

In Vivo Pharmacodynamic Profiling of API-1252 Against *Staphylococcus aureus* in a Murine Thigh Model

F1-0759

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ABSTRACT

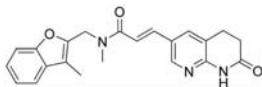
Background: API-1252 is a novel antimicrobial with a mechanism of action targeted against fatty acid biosynthesis. Pharmacodynamic (PD) studies with this novel agent have not been conducted. The objective of this study was to assess the in vivo PD profile of API-1252 after the completion of pharmacokinetic (PK), protein binding (PB), dose-ranging and dose-fractionation studies. **Methods:** MIC was determined by CLSI microdilution methods. Single dose PK was conducted using 5 oral doses ranging from 2.5 – 100 mg/kg. Protein binding studies were performed over an API-1252 concentration range of 0.1 – 2 µg/ml. The thighs of neutropenic mice were inoculated (~10⁷) with *S. aureus* (SA) ATCC 29213. API-1252 was administered orally in doses ranging from 5-100 mg/kg given 1-4 times daily. Dose fractionation studies of 10 and 20 mg/kg were conducted. PK modeling techniques were used to characterize the free API-1252 drug exposure after various single and multiple dose regimens. Efficacy was expressed as the change in log CFU in thighs after 24h of drug exposure. An E_{max} model was used to characterize the PD profile. **Results:** MIC of SA was 8 ng/ml. The mean protein binding was 98.5% with no concentration dependence. The PK profile of oral API-1252 appeared linear but non-proportional over the dose range tested. Bacterial kill of 1 - 1½ logs was observed with a total daily dose range of 20-400 mg/kg. Correlation of PD parameters was AUC₀₋₂₄/MIC (r²=0.9602), %T_{max}>MIC (r²=0.9448) and Cmax₀₋₂₄/MIC (r²=0.7917). For the AUC₀₋₂₄/MIC, the 80% maximally effective exposure (ED₈₀), ED₅₀, and ED₁₀ were 22.3, 17.0 and 9.6, respectively. **Conclusion:** These data demonstrate the in vivo potency of API-1252 against SA. AUC₀₋₂₄/MIC appears to best characterize the PD profile of this novel agent. The low efficacious AUC₀₋₂₄/MIC values support the further development of API-1252 as a novel oral and intravenous agent for staphylococcal infections.

MATERIALS and METHODS

Antimicrobial Test Agents

API-1252 was provided by Affinium Pharmaceuticals, Toronto, ON, Canada. The chemical structure of API-1252 is presented in Figure 1.

Figure 1. The chemical structure of API-1252.



Bacterial Isolate and Susceptibilities

MICs were determined in triplicate using standard CLSI methods for broth microdilution.
 MIC of API-1252 for *S. aureus* ATCC 29213 was 8 ng/ml.

Murine Thigh Model

Specific pathogen-free female ICR mice (~18-20g) were rendered neutropenic by intraperitoneal (IP) injection of 150- and 100-mg/kg doses of cyclophosphamide at -4 and -1 days in relation to bacterial inoculation (day 0).
 A bacterial suspension of ~10⁸ CFU/ml was prepared from a fresh subculture.
 Thigh infection was produced by injecting 0.1ml of the inoculum into each mouse thigh.

Protein Binding Studies

Five concentrations of API-1252 (0.1, 0.5, 0.75, 1, and 2 µg/ml) were tested in triplicate with freshly collected mouse plasma using an ultrafiltration method (Amicon Centrifree[®] Micropartition device, Millipore, Bedford, MA).
 Drug concentrations in ultrafiltrate and control samples were determined at Affinium Pharmaceuticals using a validated HPLC/MS method.

Pharmacokinetic Studies

One hour after infection, animals were administered a single oral dose of 2.5, 5, 10, 30, or 100mg/kg of API-1252.
 Blood was obtained from 6 mice at each time point: 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours following drug administration.
 Plasma was separated by centrifugation and stored at -80°C until analysis.
 Drug concentrations in plasma samples were determined at Affinium Pharmaceuticals using a validated HPLC/MS method.

Bacterial Density Studies

API-1252 exposures ranged from 5-100 mg/kg given 1-4 times daily.
 Dose fractionation studies of 10 and 20 mg/kg were given 1-4 times daily.
 Control animals received 80% PEG 400 in the same volume and schedule as API-1252.
 Untreated control mice were sacrificed at 0 and 24 hours.
 Treated mice were sacrificed at 24 hours.
 Efficacy was assessed by bacterial density (log CFU/thigh) of treated or untreated mice at 24 hours compared with the CFU of 0 hour control mice.

Data Analysis

Pharmacokinetic data derived from the single dose studies was obtained using compartmental modeling methods (WinNonlin). Parameters obtained from these studies were utilized to predict drug exposures observed during the multi-dose regimens.
 Spearman's rank correlation coefficient was used to evaluate correlation between CFU and AUC₀₋₂₄/MIC, Cmax₀₋₂₄/MIC and %T_{max}>MIC.
 Sigmond Emax model was applied to further evaluate the relationship between these variables.

RESULTS

Protein Binding Studies

The protein binding of API-1252 in murine plasma was 98.5% over a concentration range of 0.1 – 2 µg/ml.

