Effects of AFN-1252 on In Vitro and In Vivo S. aureus Virulence Gene Expression


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Abstract

Background: AFN-1252 (AFN) is a novel antibiotic currently in clinical development for staphylococcal infections, blocks type II fatty acid synthase (FAS II) by inhibiting early and late FAS II in S. aureus. The current study describes the effects of AFN on bacterial gene expression in S. aureus lab cultures, and the pharmacokinetics (PK) and efficacies of AFN in a mouse granuloma (MG) model of S. aureus infection.

Methods: Affymetrix gene array and qRT-PCR were used to determine gene expression changes in AFN treated S. aureus cultures. Experimental phase laboratory cultures of S. aureus were treated with either sub-inhibitory control or 50 ng/ml of AFN for 15 minutes, and total RNA was extracted from the cells for analysis. In vivo experiments involved inoculating S. aureus into 5-day-old granulomas that were formed in the subcutaneous area of C57 mice, and weekly during 100 mg/kg of AFN 2 hours at 0, 3, 24, and 48 hours after inoculation. Granuloma fluid was collected at multiple timepoints (0, 14, and 24 hr) from the hour following dosing for CFU counting, mRNA profiling and determining AFN concentration.

Results: Exposure of AFN in S. aureus cultures resulted in the anticipated upregulation of genes involved in the FAS pathway associated with the FasR regulon and the unpredicted derepression of virulence genes that are controlled by the SarA two-component regulator. In the MG infection model, the relative exposure (AUC) of AFN in granuloma fluid when compared to plasma ranged from 60%-75%, with a calculated T1/2 of 4 hours. A single dose of AFN at 100 mg/kg at 2 hours post-infection resulted in mean log CFU reductions of 2.9-3.1 between 24-48 hours, while concurrent doses of AFN (100 mg/kg) at 2, 26, and 48 hours resulted in a maximum log CFU reduction of 5.3 at 72 hours.

Conclusions: AFN exposure led to the unexpected effect of decreasing the expression of S. aureus genes encoding virulence factors that belong to the SaeRS regulation. In the MG model, AFN had favorable penetration in granuloma fluid and high efficacy against fluid-associated S. aureus.

Introduction

Staphylococcal aureus has the ability to produce a number of virulence factors (virFs) that are thought to be important during the infection process resulting disease states in the host. S. aureus coordinates the expression of virulence factors through a network of regulators that include sarA, and the two-component regulator saerf. The results from these studies suggest that antibiotics could impact the severity of disease by modulating virulence factor expression in S. aureus, even at sub-inhibitory levels.

The focus of our work was to evaluate the modulating effects of AFN-1252, a novel fatty acid synthase inhibitor (FAS II), on S. aureus virulence factor expression in lab cultures and in a subcutaneous granuloma pouch animal model infected with S. aureus. Here, we describe the results from the gene expression analysis, as well as the pharmacodynamic (PD) and efficacy results of AFN-1252 in the granuloma pouch model.

Methods and Materials

Bacterial strains: S. aureus strain RN4220 employed for in vitro work was obtained from the American Type Culture Collection (ATCC). S. aureus strain RN4220 (ΔsasA) was constructed by the inversion of sasA into the sasS gene. The G1542Δ and Woodlief strains were selected through the use of protoplast intolerance in Diplococcus pneumoniae (Diplo) array program supported under NIDDK/National Center for Research Resources 5R01RR018713-11S2 (Rock), and the antibiotic enzyme was kindly provided by Dr. Nick Mair (VTCN; Affinnova, NJ).

Gelation conditions and inoculation preparation. For in vitro studies, S. aureus strains were grown in nutrient broth (NB) to mid log phase, and then split into 2 aliquots for treatment with solvent control (DMSO) or treatment with formulated AFN125, sarAerf, or chloramphenicol. For in vivo studies, S. aureus Woodlief was cultured overnight on TSA (tryptic soy agar), and plated grown on TSA plates with kanamycin (15 mg/ml) to generate an estimate inoculum of 1x10⁸ CFU/ml. Antimicrobial assay analysis. The abundance of gene transcription was analyzed using the S. aureus Affymetrix array technology. RNA was isolated from control treated bacteria, 100 mg/kg AFN1252, treated, labeled and hybridized to the chip. The complete protocol was carried out using arrays from six biological samples.

Determination of virulence factor mRNA levels in S. aureus lab cultures and during the infection process was performed using a wide range of qRT-PCR using gene-specific primers and SFAM, a microarray platform.

Summary and Conclusions

The results of this investigation indicate that AFN-1252 modulates S. aureus virulence gene expression in vitro, suggesting that AFN-1252 could aid disease outcomes by affecting the virulence of S. aureus during the infection process.

Acknowledgments

This work was supported by National Institutes of Health Grant GM074496 (J.P.S.) and Centers for Support Grant CA121755 and the American Japanese Syringe Associated Charities.