Identification of the In Vivo Pharmacokinetics and Pharmacodynamic Drivers of Iclaprim

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Abstract

The neutropenic, murine thigh infection model was used to define the PK/PD index linked to efficacy of iclaprim against *S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 10813. The 24h AUC/MIC index was most closely linked to efficacy for *S. aureus* ($R^2=0.65$), while both the 24h AUC/MIC and the %T>MIC were both strongly associated with effect ($R^2=0.86$ for both parameters) for *S. pneumoniae*.

Keywords: iclaprim, trimethoprim, pharmacodynamics, thigh infection model

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Preclinical PK/PD studies using murine infection models have become standard in the development of antimicrobials to aid in selection of dosing regimens for clinical trials (1-3). These studies have been challenging for the dihydrofolate reductase inhibitors (DHFRi) as high levels of thymidine in rodents antagonized drug activity (4). As such, use of these models to define the magnitude of the linked PK/PD index would greatly overestimate the drug exposure needed for efficacy in humans, which have thymidine concentrations 100-fold lower than rodents (4). Certain bacteria, including S. aureus, have the capability of uptake of exogenous thymidine and its conversion into thymidylate by thymidine kinase, which interferes with the antimicrobial activity of the diaminopyrimidine drug TMP in vitro (5). However, it should still be possible to use these infection models to define the optimal PK/PD index. Iclaprim is a novel, broad-spectrum, DHFRi (6). The present studies were designed to determine the PK/PD driver for iclaprim. Studies were also performed to investigate the impact of mouse and human serum on in vitro activity of iclaprim.

We utilized the neutropenic, murine thigh infection model (CD-1 mice) for both pharmacokinetic and pharmacodynamics studies (2). A strain of S. aureus (ATCC 29213) and S. pneumoniae (ATCC 10813) were used. In vitro susceptibility testing was performed twice in duplicate using CLSI methodology; modal MIC values are reported. In broth, the MIC values were 0.12 and 0.06 mg/L, for S. aureus and S. pneumoniae, respectively. The MIC values for iclaprim were significantly higher in serum than in broth for both S. aureus and S. pneumoniae; MIC values were also markedly higher in mouse serum than in human serum. In 95% mouse serum, the MIC values were >80 and 80 mg/L, for S. aureus and S. pneumoniae, respectively. The MIC values of iclaprim was 10 times higher in 95% human serum than observed in broth (0.12 versus 1.25 mg/L for S. aureus and 0.06 versus 0.62 mg/L for S. pneumoniae), consistent...
with the reported 90% protein binding of iclaprim in human serum. As anticipated from the increased circulating thymidine concentrations in rodents, MICs in mouse serum were more than a 100-times higher than the MICs in broth.

The organism burden in the thighs of mice at the start of pharmacokinetic and pharmacodynamics studies was $10^{6.4-7.8}$ CFU/thigh. Single-dose plasma pharmacokinetics were performed following subcutaneous doses of iclaprim ranging from 20 to 320 mg/kg. Groups of six mice were sampled seven times at 0.25 to 120 minute intervals over six hours. Iclaprim concentrations were determined using a bioassay using *Bacillus subtilus* ATCC 6142 as the assay organism. The lower limit of detection for this assay was 0.5 ug/ml and the coefficient of variation was less than 10%. Protein binding was determined by ultrafiltration. The beta-elimination half-life, determined by linear least-squares regression (2, 7), was 9.85 minutes as was similar over the dose range. Protein binding of iclaprim was 89% over the concentration range.

Efficacy studies utilized five total doses of iclaprim ranging from 80 to 1280 mg/kg/8hours and 40 to 2560 mg/kg/8hours total dose over the treatment period for *S. pneumoniae* ATCC 10813 and *S. aureus* ATCC 29213, respectively. These dose-ranging doses were chosen based on other iclaprim animal infection models and to establish which dose works best in this model (unpublished data). These dose levels were fractionated using four dosing intervals (every 1, 2, 4, or 8 hours). We utilized an 8 hour treatment duration, as preliminary studies using a 24 hour treatment duration demonstrated limited treatment efficacy due to inhibition of drug (presumably due to elevated thymidine concentrations in mice). The dose response relationships for iclaprim against *S. pneumoniae* ATCC 10813 and *S. aureus* ATCC 29213 at various dosing frequencies are shown in Figure 1A and 1B, respectively. Each of the
dosing regimens was also expressed as either AUC/MIC, Cmax/MIC, or %T>MIC using both free and total iclaprim concentrations. The sigmoid Emax model was used for assessment of data fit with each of these PK/PD indexes. Both visual examination and the coefficient of determination were used to determine the linked PK/PD index. The 8-hour AUC/MIC demonstrated the best fit and $R^2$ for the studies with *S. aureus* ($R^2$ AUC/MIC 0.65, %T>MIC 0.53, Peak/MIC 0.40) (Figure 2B). The treatment data with *S. pneumoniae* also demonstrated a strong relationship for AUC/MIC, but %T>MIC was similarly predictive ($R^2$ AUC/MIC 0.86, T>MIC 0.86, Peak/MIC 0.40) (Figure 2A). There was no order by dosing interval that suggested AUC/MIC was better. These data are consistent with historical PK/PD data of AUC/MIC and %T/MIC correlating best with trimethoprim efficacy against *S. pneumoniae* tested in in normal mice (unpublished data).

The important task of identifying the PK/PD magnitude is not possible in this model with clinical isolates. It may be possible to define the PK/PD target using strains that have been genetically modified to thymidine-kinase deficient isolates (8). In the absence of these more laborious studies it seems that in vitro PK/PD target analysis will be most fruitful. Specifically, conducting experiment using in vitro infection models to determine PK/PD targets for efficacy, and then using these targets together with a population PK model, and Monte Carlo Simulation to conduct PK/PD target attainment analyses to support dose selection.

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**Figure Legends**

**Figure 1.** The dose response relationships for iclaprim against *S. pneumoniae* ATCC 10813 (Figure 1A top panel) and *S. aureus* ATCC 29213 (Figure 1B bottom panel) at various dosing frequencies in the neutropenic mice model.

**Figure 2.** Relationships between PK/PD parameters and efficacy of iclaprim against *S. pneumoniae* ATCC 10813 (Figure 2A top panel) and *S. aureus* ATCC 29213 (Figure 2B bottom panel) in thighs of neutropenic mice. Each data point represents data from one mouse (mean of two thighs).

**References**


