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Assessment of In Vivo Efficacy of CF-296 in addition to Vancomycin (VAN) and Daptomycin (DAP) against *Staphylococcus aureus* in the Neutropenic Murine Thigh Infection Model

Tomefa E. Asempa[1], Nicole A. DeRosa[1], Cara Cassino[2], Dario Lehoux[2], Raymond Schuch[2], David P. Nicolau[1,3]

[1]Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, CT; [2]Contrafect Corporation, Yonkers, NY, USA

[3]Division of Infectious Diseases, Hartford Hospital, Hartford, CT

David P. Nicolau, PharmD, FCCP, FIDSA
Center for Anti-Infective Research and Development
Hartford Hospital
80 Seymour Street
Hartford, CT 06102, USA
Email: david.nicolau@hhchealth.org
Telephone: 860-972-3941

ABSTRACT

Background: CF-296 is a novel lysin in pre-clinical development for the treatment of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* infections, used in addition to standard of care antibiotics including VAN and DAP. We evaluated the *in vivo* efficacy of CF-296 alone and in addition to VAN and DAP against *S. aureus*.

Methods: Eight isolates (1 MSSA and 7 MRSA) were studied. Murine ICR MIC (100% serum) and human MIC (100% serum) for CF-296 ranged from 32-256 mg/L to 0.5-1 mg/L respectively. Broth microdilution MICs for DAP ranged from 0.5-1 mg/L while all isolates exhibited a VAN MIC of 1 mg/L. Neutropenic ICR mice were thigh inoculated with bacterial suspensions (10^7 CFU/mL). Mice were administered three monotherapy regimens subcutaneously (SC) or intravenously (IV): i) sub-therapeutic VAN, SC (i.e., a dose that yielded bacteria stasis or growth in order to evaluate further bacterial killing), ii) sub-therapeutic DAP, SC, or iii) CF-296 50 mg/kg, IV. Combination of sub-therapeutic VAN or DAP in addition to 5 escalating CF-296 doses ranging from 0.5 to 50 mg/kg were also examined. Control mice were vehicle-dosed. Efficacy was measured as the change in mean thigh bacterial density at 24h relative to 0h controls.

Results: Relative to starting inoculum (5.71 ± 0.27 at 0h), bacterial density in controls increased by +2.49 ± 0.98 $\log_{10}$ CFU/thigh across all 8 strains. On average, VAN, DAP, and CF-296 monotherapy resulted in +0.90 ± 1.21, +1.47 ± 0.80, and +0.87 ± 1.39 $\log_{10}$ CFU/thigh bacteria growth, respectively. In addition to VAN, escalating CF-296 exposures (0.5 – 50 mg/kg) resulted in an augmented dose-response, ranging from bacterial reduction of -0.26 ± 1.10 (with addition of CF-296 0.5 mg/kg) to -1.01 ± 0.41 $\log_{10}$ CFU/thigh (with addition of CF-296 50 mg/kg). Similarly, escalating CF-296 exposures in addition to DAP resulted in an augmented dose-response, ranging from bacterial density of +0.80 ± 1.19 to -0.72 ± 0.59 $\log_{10}$ CFU/thigh.

Conclusion: Compared with 24h control, VAN, DAP, and CF-296 alone displayed modest CFU reduction while CF-296 synergized with VAN and DAP to cause further bacterial killing highlighting a potential role for CF-296 adjunctive therapy against MSSA and MRSA isolates.

INTRODUCTION

- CF-296 is a novel anti-staphylococcal lysin that is being developed as an antimicrobial agent to lyse pathogenic bacteria by hydrolyzing peptidoglycan from outside the cell.
- CF-296 is in pre-clinical development as an adjunctive treatment to improve clinical cure rates of *S. aureus* infections, used in addition to standard of care antibiotics including vancomycin (VAN) and daptomycin (DAP).

OBJECTIVES

- To asses the *in vivo* efficacy of CF-296 alone and in addition to VAN and DAP against *S. aureus* isolates using a murine thigh infection model.