Pharmacokinetic Studies

Pharmacokinetic parameters based on calculated free drug concentrations following single oral doses of API-1252 are reported in Table 1.
 Pharmacokinetic profile for API-1252 appeared linear but non-proportional over dose range tested (Figure 2).

Bacterial Density Studies

At initiation of treatment, animals had 10^{8.47} CFU/thigh of *S. aureus* ATCC 29213. The mean change in bacterial density among untreated control animals at 24 h was 2.32 (±0.15) log CFU.
 Bactericidal effects of 1 log and greater begin at exposures starting at 20mg/kg. Partial growth inhibition of *S. aureus* 29213 began at 5 mg/kg (Figure 3).
 For dose ranging studies, significant correlations were observed for the relationships between the change in bacterial density (log CFU change) and all the pharmacodynamic parameters; AUC₀₋₂₄/MIC, %T_{max}>MIC and Cmax₀₋₂₄/MIC, refer to Figures 4-6.
 Dose fractionation of the lower doses of 10 and 20 mg/kg demonstrated bactericidal effects with minimal differences between the different regimens, thus suggesting that AUC₀₋₂₄/MIC best characterizes the PD profile (Figure 7).
 For the AUC₀₋₂₄/MIC, the 80% maximally effective exposure (ED₈₀), ED₅₀, and ED₁₀ were 22.3, 17.0 and 9.6, respectively.

Table 1. Pharmacokinetic parameters based on calculated free drug concentrations following single oral doses of API-1252 in infected neutropenic mice

API-1252 (mg/kg)	AUC ₀₋₂₄ (ng·h/ml)	Cmax (ng/ml)	Tmax (hr)	T 1/2 (hr)
2.5	51.4	5.02	0.72	7.30
5	113	12.32	0.48	6.30
10	141	12.32	0.81	7.97
30	160	13.23	1.70	7.60
100	285	22.14	2.70	8.13

Figure 2. Pharmacokinetic profile of free API-1252 following single oral dose in immunocompromised *S. aureus* ATCC 29213 infected mice.

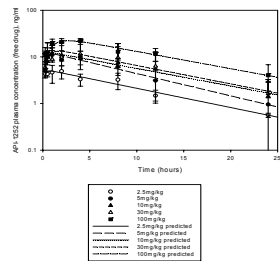


Figure 3. Dose response relationship for API-1252 at dose exposure range of 5-400mg/kg.

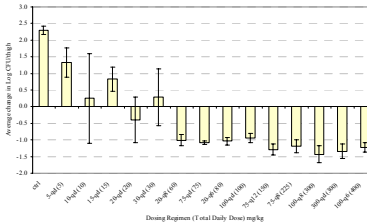


Figure 4. Relationship between AUC₀₋₂₄/MIC and change in bacterial density at 24 hours following treatment with API-1252.

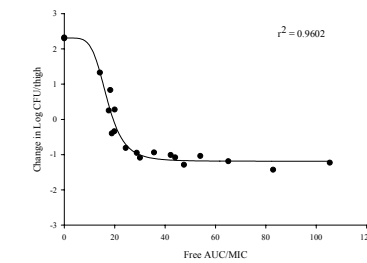


Figure 5. Relationship between %T_{max}>MIC and change in bacterial density at 24 hours following treatment with API-1252.

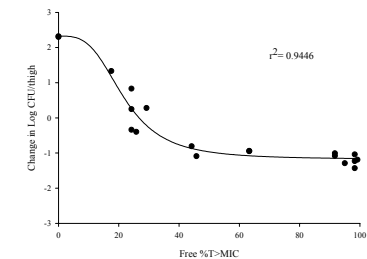


Figure 6. Relationship between Cmax₀₋₂₄/MIC and change in bacterial density at 24 hours following treatment with API-1252.

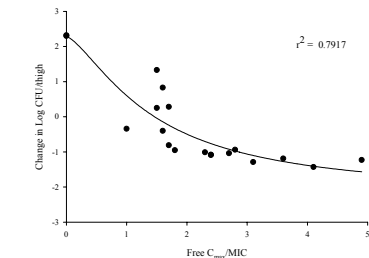
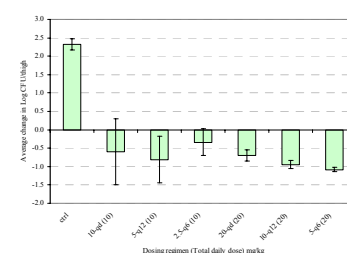


Figure 7. Dose fractionation studies of API-1252 10 and 20 mg/kg.



CONCLUSIONS

- The pharmacokinetic profile of orally administered API-1252 in the mouse appears to be linear but non-proportional over the dose range tested.
- The current in vivo data display the potency of API-1252 against *S. aureus* 29213 when studied over a wide range of both single and dose-fractionated doses.
- Pharmacodynamic data derived from this study suggest that the AUC₀₋₂₄/MIC ratio is the pharmacodynamic index that best correlates with the antibacterial activity of API-1252.
- The low efficacious AUC₀₋₂₄/MIC values support the further development of API-1252 as a novel oral and intravenous agent for staphylococcal infections.