initial}$	1	8	1	2	1	16
	MIC_{final}	0.25	0.5	0.125	0.25	0.125	4
	FICI	0.313		0.250		0.133	
HPV 107	$MIC_{initial}$	1	8	1	2	1	16
	MIC_{final}	0.25	0.5	0.25	0.25	0.125	2
	FICI	0.313		0.375		0.250	
CAIRD 426	$MIC_{initial}$	1	4	1	16	1	8
	MIC_{final}	0.25	1	0.25	1	0.25	0.5
	FICI	0.313		0.313		0.313	
JMI 227	$MIC_{initial}$	1	16	1	4	1	2
	MIC_{final}	0.25	1	0.25	0.5	0.25	0.5
	FICI	0.313		0.375		0.500	
JMI 1280	$MIC_{initial}$	1	256	1	256	1	32
	MIC_{final}	0.25	1	0.25	2	0.25	0.5
	FICI	0.254		0.258		0.266	
JMI 4789	$MIC_{initial}$	1	64	1	4	1	4
	MIC_{final}	0.25	2	0.125	0.5	0.125	1
	FICI	0.281		0.250		0.375	
MW2	$MIC_{initial}$	1	64	2	4	1	4
	MIC_{final}	0.25	2	0.5	0.031	0.125	1
	FICI	0.281		0.258		0.375	
ATCC 33591	$MIC_{initial}$	1	256	1	64	1	128
	MIC_{final}	0.25	1	0.125	2	0.25	0.5
	FICI	0.254		0.156		0.254	

Key: resensitization; synergy

- All combinations of CF-301 and β-lactam antibiotics exhibited synergy against the set of 9 MRSA strains
- BL sensitivity was restored to MRSA strains as demonstrated by the reduction of MIC values to below established BL breakpoints for *S. aureus*

In Vitro CF-301 Exposure Increases OXA Susceptibility

MRSA strain MW2 was serially passaged (using multiple lineages) on a daily basis for 21 days using both a 1.1-fold and 2-fold CF-301 dilution series. Only modest 2-fold shifts in CF-301 MIC values were observed (below), as previously observed (1). CF-301 exposures resulted in a seesaw effect, with reduced OXA MICs. Colored arrows indicated isolates that were analyzed by WGS.

Fig. A

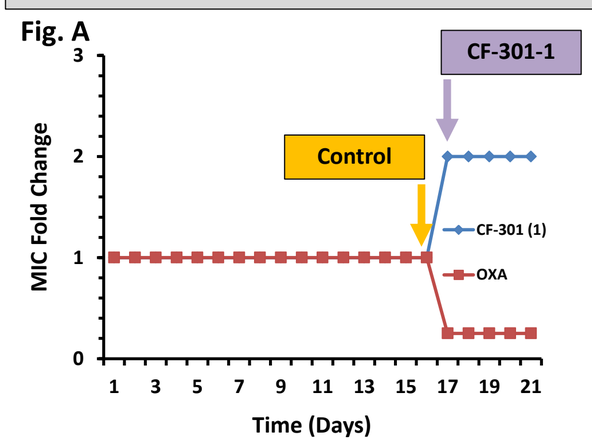


Fig. B

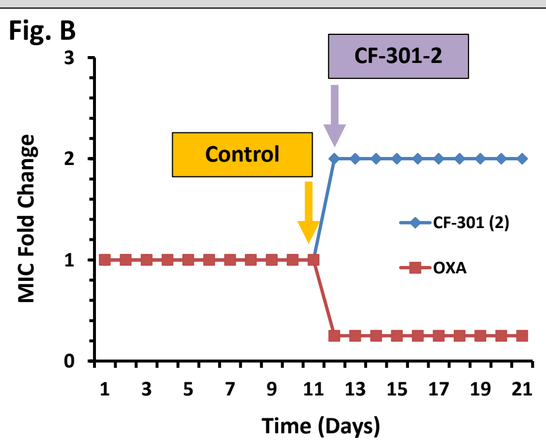
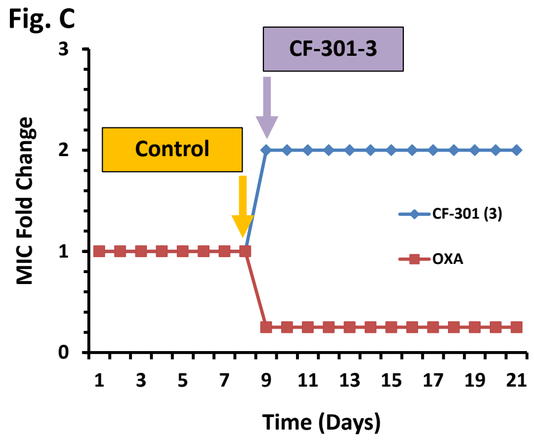


