

# Pharmacokinetic and Pharmacodynamic Evaluation of P-873 versus *Klebsiella pneumoniae* in a Neutropenic Murine Thigh Infection Model

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**P-873 is a novel compound in the RX-04 pyrrolocytosine series of protein synthesis inhibitors currently under development by Rib-X Pharmaceuticals. We evaluated the pharmacodynamic and pharmacokinetic properties of this compound against *Klebsiella pneumoniae* using a murine neutropenic thigh infection model. P-873 demonstrated potent and rapid *in vivo* activity against this organism with enhanced penetration and duration of exposure in thigh tissue.**

The increase in resistance to currently available antibiotics underscores the need to develop new therapies, particularly against Gram-negative organisms, including *Klebsiella pneumoniae* (1, 2). To address this unmet medical need, Rib-X Pharmaceuticals has designed and developed novel antibacterial scaffolds that inhibit protein synthesis by binding to the 50S ribosomal subunit (RX-04 program). The pyrrolocytosine (P) series has been shown to have broad-spectrum activity against clinically relevant organisms, including *K. pneumoniae* (3, 4, 5, 6). The pharmacodynamic and pharmacokinetic properties of P-873 were evaluated against *K. pneumoniae* using a murine neutropenic thigh infection model.

P-873 (Rib-X Pharmaceuticals, New Haven, CT) was used throughout these studies. Prior to each experiment, P-873 was reconstituted in 10% Captisol-50 mM sodium phosphate buffer, pH 7.2 (vehicle), and further diluted to achieve the desired concentrations. Dosing solutions were held on ice throughout the dosing period. Specific-pathogen-free female ICR (CD-1) mice (25 to 30 g) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). All mice were provided food and water *ad libitum*. The study was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee.

A previously described murine neutropenic thigh infection model was used for both bacterial density and pharmacokinetic experiments (7). Briefly, mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide (Baxter, Deerfield, IL) at 150 mg/kg and 100 mg/kg of body weight given 4 days and 1 day, respectively, prior to inoculation. Two hours prior to the initiation of antimicrobial therapy, each thigh was inoculated intramuscularly with 0.1 ml of *Klebsiella pneumoniae* 457 (P-873 MIC, 0.06 µg/ml by broth microdilution [8]) containing approximately 10<sup>7</sup> CFU/ml in normal saline. Groups of 3 animals were administered total daily doses of P-873 ranging from 0.625 to 15 mg/kg subcutaneously as 0.2-ml injections. P-873 dose fractionation studies were conducted using total daily doses of 0.625, 1.25, 2.5, 5, and 10 mg/kg. Doses were administered 1, 2 (every 12 h [q12h]), or 4 (q6h) times over a 24-h period. Control mice received vehicle as sham treatment. A group of untreated control mice ( $n = 3$ ) was euthanized just prior to antibiotic treatment initiation to establish the initial bacterial burden (0 h). At 24 h postinfection, sham-treated control mice and all treated mice were euthanized. Both thighs were harvested aseptically and indi-

vidually homogenized in 5 ml sterile normal saline. Serial dilutions of thigh homogenate were subcultured onto Trypticase soy agar with 5% sheep blood for bacterial density determinations. Antibacterial efficacy was calculated as the change in average bacterial density in treated or control animals after 24 h compared to the average in the control animals at the initiation of dosing (0 h).

For pharmacokinetic studies, single doses of P-873 at 5, 1.25, or 0.3125 mg/kg were administered 2 h after bacterial inoculation. Terminal blood samples were collected via cardiac puncture into lithium heparin tubes (Terumo, Elkton, MD) at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h postdose. Plasma was separated by centrifugation. In conjunction with the plasma pharmacokinetic studies, time-kill studies were conducted in thigh tissue at 1, 2, 4, and 8 h postdose. Thigh tissue was processed as described above for bacterial density determination, and an aliquot of the thigh homogenate was reserved to evaluate P-873 pharmacokinetic distribution into the site of infection. While tissue homogenates can provide an early look into drug concentrations at the site of infection, it should be noted that their use does not allow differentiation between interstitial, intracellular, and vascular compartments. Plasma and thigh homogenate samples were stored at  $-80^{\circ}\text{C}$  until analyzed. Drug concentrations in plasma and thigh homogenate samples were analyzed by a validated liquid chromatography-tandem mass spectrophotometry assay.

