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LETTER TO THE EDITOR

Activity of Lysin CF-296 Alone and in Addition to Daptomycin in a Rat Model of Experimental Methicillin-Resistant *Staphylococcus aureus* Osteomyelitis

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Staphylococcal osteomyelitis is a complex, costly disease that is difficult to treat, with high failure rates and often poor outcomes (1, 2). Staphylococci form biofilms and survive intracellularly, compromising the activity of traditional antibiotics, often requiring debridement/resection of infected and necrotic tissue (2–7). There is an unmet need for more effective, rapidly bactericidal treatments, including those that can be used with standard of care (SOC) therapeutics.

Lysins are recombinantly expressed and purified cell wall hydrolytic enzymes under development as novel antimicrobial agents. We previously showed that the lysin exebacase, targeting *Staphylococcus aureus*, has rapid bactericidal and antibiofilm activity *in vitro* (8) and, when given in a single dose with daptomycin, reduced methicillin-resistant *S. aureus* (MRSA) in a rat model of acute osteomyelitis (9). Lysin CF-296 is an engineered variant of exebacase, developed to maintain activity against biofilms and to be administered in repeated and/or higher doses than exebacase. Due to the complexity of staphylococcal osteomyelitis and poor activity of SOC antibiotics, addition of CF-296 to treatment regimens may offer a more robust antimicrobial response, potentially decreasing morbidity and time to recovery. Here, we tested the activity of CF-296 *in vitro* and in a rat model of MRSA acute osteomyelitis.

S. aureus MICs and minimum biofilm eradication concentrations (MBECs) of CF-296 were 0.5 to 1 μg/ml, including IDRL-6169, the strain studied *in vivo* (Table 1) (10, 11). The daptomycin MIC of IDRL-6169 was 0.5 μg/ml.

A previously described model of rat acute osteomyelitis of the left tibiae used to test the *in vivo* activity of exebacase was used to evaluate CF-296 (9). A week after infection was established, rats were assigned to one of six groups: no treatment, 60 mg/kg daptomycin subcutaneously twice daily (12), 40 mg/kg CF-296 intravenously daily, 40 mg/kg CF-296 daily plus daptomycin, 100 mg/kg CF-296 intravenously once, or 100 mg/kg CF-296 once plus daptomycin. After 4 days, rats were euthanized and the left tibiae collected and analyzed, as previously described (9).

Results of bone cultures are shown in Fig. 1. Compared with no treatment, there were median 0.52-, 0.04-, and 0.33-$\log_{10}$ CFU/g reductions in MRSA for the daptomycin, CF-296 daily, and CF-296 single-dose animals, respectively, and 0.60- and 1.34-$\log_{10}$ CFU/g reductions in animals dosed with CF-296 daily or single-dose plus daptomycin, respectively. A CF-296 single 100 mg/kg dose plus daily daptomycin provided the most activity compared to no treatment ($P = 0.003$) and CF-296 single and daily doses

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Table 1 *In vitro* antistaphylococcal activity of CF-296

Strain[a]	Type[b]	MIC (μg/ml)	MBEC (μg/ml)
IDRL-6169	MRSA	0.5	1
MW2	MRSA	1	1
ATCC BAA-42	MRSA	1	1
JMI 227	MRSA	0.5	1
JMI 21489	MRSA	1	1
ATCC 29213	MSSA	1	0.5
JMI 7141	MSSA	1	1
JMI 3830	MSSA	1	1
JMI 7140	MSSA	1	1
JMI 7441	MSSA	1	1

[a]Strain MW2 (NRS 123) was obtained from BEI Resources (NIAID, NIH); ATCC strains were obtained from the American Type Culture Collection (Manassas, VA); JMI strains were obtained from JMI Laboratories (North Liberty, IA) and are described in Watson et al. (13).
[b]MSSA, methicillin-susceptible *S. aureus*.

alone (P = 0.0210 and 0.0175, respectively). Although not statistically significant, there was a trend toward the single 100 mg/kg dose of CF-296 administered plus daptomycin having more activity than daptomycin (additional 0.82 $\log_{10}$ CFU/g reduction). Despite reductions in CFUs, there was no cure of infection in the model studied.

Previously, we showed exebacase activity in our osteomyelitis model when given with daptomycin (9), and we now show similar activity using CF-296. Although different concentrations of exebacase and CF-296 were tested, the current and previous study showed a single dose of lysin administered in conjunction with daptomycin to result in a reduction in MRSA compared with untreated animals. Whether lysins might prevent emergence of daptomycin (or other antibiotic) resistance and possibly vice versa remains to be determined. In conclusion, CF-296 has *in vitro* antistaphylococcal activity and, when used with daptomycin, is active and well tolerated in rat MRSA acute osteomyelitis.

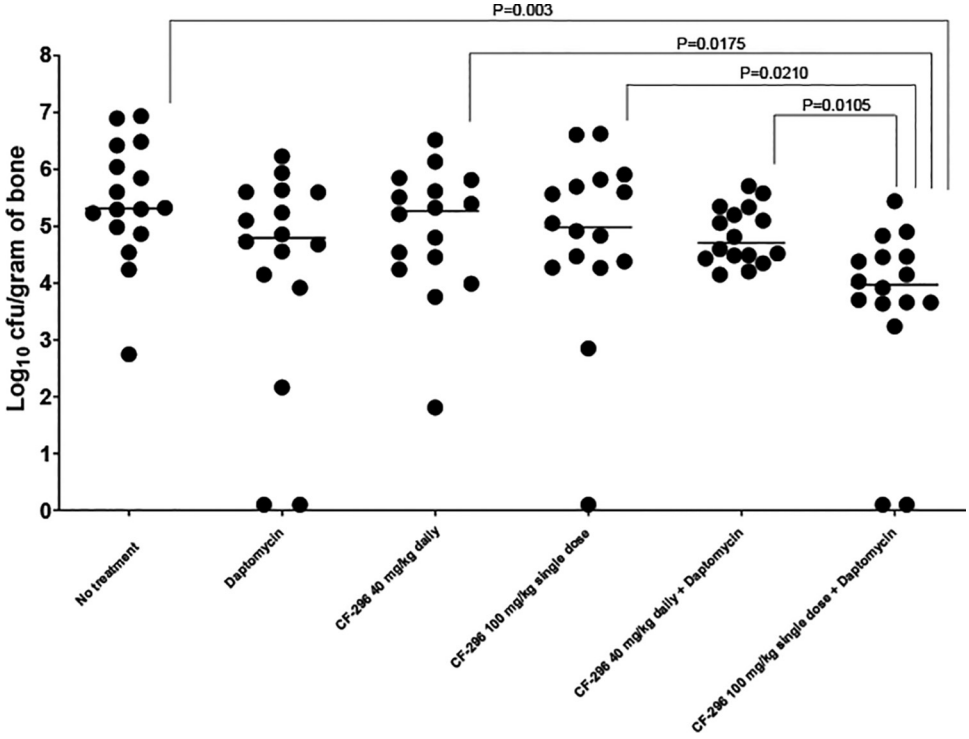

FIG. 1 Results of quantitative cultures (n = 16/group) of the tibiae after treatment ($\log_{10}$ CFU MRSA/g of bone). Bar represents median values.

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