F1-833

Oral Pharmacokinetics and Efficacy of AFN-1252 in a Murine Septicemia Infection Model with *S. aureus*

50th ICAAC Meeting
Boston, MA
September 12-15, 2010

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Abstract

Background: AFN-1252 (AFN), a novel antibiotic inhibitor of the bacterial fatty acid biosynthesis pathway (specifically FabI), is in clinical development for susceptible and multi-drug resistant staphylococcal infections. As no previous data exist for the efficacy of AFN in bloodstream infections, the pharmacokinetics (PK) and oral efficacy of AFN were determined in a murine septicemia model. **Methods:** For the PK and efficacy studies, Female CD-1 mice were administered escalating single oral doses of AFN in either carboxymethylcellulose (CMC) or Poloxamer (PLX). Plasma samples were analyzed by LCMS and PK parameters determined. Female CD-1 mice were infected with a bacterial inoculum of S. aureus Smith resulting in the death of untreated controls within 48 hr. A single oral treatment of AFN or linezolid (LNZ) was initiated 30 min post-infection and survival ratios were determined at 7 days for ED50 determination by Probit analysis. **Results**: The PLX formulation achieved 2-3x higher exposure of AFN than the equivalent doses in CMC. At doses of 0.3 – 1 mg/kg C_{max} values were 63 – 106 ng/mL in the CMC formulation and 154 – 304 ng/mL for PLX. The AUC_{0-t} values in CMC and PLX were 224 – 458 and 367 - 1030 ng-hr/mL, respectively. AFN administration resulted in survival of all animals at doses of 1 - 3 mg/kg which was 12-24x more efficacious than LNZ by the oral route. AFN exhibited ED_{50}'s of 0.29 mg/kg and 0.15 mg/kg in CMC and PLX, respectively. LNZ exhibited an ED_{50} of 3.6 mg/kg. Modeling AFN dose vs AUC showed that similar AUC_{0-t} values of 204.5 ng-hr/mL and 203 ng-hr/mL were observed at the ED_{50} for CMC and PLX, respectively. **Conclusion:** Plasma levels of AFN following oral administration can be enhanced based on formulation. Oral AFN is highly effective in the lethal S. aureus murine septicemia model and exhibits much greater efficacy than LNZ. These data support the potential utility of AFN as a therapeutic treatment for bacterial bloodstream infections.

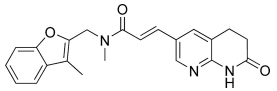
Introduction

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 (enoyl-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 following oral administration in a mouse septicemia model with *S. aureus*.

Methods and Materials

Mice: Female 5 - 6 week old CD-1 mice (18-22 gm) were used in the studies.
Acute Lethal Infection Model: Mice were challenged by injecting 0.5 ml intraperitoneally of a predetermined *S. aureus* Smith bacterial inoculum suspended in hog gastric mucin. The bacterial inoculum was equivalent to 10 - 100 LD_{50}s of the specific infecting strain and resulted in the death of the non-treated control animals within 24 - 48 hrs. Antibacterial doses of AFN-1252 were prepared in both aqueous 0.5% carboxymethylcellulose and an aqueous 1% Poloxamer 407 formulation. Linezolid stock solution was diluted in 5% dextrose water and was used as the positive control compound in all tests. Compounds were administered as a single oral dose at 0.5 hrs post-infection. Animals were observed for 5 days after treatment. A census of survivors was taken and the results of these tests were used for the determination of the median effective dose (ED_{50}) using a computerized program for Probit analysis.
Pharmacokinetics: The pharmacokinetics of AFN-1252 were determined at 3 different dose levels (0.3, 0.6 and 1 mg/kg) for both the 0.5% CMC and 1% Poloxamer 407 formulations. The compound was prepared at equivalent concentrations in both formulations and administered by oral gavage (comparable to efficacy study concentrations and dose volumes). Blood was collected by cardiac puncture at 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 hrs into Na-heparin tubes (3 mice/time point, collected individually), stored on ice and centrifuged to collect plasma. Plasma samples were analyzed by LCMS.

Panel 1: Chemical Structure of AFN-1252



AFN-1252

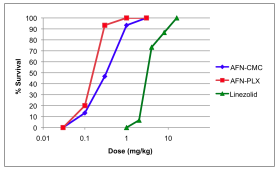
Panel 2: Minimum Inhibitory Concentration (MIC) for AFN-1252 and Selected Agents Against *S. aureus* Smith

Compound	MIC (ug/mL)
AFN-1252	0.004
Linezolid	1
Ciprofloxacin	0.125
Erythromycin	1
Penicillin	0.0625
Gentamicin	2
Vancomycin	1

- Microtiter broth MICs were performed in accordance with CLSI guidelines.
- AFN-1252 exhibited potent activity against *S. aureus* Smith and was 15-500x more active than all other agents tested, including Linezolid and Vancomycin.