Fig. C



- CF-301 exposures resulted in 4-fold reductions in OXA MIC values (from 64 µg/mL to 16 µg/mL)
- Corresponding increases in CF-301 MIC were only 2-fold, thus susceptibility to CF-301 was maintained
- Seesaw effects similar to that described here are associated with resensitization of MRSA to OXA after exposure to DAP (3)
- Select isolates (Purple and Orange arrows, Fig. A-C) were chosen for WGS and mutagenesis to determine if the molecular basis of resensitization by CF-301 is similar to that identified for DAP

Molecular Basis of In Vitro OXA Effect

The ability of DAP to resensitize MRSA to OXA is driven by *mprF*-mediated cell membrane modifications which result in mislocalization of factors required for maturation of PBP 2a (*mecA* product) (**3**). To initiate similar studies of the CF-301 effect, 3 mutant derivatives obtained just after the shift in CF-301 and OXA MICs (see Fig. A-C) were analyzed by WGS and SNP/INDELs associated with the seesaw effect were identified in comparisons to control strains. Three distinct mutations were implicated (purple columns, Table 2) and the impact of each on CF-301 and OXA MICs was confirmed using a two-step allelic exchange process (**6**) in a clean genetic background.

TABLE 2. Mutations associated with CF-301-mediated reductions in OXA MICs (SNP/INDEL analysis of in vitro-derived isolates)

Reference Position(s)[a]	Overlapping Annotation[b]	Ref	Allele	Amino Acid Change	CF-301 Control	CF-301 1	CF-301 2	CF-301 3
2180631	*murA*	G	T	R95S	-	-	+	-
2403752	*lyrA*	C	A	Y245*	-	-	-	+
2658191	*oatA*	C	A	*oatA* promoter	-	+	-	-

[a]Position in the reference genome of *Staphylococcus aureus* MW2 (GenBank accession: NC_003923.1)

[b]Annotated open reading frames overlapping computationally predicted polymorphisms

- Mutations in/near loci encoding 3 different cell wall modifying enzymes (i.e., *murA*, *lyrA*, and *oatA*) are each sufficient to reduce OXA MICs
- Our findings are consistent with a model (3) in which cell wall perturbations (mediated through MurA, LyrA, or OatA for CF-301) reduce membrane amounts of PBP 2a, as was observed for MprF and DAP
- A reduction in PBP 2a, mediated by exposure to CF-301, has not yet been confirmed

In Vivo CF-301 Exposures Enhance the Increase in OXA Susceptibility

We used the standard rabbit IE model (5) to confirm the impact of CF-301 treatment on OXA MICs. After exposures to CF-301, isolates were recovered from valvular vegetations and plated on TSAB (non-selective condition, A) and TSAB supplemented with CF-301 over a range of concentrations (selective conditions, B). CF-301 and OXA MICs were determined for isolates obtained from both conditions.

Table 3.

Treatment Groups[a]	Log_{10} CFU/g of Vegetation[b]	n[c]	CF-301 MIC (µg/mL)[d]: 0.25	0.5	1	2	4	OXA MIC (µg/mL)[e]: ≤2	4	8	16	32	64
Pre-Treatment Control	7.02±1.47	24			24							24	
Buffer treatment Control	7.26±1.54	32			31	1				2		30	
CF-301 (1.4)	8.24±0.02	24			24			7				17	
CF-301 (0.7)	7.93±0.12	24		1	23						1	23	
CF-301 (0.35)	8.17±0.58	24		3	21			6				18	
CF-301 (0.18)	8.65±0.58	16			16							16	

[a]Number in parenthesis represents dose in mg/kg
[b]Limit of detection is indicated by the less than sign for each group
[c]Number of isolates analyzed in MIC assays from each group
[d]The CF-301 MIC of *S. aureus* strain MW2 is 1 µg/mL
[e]The OXA MIC of *S. aureus* strain MW2 is 32 µg/mL

CF-301 MICs are stable

CF-301 exposure resulted in increased OXA susceptibility

Table 4.