The average ( $\pm$ standard deviation [SD]) bacterial density at the onset of therapy was  $6.00 \pm 0.18 \log_{10}$  CFU and increased to  $8.83 \pm 0.27 \log_{10}$  CFU at 24 h in untreated mice. Single daily doses of P-873 of  $\geq 5$  mg/kg resulted in reductions of thigh bacterial burden of  $\geq 0.5 \log_{10}$  CFU.

During dose fractionation studies, a general trend toward greater activity was noted with increases in dose fractionation, suggesting the importance of duration of exposure for P-873 activity.

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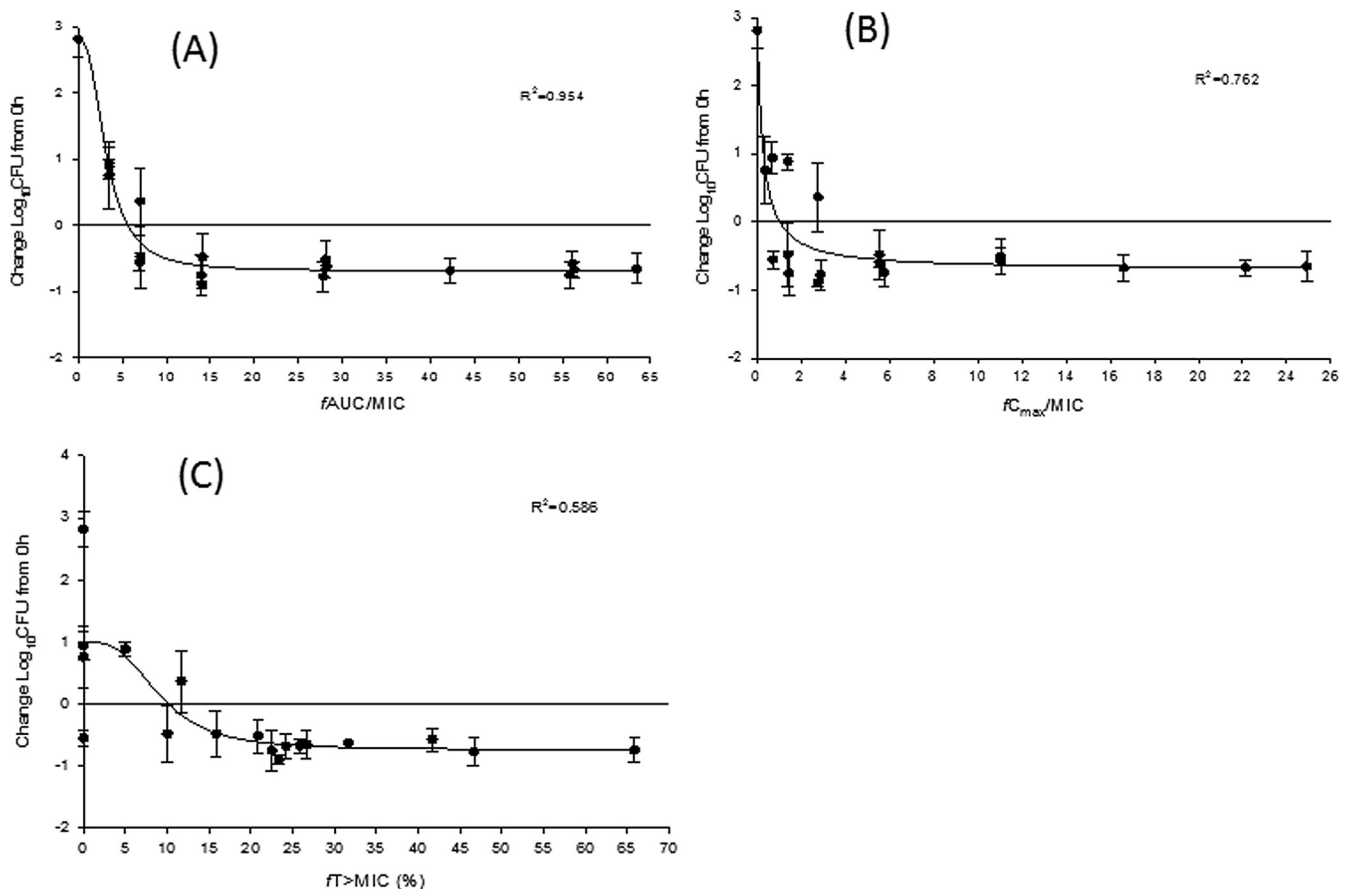


FIG 1 Free drug pharmacodynamic profiles of P-873 versus *Klebsiella pneumoniae* 457 (MIC, 0.06  $\mu\text{g}/\text{ml}$ ) in the neutropenic thigh infection model. (A)  $fAUC/MIC$ ; (B)  $fC_{max}/MIC$ ; (C)  $fT > MIC$ . Symbols represent means  $\pm$  standard deviations.