METHODS

Antimicrobial Test Agents

- CF-296 (10.5 mg/mL; Lot # 30180157, ContraFect Corporation, Yonkers, NY) was used for *in vivo* testing.
- VAN 1000 mg (Lot # YV822 and YV827, Alvogen, Pine Brook, NJ, USA) and DAP 500 mg (Lot # 931096, TEVA, North Wales, PA) commercial intravenous-use vials were used for *in vivo* testing.

Bacterial isolates and susceptibility testing

- Eight *S. aureus* isolates (1 MSSA and 7 MRSA) were used in this study (**Table 1**).
- MICs of VAN and DAP were determined using broth microdilution in triplicate as outlined by CLSI.[1]
- CF-296 MICs determined in human and ICR mouse serum (100%) were provided by ContraFect Corp.

Neutropenic Thigh Infection Model

- Pathogen-free, female ICR mice (20-22g) were obtained from Envigo RMS, Inc. (Indianapolis, IN). All studies were in accordance with the Institutional Animal Care and Use Committee at Hartford Hospital.
- Mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide (150 mg/kg on Day-4, 100 mg/kg on Day-1). Uranyl nitrate (5 mg/kg on Day-3) was administered to produce a controlled degree of renal impairment to assist with achieving appropriate VAN and DAP exposures.
- Isolates were transferred twice on TSA II™ plates (BD BioSciences, Sparks, MD) and incubated at 37°C. After 18 to 24h incubation of the isolate second transfer, a bacterial suspension of approximately 10^7 CFU/mL was made for inoculation.
- Thigh infection was produced by intramuscular inoculation of 0.1 mL of inoculum 2h prior to therapy initiation.

Antimicrobial Dosing

- CF-296 doses evaluated: 0.5 mg/kg, 2.5 mg/kg, 5 mg/kg, 25 mg/kg, and 50 mg/kg administered intravenously (IV tail) once over 24 hours.
- To obtain a VAN regimen appropriate for evaluating synergy upon addition of CF-296, VAN dose-ranging studies (3.125 mg/kg to 25 mg/kg q12h SC) were conducted against each isolate to obtain a regimen that would yield stasis or growth of the isolate at 24h.
- A VAN dose of 3 mg/kg q12h SC was ultimately determined to result in a dose-response profile appropriate for administration in subsequent CF-296 synergy studies.
- A previously developed sub-therapeutic DAP dosing regimen was utilized.[2] This regimen yielded net stasis or bacterial growth across all isolates at 24h relative to the 0h untreated controls.

In vivo Synergy Assessment Studies

- For each isolate tested, 3 untreated mice (six thighs) were used as 0h controls, 3 additional mice (receiving normal saline) as 24h controls, and 3 mice per each treatment dosing regimens was utilized.
- Treatments were initiated 2h post inoculation and continued for 24 hours.
- Mice were administered one of the following regimens subcutaneously:
  - (i) Escalating doses of CF-296 (0.5 to 50 mg/kg/day)
  - (ii) VAN alone
  - (iii) DAP alone
  - (iv) Escalating doses of CF-296 in addition to VAN
  - (v) Escalating doses of CF-296 in addition to DAP
- Efficacy was measured as the change in thigh bacterial density at 24h relative to 0h controls.

RESULTS

- On average, bacterial density in controls increased from 5.71 ± 0.27 at 0h to 8.20 ± 0.98 $\log_{10}$ CFU/thigh at 24h.
- Compared with 24h controls, a modest dose-response in bacterial burden was observed with increasing CF-296 doses (**Figure 1**).
- In addition to VAN, escalating CF-296 exposures (0.5 – 50 mg/kg) resulted in an augmented dose-response, ranging from bacterial reduction of -0.26 ± 1.10 to -1.01 ± 0.41 $\log_{10}$ CFU/thigh (**Figure 2**).
- Similarly, escalating CF-296 exposures in addition to DAP resulted in an enhanced dose-response, ranging from bacterial density of +0.80 ± 1.19 to -0.72 ± 0.59 $\log_{10}$ CFU/thigh (**Figure 3**).

