Antimicrobial Original Research Paper

# Activity of AFN-1252, a novel FabI inhibitor, against *Staphylococcus aureus* in an *in vitro* pharmacodynamic model simulating human pharmacokinetics

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AFN-1252, a potent enoyl-ACP reductase (FabI) inhibitor, is under development for the treatment of *Staphylococcus aureus* infections. The activity of AFN-1252 against two isolates of *S. aureus*, MSSA 26213 and MRSA S186, was studied in an *in vitro* pharmacodynamic model simulating AFN-1252 pharmacokinetics in man. Reductions in bacterial viable count over the first 6 hours were generally 1–2 logs and maximal reductions in viable count were generally achieved at *f*AUC/MIC ratios of 100–200. Maximum reductions in viable count against MSSA 29213 and MRSA S186 were approximately 4 logs, achieved by 450 mg q12h (*f*AUC/MIC=1875) dosing at 28 hours. Staphylococcal resistance to AFN-1252 did not develop throughout the 48-hour experiments. As multidrug resistance continues to increase, these studies support the continued investigation of AFN-1252 as a targeted therapeutic for staphylococcal infections.

**Keywords:** *Staphylococccus aureus*, FabI, AFN-1252, Pharmacodynamics, MRSA, MSSA, Bactericidal

## Introduction

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) to newer antibacterial agents, in both community and healthcare settings, is a serious worldwide concern as therapeutic options are limited. Hence, there is a need for new antimicrobials possessing potent activity against this pathogen.

Bacterial fatty acid biosynthesis (FASII) is a relatively new and unexploited target for antimicrobial treatment of *S. aureus*.[1] AFN-1252, a potent inhibitor of staphylococcal enoyl-acyl carrier protein (enoyl-ACP) reductase (FabI), a critical enzyme required for bacterial FASII, is being developed by Affinium Pharmaceuticals, Inc. (Toronto, ON, Canada), in both oral and intravenous formulations, for the treatment of *S. aureus* infections. *In vitro*, AFN-1252 exhibits targeted and highly potent activity against *Staphylococcus* spp., with typical minimum inhibitory concentration ($MIC_{90}$) values of 0.008–0.015 mg/l, up to 3 log reductions in bacterial viable count over 24 hours and low potential for resistance development.[2–4] Murine studies indicate a long elimination half-life 5–7 hours and excellent efficacy in models of infection.[5,6] Pharmacokinetic studies in man indicate the potential for once or twice daily dosing.[7]

The objective of this study was to utilize an *in vitro* pharmacodynamic model simulating human pharmacokinetics to evaluate potential therapeutic regimens of AFN-1252 against *S. aureus*, including both methicillin-susceptible *S. aureus* (MSSA) and MRSA.

## Materials and Methods

### *Bacterial isolates, antibiotics, media, and susceptibility testing*

Bacterial *S. aureus* isolates utilized were ATCC 29213, a standard reference MSSA and S186, a clinical MRSA isolate obtained from the bloodstream of an infected patient at the Buffalo Veterans Affair Health System of Western New York. Stock solutions of AFN-1252, provided by Affinium Pharmaceuticals, Inc., were prepared in 100% dimethyl sulphoxide and diluted at least 100-fold in Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) supplemented with calcium (25 μg/ml) and magnesium (12.5 mg/l)

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for MICs determinations[8] and use in the *in vitro* pharmacodynamic model. Bacterial quantification of all samples was determined using tryptic soy agar with 5% sheep blood (Becton-Dickinson, Mississauga, ON, Canada).

### In vitro *pharmacodynamic model*

The *in vitro* pharmacodynamic model was as previously described.[8] Human pharmacokinetic profiles of AFN-1252 were based on phase 0 studies.[9] Simulated therapeutic regimens of AFN-1252 were based on free drug area under the concentration time curve to MIC ratio over MIC (*f*AUC/MIC) as detailed in Table 1. The initial bacterial inoculum was adjusted spectrophotometrically to achieve a final concentration of $\sim 10^6$ colony-forming units/ml. Bacterial samples were taken at 0, 2, 4, 6, 8, 24, 28, 32, and 48 hours and the viable counts were determined. To investigate the impact of escalating exposures on selection of resistant isolates, samples taken at 0, 24, and 48 hours were also plated on tryptic soy agar containing 4× and 6× MICs of AFN-1252 to detect and amplify resistant subpopulations.