For pharmacodynamic analyses, plots of the change in log<sub>10</sub> CFU versus the free drug (P-873, 95% protein bound) pharmacodynamic parameters ( $fAUC/MIC$  [ratio of the free drug area under the concentration-time profile to MIC],  $fC_{max}/MIC$  [ratio of the free peak drug concentration to the MIC], and  $fT > MIC$  [percentage of the dosing interval in which free drug concentrations remained above the MIC]) were constructed and analyzed using the sigmoidal  $E_{max}$  model:  $E = E_0 - (E_0 - E_{max}) \times [I^\gamma / (I^\gamma + EI_{50}^\gamma)]$ , where  $E_{max}$  is maximum efficacy,  $I$  is pharmacodynamic index of interest,  $\gamma$  is the slope of the inhibitory-effect curve, and  $EI_{50}$  is the value of the pharmacodynamic index associated with 50% of maximal efficacy (WinNonlin version 5.0.1; Pharsight, Mountain View, CA). As noted in Fig. 1A to C, the respective  $R^2$  values for  $fAUC/MIC$ ,  $fC_{max}/MIC$ , and  $fT > MIC$  were 0.954, 0.762, and 0.586, suggesting that  $fAUC/MIC$  was the predominant driver of *in vivo* efficacy. The calculated exposure index values required for stasis, 50% of maximal efficacy ( $EI_{50}$ ), and 80% of maximal efficacy ( $EI_{80}$ ) for  $fAUC/MIC$  were determined to be 5.6, 3.2, and 5.5, respectively.

Over the time course tested, thigh tissue concentrations ( $\mu\text{g}/\text{g}$  tissue) of P-873 for all doses exceeded the MIC (0.06  $\mu\text{g}/\text{ml}$ ) of the infecting *K. pneumoniae* isolate for the 8-h sampling period. When comparing relative 8-hour AUCs, the penetration of P-873 was  $\sim 4$  to 6 times higher in thigh tissue than in free plasma. The efficacy of a single 5-mg/kg dose of P-873 as determined during

time-kill experiments, compared with the free plasma and thigh tissue concentrations over the same time period, is shown in Fig. 2. Of note, reductions in thigh bacterial burden were observed at 1 h postdose and reached maximal activity at 4 h postdose. Further, while free plasma concentrations of P-873 exceeded the MIC for 5 h postdose, thigh tissue concentrations not only exceeded those

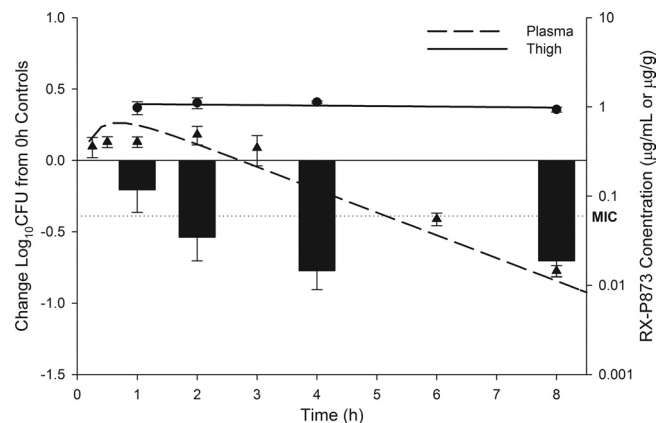


FIG 2 Efficacy of a single 5-mg/kg dose of P-873 (bars) compared with tissue homogenate (circles, solid line) and free drug plasma concentrations (triangles, dashed line). *Klebsiella pneumoniae* 457 MIC, 0.06  $\mu\text{g}/\text{ml}$  (dotted line).

found in plasma but remained above the MIC for the duration of the experiment.

P-873 was shown to exhibit potent and rapid *in vivo* activity against *K. pneumoniae* 457 in a murine neutropenic thigh infection model. While dose fractionation studies showed a trend toward enhanced activity with increasing duration of exposure, plasma  $E_{\max}$  profiles found  $fAUC/MIC$  to be the parameter most predictive of *in vivo* activity. This discordance in the plasma-defined pharmacodynamic profile appears to be explained by the enhanced penetration and duration of exposure in tissue.

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