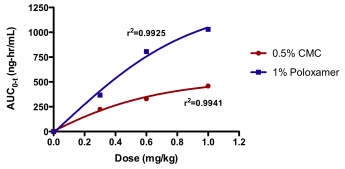
Panel 4: Oral Efficacy of AFN-1252 in Two Formulations Against *S. aureus* Smith in the Murine Septicemia Model

Compound	ED_{50} (mg / kg)	95% Conf. limts
AFN-1252 : CMC	0.29	0.21 - 0.39
AFN-1252 : PLX	0.15	0.11 - 0.21
Linezolid	3.6	2.8 - 4.7
LD_{50}	5.07×10^3	



- AFN-1252 exhibits excellent *in vivo* efficacy against *S. aureus* in the mouse acute lethal septicemia model with oral ED_{50} values that were 12 – 24 times lower than Linezolid.

Panel 5: Comparison of AFN-1252 Formulation Pharmacokinetics and Efficacy in the Mouse Septicemia Model



AFN-1252 Formulation Comparison – ED_{50} and Corresponding Plasma AUC

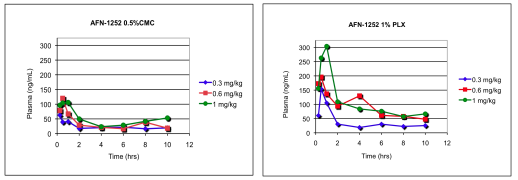
Parameter	CMC	PLX
ED_{50} (mg/kg)	0.29	0.15
AUC_{0-t} (ng-hr / mL)	204.5	203.0

- Analysis of AFN-1252 plasma levels demonstrated that although the AFN-1252 oral ED_{50} for the PLX formulation was 2-fold lower than for the CMC formulation, total plasma exposure (AUC) at the ED_{50} value for both formulations was equivalent.

Panel 3: Pharmacokinetics of AFN-1252 Following Oral Administration in 0.5% Carboxymethylcellulose and 1% Poloxamer Formulations to Female CD-1 Mice

	Plasma (ng/mL)					
	0.5% CMC			1% Poloxamer		
Time (hrs)	0.3 mg/kg	0.6 mg/kg	1 mg/kg	0.3 mg/kg	0.6 mg/kg	1 mg/kg
0.25	62.7	78.5	96.6	60.8	173	156
0.5	39.3	120	104	154	196	263
1	40.8	66.9	106	105	137	304
2	17.7	28.8	49.8	30.7	93.6	109
4	20.3	21.2	23.4	18.8	130	84
6	21.8	16.6	28.7	30.5	61.2	75.9
8	16.4	38.7	41.8	22.5	57.8	57.2
10	19.5	18.5	53.2	25.5	47.7	66.5
C_{max} (ng/mL)	62.7	120	106	154	196	304
T_{max} (hr)	0.25	0.5	1	0.5	0.5	1
AUC_{0-t} (ng-hr/mL)	224.1	329.5	458.4	366.9	805.6	1029.8
AUC_{0-inf} (ng-hr/mL)	361.1	379.6	nd	534.5	1402.2	1683
MRT (hr)	9.2	5.2	nd	7.7	9.8	9.9

Effect of the formulation on the pharmacokinetics of AFN-1252 following oral administration in female CD-1 mice



- Poloxamer (Pluronic F127; Polyoxyethylene-Polyoxypropylene Block Copolymer) and carboxymethylcellulose (1500 cps) formulations of AFN-1252 achieve different overall plasma exposure following oral administration.
- Both the observed C_{max} and AUC exposure obtained with the Poloxamer formulation were 3 x higher than that achieved with the carboxymethylcellulose formulation of AFN-1252.
- The Time to C_{max} (T_{max}) and mean residence time (MRT) for both formulations were comparable.

Summary

- The Poloxamer formulation of AFN-1252 achieved 2-3x higher plasma exposure following oral administration than the equivalent doses formulated in methylcellulose.
- At doses of 0.3 - 1 mg/kg, C_{max} values were 63 - 106 ng/mL in the CMC formulation and 154 - 304 ng/mL for PLX.
- The AUC_{0-t} values in CMC and PLX were 224 - 458 and 367 - 1030 ng-hr/mL, respectively.
- In the acute lethal septicemia infection model with *S. aureus*, AFN-1252 exhibited ED_{50}s of 0.29 mg/kg and 0.15 mg/kg in CMC and PLX formulations, respectively, while LNZ exhibited an ED_{50} of 3.6 mg/kg.
- Modeling of AFN-1252 dose vs AUC showed that similar AUC_{0-t} values of 204.5 ng-hr/mL and 203 ng-hr/mL were observed at the calculated ED_{50} values for CMC and PLX formulations, respectively.

Conclusion

- Plasma levels of AFN-1252 following oral administration can be enhanced based on formulation.
- Oral AFN-1252 is highly effective in the lethal *S. aureus* murine septicemia model and exhibits much greater efficacy than LNZ , supporting the potential utility of AFN -1252 as a therapeutic treatment for bacterial bloodstream infections.

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Acknowledgments

This study was funded and supported by Affinium Pharmaceuticals, Toronto, Canada. The authors would like to acknowledge Jessica Pierce and Maciej Kukula of UNTHSC for technical assistance and MPI Research, Matawan MI for analysis of plasma samples.