Identification of the *In Vivo* Pharmacokinetics and Pharmacodynamic Driver of Iclaprim

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ABSTRACT The neutropenic murine thigh infection model was used to define the pharmacokinetic/pharmacodynamic index linked to efficacy of iclaprim against *Staphylococcus aureus* ATCC 29213 and *Staphylococcus pneumoniae* ATCC 10813. The 24-h area under the curve (AUC)/MIC index was most closely linked to efficacy for *S. aureus* (R^2 , 0.65), while both the 24-h AUC/MIC and the percentage of time that drug concentrations remain above the MIC (%T>MIC) were strongly associated with effect (R^2 , 0.86 for both parameters) for *S. pneumoniae*.

KEYWORDS iclaprim, trimethoprim, pharmacodynamics, thigh infection model

Preclinical pharmacokinetic/pharmacodynamic (PK/PD) studies using murine infection models have become standard in the development of antimicrobials to aid in selection of dosage regimens for clinical trials (1–3). These studies have been challenging for dihydrofolate reductase inhibitors (DHFRi) because high levels of thymidine in rodents antagonize 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, who have thymidine concentrations that are 100-fold lower than those in rodents (4). Certain bacteria, including *Staphylococcus 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 PK/PD studies (2). Strains of *S. aureus* (ATCC 29213) and *Staphylococcus 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/liter for *S. aureus* and *S. pneumoniae* isolates, respectively. The MIC values for iclaprim were significantly higher in serum than in broth for both *S. aureus* and *S. pneumoniae* isolates; 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/liter for *S. aureus* and *S. pneumoniae*, respectively. The MIC values of iclaprim were 10 times higher in 95% human serum than in broth (0.12 versus 1.25 mg/liter for *S. aureus* and 0.06 versus 0.62 mg/liter for *S. pneumoniae*), consistent with the reported 90% protein binding of iclaprim in human serum. As anticipated

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FIG 1 The dose-response relationships for iclaprim against *S. pneumoniae* ATCC 10813 (top) and *S. aureus* ATCC 29213 (bottom) at various dosing frequencies in the neutropenic mouse model.

from the increase in circulating thymidine concentrations in rodents, MICs in mouse serum were >100 times higher than the MICs in broth.

The organism burden in the thighs of mice at the start of PK/PD studies was $10^{6.4-7.8}$ CFU/thigh. Single-dose plasma pharmacokinetics was performed following administration of subcutaneous doses of iclaprim ranging from 20 to 320 mg/kg. Groups of six mice were sampled seven times at 0.25- to 120-min intervals over 6 h. Iclaprim concentrations were determined by using a bioassay with *Bacillus subtilis* ATCC 6142 as the assay organism. The lower limit of detection for this assay was 0.5 $\mu\text{g/ml}$, and the coefficient of variation was $<10\%$. Protein binding was determined by ultrafiltration. The β -elimination half-life, determined by linear least-squares regression (2, 7), was 9.85 min and 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 1,280 mg/kg/8 h and 40 to 2,560 mg/kg/8 h total dose over the treatment period for *S. pneumoniae* ATCC 10813 and *S. aureus* ATCC 29213, respectively. The dose ranges 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 into four dosing intervals (every 1, 2, 4, and 8 h). We utilized an 8-h treatment duration, as preliminary studies using a 24-h treatment duration demonstrated limited treatment efficacy due to drug inhibition (presumably resulting from 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 Fig. 1A and B, respectively. Each of the dosage regimens was also expressed as AUC/MIC, the maximum concentration of drug in serum (C_{max})/MIC, or the percentage of time that drug concentrations remain above the MIC (%T>MIC) using both free and total iclaprim concentrations. The sigmoid

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

maximum effect ($E_{\max}$) model was used to assess the data fit with each of these PK/PD indexes. Visual examination and the coefficient of determination were used to determine the linked PK/PD index. The 8-h 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) (Fig. 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) (Fig. 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 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 the most fruitful, specifically, conducting experiments with *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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