

Efficacy of AFN-1252 and Vancomycin in the Mouse Subcutaneous Abscess Model with a Methicillin-Resistant *S. aureus*

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Abstract

Background: AFN-1252, a novel antibiotic inhibitor of the bacterial fatty acid biosynthesis (FAS II) pathway, is currently under clinical development as an oral and intravenous agent for susceptible and multi-drug resistant staphylococcal infections. AFN-1252 specifically targets the essential enzyme FabI (enoy-ACP reductase). The current study was performed to determine the efficacy of AFN-1252 (AFN) and Vancomycin (Vanco) in a mouse model of skin and skin structure MRSA infection.

Methods: Female CD-1 mice were rendered neutropenic by a single IP injection of cyclophosphamide (150 mg/kg) on day -4 prior to infection. Abscesses were formed on the flanks of mice by the subcutaneous injection of 10⁷ CFU of an *S. aureus* (MRSA) culture mixed with Cytodex (dextran) beads. Treatment with AFN (orally) or Vanco (intraperitoneal) was initiated 2 hrs post-infection and continued for three days either once-per-day (qd) or twice-per-day (bid). Abscesses were removed, homogenized and plated 18 hrs after the last dose. Efficacy was determined as the change in CFU/abscess as compared to vehicle treated controls.

Results: The MICs of AFN and Vanco for the MRSA strain used in this study were determined to be 0.008 and 1 µg/mL, respectively. Abscesses in vehicle treated control animals exhibited a mean bacterial density of 8.7 log CFU/abscess at the end of the study. AFN, administered orally at 100, 30 or 10 mg/kg, demonstrated log reductions of 5.9, 5.2 and 2.5 CFU when administered bid and 5.2, 4.1 and 2.4 CFU when dosed qd, respectively, as compared to the vehicle treated control animals. Vanco dosed at 30 mg/kg IP bid exhibited a 4.4 log CFU reduction.

Conclusion: The results of this study demonstrate the *in vivo* efficacy of AFN-1252 in a murine subcutaneous abscess model with an MRSA strain and support its further study and development for both susceptible and resistant *S. aureus* skin infections.

Introduction

The incidence as well as prevalence of MRSA continues to rise with 60-70% of all *S. aureus* strains from hospitals being multi-drug resistant MRSA. Concerns have been accelerated when MRSA isolates began to appear in the community setting including day care facilities, athletic teams, prison populations and the military. Coupled with both vancomycin and fluoroquinolone resistance, hospital and community acquired MRSA infections pose a therapeutic challenge.

FabI (enoy-ACP reductase) catalyzes the final step in the FASII chain elongation cycle and is essential for bacterial growth and survival. AFN-1252 was optimized against staphylococcal FabI, and by inhibiting this enzyme, disrupts fatty acid biosynthesis thereby inhibiting growth. It exhibits potent activity against MRSA strains with no cross resistance and a low frequency of resistance due to this novel mechanism of action.

The current study was performed to evaluate the efficacy of AFN-1252 in a mouse model of an MRSA subcutaneous infection.

Methods and Materials

Bacteria: Staphylococcus aureus (UNT002-3) clinical isolate that is both fluoroquinolone-resistant and methicillin-resistant.

Mouse: Female 5-6 week old CD-1 mice were used in the studies.

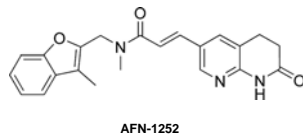
Pre-treatment: Mice were rendered neutropenic by a single IP administration of cyclophosphamide (Cytodex) given at 150 mg/kg day -4 prior to infection.

Inoculum: *S. aureus* was grown on appropriate agar media at 37C for 12 - 18 hr and resuspended in Trypticase Soy Broth (TSB) medium to an absorbance of 1.37 (800 nm). This suspension was diluted to the target CFU range in TSB (approx. 5 x 10⁷ CFU/mL). Diluted cells in TSB were further diluted 4 fold (v/v) into 20 mg/mL sterile dextran beads to generate infecting inocula. Plate counts performed on the inoculum determined there to be 2.38 x 10⁷ CFU/abscess.