## Results

The AFN-1252 MICs for MSSA 29213 and MRSA S186 were both 0.008 μg/ml. The activity of AFN-1252 in the *in vitro* pharmacodynamic model is shown in Fig. 1A–D. For all dosage regimens, reductions in viable count over the first 6 hours were generally 1–2 logs and maximal reductions in viable count (e.g. −2 to −3 logs) generally achieved at *f*AUC/MIC of 100–200. Greater reductions in viable count were observed with q12h regimens than corresponding q24h regimens, against both MSSA 29213 and MRSA S186 at 24 and 48 hours. The greatest reductions in viable count against MSSA 29213 and MRSA S186 were approximately 4 logs, achieved by 450 mg q12h (*f*AUC/MIC=1875) dosing at 28 hours. Further analysis of the pharmacodynamic responses revealed an excellent correlation in the Hill model[10,11] between log reduction in viable count and *f*AUC/MIC fits ($R^2$ values 0.998–0.999). AFN-1252 did not develop resistance at any time point throughout the 48-hour experiment as no growth was present on AFN-1252-containing agar.

**Table 1 Simulated AFN-1252 doses and corresponding free drug area under the concentration time curve to minimum inhibitory concentration (MIC) ratio over MIC (*f*AUC/MIC) parameters in the *in vitro* pharmacodynamic model**

| | *f*AUC/MIC | |
|---|---|---|
| Dose (mg) | Dosing q12h | Dosing q24h |
| 4.2 | 39 | — |
| 9.4 | 78 | 39 |
| 18.8 | 156 | 78 |
| 37.5 | 312 | 156 |
| 56.2 | 468 | — |
| 75.0 | 625 | 312 |
| 112.5 | 938 | 468 |
| 225 | — | 938 |
| 450 | — | 1875 |

## Discussion

FabI is the sole form of enoyl-ACP reductase present in *S. aureus*, *S. epidermidis*, and other staphylococci. No alternative enzyme or rescue pathway, e.g. exogenous fatty acids, for FabI in staphylococci has been identified suggesting that FabI is essential to cell viability in *Staphylococcus* spp and therefore has the potential to become a significant new target for the treatment of staphylococcal infections.[4]

AFN-1252 is a highly potent and specific inhibitor of FabI with exquisite activity against staphylococci in extensive MIC studies,[2–4] and superior activity to linezolid in the MRSA murine thigh lesion model.[5] Pharmacokinetic studies in human volunteers indicate a good safety profile and the potential for once or twice a day dosing.[7] Rate of kill studies demonstrate that AFN-1252 typically achieves a 1–2 log reduction in viable count within 24 hours and therefore is not bacteriostatic but does not meet the CLSI criteria of bactericidal.[12] Other studies have also indicated that AFN-1252 can achieve a >2 log reduction in bacterial count over more than 24 hours[4] and hence its action may be best described as 'slowly bactericidal'. Although other triclosan-based FabI inhibitors CG400462, CG400549,[13] and MUT056399[14] are under investigation, they appear to be less potent than AFN-1252 and are less advanced.

In this current investigation, we studied the bactericidal activity and pharmacodynamics of AFN-1252, using an *in vitro* pharmacodynamic model, against two strains of *S. aureus* to determine optimal therapeutic regimens including comparisons of once and twice daily dosing. As a result of these studies, the pharmacodynamic profile of AFN-1252 was adequately characterized with the *f*AUC/MIC well related to antibacterial killing, which is also in agreement with what others have shown.[15] Twice daily dosing achieved marginally greater reductions in bacterial viable counts than once daily dosing, approaching the CLSI definition of bactericidal. Both dosing regimens resulted in bactericidal activity starting at 28 hours. It will be interesting to perform further pharmacokinetic/pharmacodynamic studies using hollow fibre and animal infection models to see if this affects the degree of bactericidal activity.