Table 1. MICs of CF-296, VAN, and DAP against MSSA and MRSA isolates

		MIC (µg/mL)			
Isolate ID	**Bacterial Species**	**CF-296[1]**	**CF-296[2]**	**VAN**	**DAP**
STA 513	MRSA/LRSA	0.5	64	1	0.5
STA 514	MRSA	1	32	1	0.5
STA 516	MRSA	1	128	1	0.5
STA 517	MRSA	1	32	1	0.5
STA 518	MRSA	1	64	1	0.25
STA 520	MRSA	1	256	1	0.5
STA 521	MRSA	1	256	1	0.5
STA 523	MSSA	1	256	1	0.5

LRSA, Linezolid-resistant *Staphylococcus aureus;* [1]*Determined in human serum (100%);* [2]*Determined in ICR mouse serum (100%)*

Figure 1. *In vivo* efficacy of escalating CF-296 doses (0.5 mg/kg – 50 mg/kg).

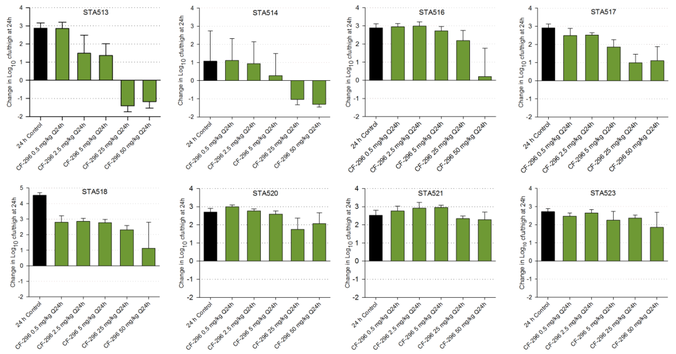


Figure 2. Efficacy of escalating CF-296 doses (0.5 mg/kg – 50 mg/kg) in addition to VAN compared with VAN and CF-296 alone.

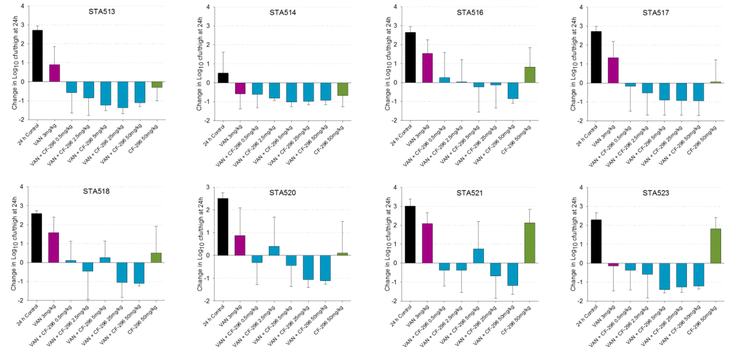
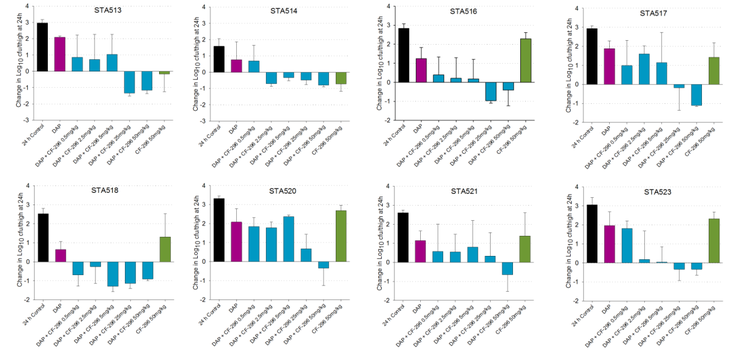


Figure 3. Efficacy of escalating CF-296 doses (0.5 mg/kg – 50 mg/kg) in addition to DAP compared with DAP and CF-296 alone.



CONCLUSIONS

- CF-296 administered with sub-therapeutic VAN and DAP resulted in further bacterial killing compared with either agent alone against all tested strains.
- Data supports a potential role for CF-296 in addition to standard of care anti-staphylococcal antimicrobials.
- Further pre-clinical *in vitro* and *in vivo* development of CF-296 for *S. aureus* infections is warranted.

ACKNOWLEDGEMENTS

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