Abscesses: Mice were anesthetized, shaved, sterilized and 0.2 mL of infecting inocula injected into 2 separate subcutaneous sites on the flanks of the mice. All treatments began 2 hr after infection and administered by either 0.4 mL oral gavage (PO) or 0.2 mL intraperitoneal (IP) injection dose. All groups were treated beginning 2 hrs post-infection and then either twice-a-day (bid) or once-a-day (qd) for a period of three days as indicated for each group.

Sampling: The mice were euthanized by CO₂ inhalation at the completion of the study (+18 hrs post-final dose). The flanks of the mice were sterilized and the skin was then gently resected, and abscesses excised into 1 mL of sterile 1xPBS and homogenized. Homogenates were serially diluted and spotted onto charcoal agar to determine CFU counts. Plates were incubated 18 - 24 hr under the appropriate strain conditions prior to counting.

Panel 1: Chemical Structure of AFN-1252



Panel 2: Mouse Subcutaneous Abscess Model



* Subcutaneous abscesses on the dorsal surface of CD-1 mice following subcutaneous injection of methicillin-resistant *S. aureus* UNT002-3 and dextran (Cytodex) beads.

Panel 3: AFN-1252 *in vitro* antimicrobial activity against *S. aureus* UNT002-3 (MRSA)*

Compound	MIC (µg/mL)
AFN-1252	0.008
Vancomycin	1
Rifampin	0.008
Daptomycin	0.5
Linezolid	4
Tigecycline	0.25
Ciprofloxacin	16
Ceftazoxime	> 16
Azithromycin	> 16

- * Microtiter broth MICs were performed in accordance with CLSI guidelines.
- AFN-1252 exhibits potent activity against *S. aureus* (MRSA), comparable to Rifampin and 64 - 512 fold more active than Daptomycin, Vancomycin or Linezolid.
- MRSA UNT002-3 was also ciprofloxacin-resistant.

*Determined by growth on 6 µg/mL 4% NaCl plate

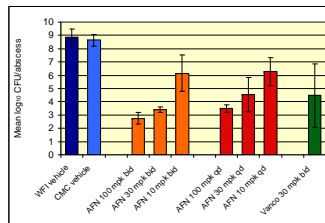
Panel 4: AFN-1252 Efficacy in the Mouse Subcutaneous Abscess Model

Treatment	Mean log ₁₀ CFU/abscess ^a	Std. dev	Log reduction ^b
WFI vehicle ^c	8.88	0.62	NA
0.5% CMC vehicle	8.64	0.42	NA
AFN 100 mpk bid	2.75	0.45	5.89
AFN 30 mpk bid	3.4	0.2	5.24
AFN 10 mpk bid	6.14	1.37	2.5
AFN 100 mpk qd	3.49	0.27	5.15
AFN 30 mpk qd	4.52	1.29	4.12
AFN 10 mpk qd	6.27	1.04	2.37
Vanco 30 mpk bid	4.46	2.39	4.42

^a N=10 abscesses (5 mice w/ 2 per mouse); ^b log reduction vs. 24 hr vehicle control(s)
^c WFI = water for injection, CMC = 0.5% carboxymethylcellulose

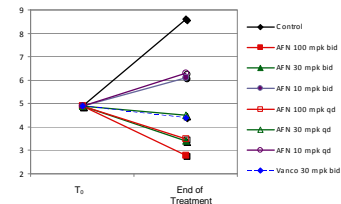
- Bacterial densities in abscesses from vehicle control animals were 8.88 and 8.64 log₁₀ CFU/abscesses for the WFI and CMC vehicle groups, respectively.
- AFN-1252, administered twice-a-day (bid) resulted in a 5.89, 5.24 and 2.5 log reduction (compared to the CMC vehicle control treated animals) for the 100, 30 and 10 mg/kg dose groups, respectively.
- When AFN-1252 was administered once-a-day (qd), log reductions of 5.15, 4.12 and 2.37 were observed for the 100, 30 and 10 mg/kg dose groups, respectively.
- Vancomycin, at 30 mg/kg IP, bid exhibited a 4.42 log reduction from the WFI vehicle control group.