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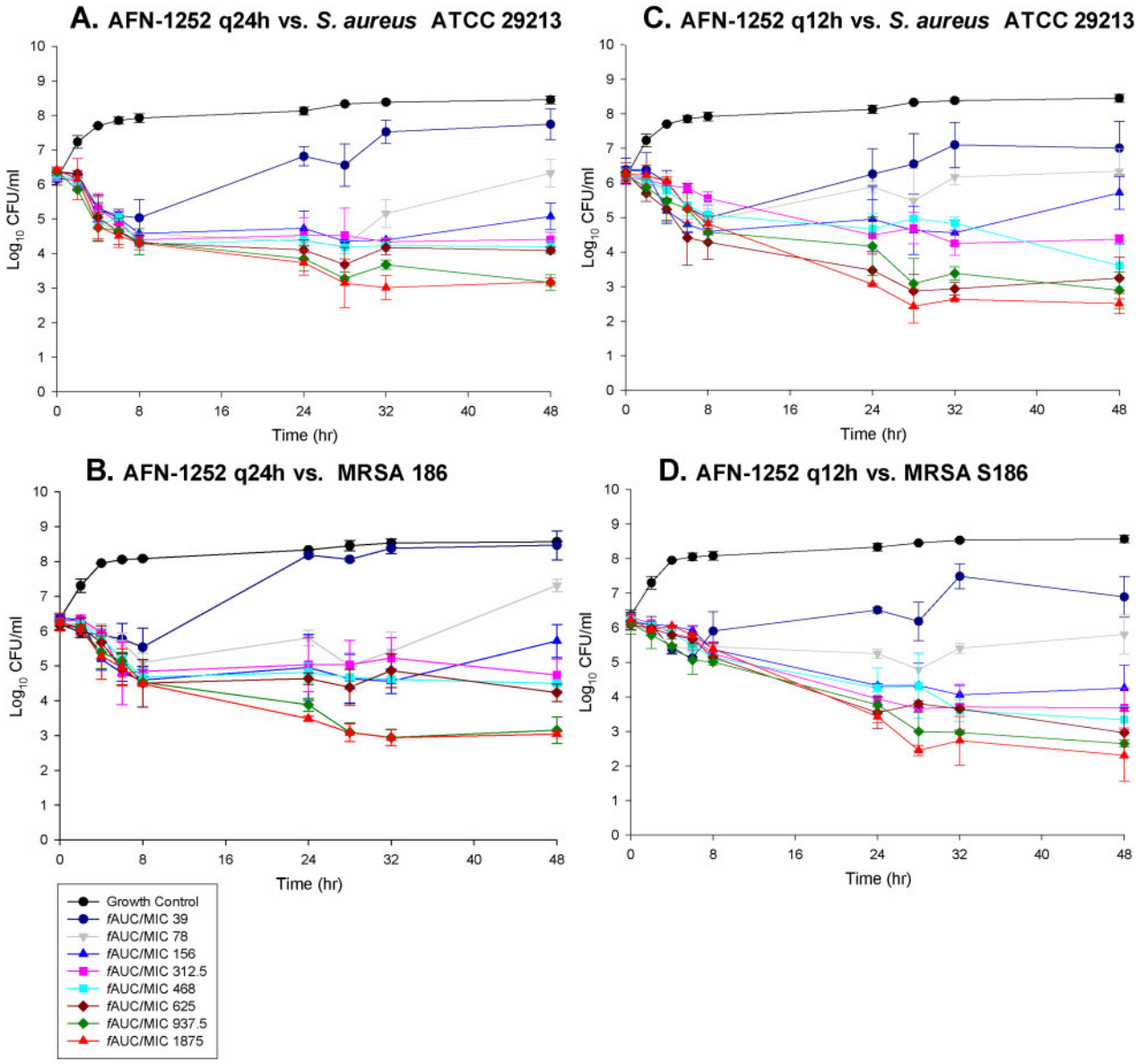


**Figure 1** ***In vitro*** **pharmacodynamic model experiments of (A) AFN-1252 q24h versus ATCC 29213, (B) AFN-1252 q12h versus ATCC 29213, (C) AFN-1252 q24h versus MRSA S186 and (D) AFN-1252 q12h versus MRSA S186.**

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