Treatment Groups[a]	Log10 CFU/g of Vegetation[b]: 0	16	32	64	128	n[c]	CF-301 MIC (µg/mL)[d]: 0.25	0.5	1	2	4	OXA MIC (µg/mL)[e]: ≤2	4	8	16	32	64
Pre-Treatment Control	7.02±1.47	4.3±2.0	<2.3±0.04	<2.3±0.04	<2.3±0.04	19		1	18							19	
Buffer treatment Control	7.26±1.54	3.3±1.1	4.9±1.8	3.1±1.6	<2.1±0.1	44			44							44	
CF-301 (1.4)	8.24±0.02	7.1±0.06	5.9±0.03	4.2±1.7	2.8±1.2	24			20	4		3				21	
CF-301 (0.7)	7.93±0.12	6.7±0.1	5.7±1.1	3.8±1.8	<2.1±0.1	24		2	19	3					3	21	
CF-301 (0.35)	8.17±0.58	6.9±0.2	6.4±0.5	5.5±0.6	3.4±1.3	24			22	2		7				17	
CF-301 (0.18)	8.65±0.58	7.1±0.1	7.1±0.1	6.6±0.9	4.6±0.7	16			14	2						16	

[a]Number in parenthesis represents dose in mg/kg
[b]Limit of detection is indicated by the less than sign for each group
[c]Number of isolates analyzed in MIC assays from each treatment
[d]The CF-301 MIC of *S. aureus* strain MW2 is 1 µg/mL
[e]The OXA MIC of *S. aureus* strain MW2 is 32 µg/mL

CF-301 MICs exhibit only 2-fold increases

CF-301 exposure resulted in increased OXA susceptibility

- CF-301 exposures resulted in up to >16-fold reductions in OXA MIC values (from 32 µg/mL to <2 µg/mL)
- The resensitization observed in vivo was greatly enhanced over that observed in vitro
- MIC increases of only up to 2-fold were observed for CF-301
- Select isolates were chosen for WGS and mutagenesis to examine the mechanism of resensitization

Molecular Basis of In Vivo Resensitization Effect

Two mutants from valvular vegetations exhibiting 32-fold decreases in OXA MICs were analyzed by WGS and SNPs/INDELs were identified in a comparison with 3 control isolates. The CF-301 and OXA MICs of each mutant and control strain are shown (Table 4). The (+) symbol indicates the presence of the indicated mutation.

TABLE 4. Mutations associated with CF-301-mediated reductions in OXA MICs (SNP/INDEL analysis of in vivo-derived isolates)

Reference Position(s)[a]	Overlapping Annotation[b]	Ref	Allele	AA Change	MIC (µg/mL): Control CF-301	Control OXA	Control 2 CF-301	Control 2 OXA	Control 3 CF-301	Control 3 OXA	Mutant 1 CF-301	Mutant 1 OXA	Mutant 2 CF-301	Mutant 2 OXA
					1	64	2	32	2	64	2	1	1	1
2492859	*hlgCB* (near)	T	C		-		-		-		+		+	
1366472	*mprF*	T	A	L291I	-		-		-		+		-	
704001	*graR*	T	G	I158S	-		-		-		-		+	
34167	*rlmH*	G	A	K159R	-		-		-		+		+	
SCC*mec*			ΔSCC*mec*		-		-		-		+		+	

- Two sets of 4 mutations each (mutant 1 and 2) were implicated in the 32-fold decrease in OXA
- Loss of SCC*mec* is likely driver of OXA resensitization in IE model, specific mutations still need to be confirmed
- The in vivo mutations associated with 32-fold decreases in OXA MICs (i.e., resensitization) were distinct from the vitro mutations associated with 4-fold decreases in OXA MICs

Conclusion

- CF-301 treatment resensitizes MRSA to penicillin derivatives and 1st generation cephalosporins in vitro and in vivo studies
- Potent synergy with CF-301 reduces BL MICs to below breakpoints without adverse impact on anticipated susceptibility to CF-301
- Exposure to CF-301 alone, selects for mutations in cell wall biosynthetic genes or SCC*mec* that decrease OXA MICs
- Acting as a "resensitizing agent" by restoring sensitivity of MRSA strains to BLs, CF-301 represent a promising mechanism to combat and reverse antimicrobial resistance.