Panel 5: Mean Log₁₀ CFU/abscess of *S. aureus* UNT002-3



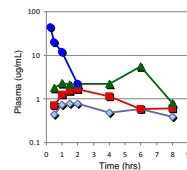
- * Comparison of mean Log₁₀ CFU/abscess for methicillin-resistant *S. aureus* UNT002-3 following administration of AFN-1252 orally (bid and qd) or vancomycin IP (bid) initiated at +2 hrs post-infection and continued for 3 days.
- Abscesses were excised approx. 18 hrs after the last dose.
- N=10 abscesses (5 mice w/ 2 abscesses per mouse) for each dose group.

Panel 6: Reduction of Mean Log₁₀ CFU From T₀ Following AFN-1252 Administration



- Compounds were administered at 2 hrs post-infection (T = 0 hr).
- Mean Log₁₀ CFU in abscesses of vehicle treated controls increased 3.7 - 3.9 Log₁₀ from 0 - 24 hrs.
- AFN-1252 exhibited a 2.15 and 1.5 Log₁₀ CFU reduction from T₀ when administered orally at 100 and 30 mg/kg bid x 3 days and a 1.4 and 0.4 Log₁₀ reduction when administered once-a-day, respectively.
- Vancomycin at 30 mg/kg IP bid reduced abscess CFU by only 0.44 Log₁₀ from T₀ counts.

Panel 7: Pharmacokinetics of AFN-1252 and Vancomycin in CD-1 Mice



Parameter	AFN 10 mg/kg	AFN 30 mg/kg	AFN 100 mg/kg	Vanco 30 mg/kg
C _{max} (µg/mL)	0.79	1.68	5.58	43.6
T _{max} (hr)	1.5	2	6	0.25
AUC ₀₋₈ (µg-hr/mL)	4.5	8	22.4	36.3
AUC ₀₋₂₄ (µg-hr/mL)	6.4	9.7	23.2	37.6
Half-life (hr)	3.3	2.1	2.7	1.7
MRT (hr)	6.5	4.8	4.6	0.7
C _{min} / dose	0.079	0.056	0.056	1.45
AUC ₀₋₈ / dose	0.45	0.27	0.22	1.21

Summary and Conclusions

- AFN-1252 is an anti-staphylococcal agent acting by a novel mechanism of action inhibiting the bacterial fatty acid biosynthesis (FAS II) pathway.
- AFN-1252 exhibited potent *in vitro* activity against the methicillin-resistant *Staphylococcus aureus* isolate used in this infection study and was more active than Vancomycin, Daptomycin, Linezolid and Tigecycline.
- Administered orally, AFN-1252 exhibits a dose response resulting in Log₁₀ reductions from 2.5 - 5.89 and 2.37 - 5.15 when dosed from 10 - 100 mg/kg either once (qd) or twice (bid) per day.
- AFN-1252, dosed qd orally, demonstrated equivalent efficacy to that of Vancomycin administered bid IP, at the same 30 mg/kg dose.
- AFN-1252 exhibited a 1.5 - 2.15 Log₁₀ CFU reduction from T₀ when administered orally at 30 - 100 mg/kg bid x 3 days and a 0.4 - 1.4 Log₁₀ reduction when administered once-a-day, respectively.
- The pharmacokinetics of orally administered AFN-1252 indicate that it attains equivalent efficacy to that of vancomycin at a lower plasma AUC and C_{max}, but much longer MRT.
- AFN-1252 exhibits efficacy in the murine subcutaneous abscess model following oral administration and warrants further investigation as a promising agent for the treatment of skin and skin structure infections caused by methicillin-resistant *Staphylococcus aureus*.

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