# Pharmacokinetics of PTK 0796 (BAY 73-6944) in Mouse, Rat, and Cynomolgus Monkey

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## ABSTRACT

**Background** PTK 0796 (7-dimethylamino, 9-(2,2-dimethyl-propyl)-aminomethylcycline) is a novel antibacterial agent of the tetracycline family that exhibits potent and enhanced activity *in vitro* against resistant and susceptible gram-positive and gram-negative pathogens and efficacy in animal models of infection. The pharmacokinetics (PK) of intravenously (IV) administered PTK 0796 was determined in the mouse, rat, and monkey.

**Methods** PTK 0796 was administered as a single IV dose escalating from 5 mg/kg to 40 mg/kg to rats, as a single IV 10 mg/kg dose to mice, and a 25 mg/kg dose to monkeys. Analyses of plasma (all), kidneys (rats), and lungs (rats) were accomplished using LC-MS/MS.

**Results** In all species, PTK 0796 exhibited a bi-exponential decline with a rapid distribution phase and an extended elimination phase. In mouse and rat, the plasma T½ (elim) were 5.6 hr and 3.8 hr, respectively. $AUC_{0\text{-}24}$ in rat and mouse were similar at similar doses, and increased linearly with increasing dose (rat). Time of plasma concentrations above 1 µg/ml ranged from 15 min in mice and 40 min in rats (10 mg/kg) to more than nine hrs in rats (40 mg/kg). In monkeys, plasma T½ (elim) was greater than in rodents (10-11 hrs) and plasma concentrations remained above 1 µg/ml for at least 24 hrs at 25 mg/kg. In rats, the kidney concentrations were more than six-fold greater than in plasma and were linearly related to dose. In the lung, concentrations were found to be 10 to 20-fold that of the plasma and similarly dose related. Time above 1 µg/gram in lung increased with increasing dose (6 hrs to 22 hrs).

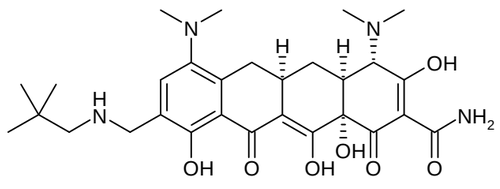
**Conclusions** PTK 0796 administered IV to mice, rats, and monkeys exhibited similar PK. PTK 0796 plasma PK in all species indicated a rapid distribution and prolonged elimination. Dose escalation studies in rats indicated linear PK in both plasma and tissue, with sustained tissue levels in both kidneys and lungs.

## INTRODUCTION

We have studied the single dose IV pharmacokinetics of PTK 0796 (BAY 73-6944) (7-dimethylamino, 9-(2,2-dimethyl-propyl)-aminomethylcycline, structure shown below) in mice, rats and Cynomolgus monkeys.

Noncompartmental analysis of tissue and plasma concentrations of PTK 0796 (BAY 73-6944) suggested a potential for PTK 0796 (BAY 73-6944) as a once-daily IV therapy against either susceptible or multiply antibiotic-resistant organisms.

### Structure of PTK 0796 (BAY 73-6944)




## METHODS

The phamacokinetic behavior of PTK 0796 was evaluated in CD-1 mice, Sprague Dawley rats, and Cynomolgus monkeys. PTK 0796 was prepared as a solution in sterile water or in PlasmaLyte (USP Multiple Electrolytes Injection Type 1). Rat ascending dose pharmacokinetic studies were conducted using a single dose of four different concentrations administered as bolus injections. Blood samples were obtained at predetermined time points in EDTA-containing tubes. The samples were centrifuged, and the plasma was withdrawn and stored at 4° C for next-day analysis, or at -20° C if analysis was delayed.

The concentration of PTK 0796 in each sample was determined by LC-MS/MS, and the data were analyzed using a noncompartmental model in WinNonlin v. 4.0.1.

Kidney and lung tissue was weighed and homogenized in three volumes of ice-cold extraction buffer (90:10 Water:Acetonitrile with 0.1 % TFA). The homogenate was centrifuged to remove solids and supernatant fluid was stored with the plasma.

Protein was precipitated from the plasma and tissue samples by addition of Acetonitrile containing 0.1% Formic Acid. The samples were centrifuged and an aliquot of the supernatant fluid was evaporated to dryness. The samples were reconstituted in Methanol: Water 1:1, and were analyzed on a Sciex 3000 LC/MS-MS. Concentration data were analyzed using a noncompartmental model in WinNonlin v.4.01.

## RESULTS

### Single IV Bolus Dose Pharmacokinetics

**Mouse.** Male CD-1 mice were injected IV with PTK 0796 via a lateral tail vein with a single dose of 10 mg/kg, and blood samples were obtained at 5, 30, 60, 120, 240, 480, 960, and 1440 min post-dosing. The samples were pooled at each timepoint for measurement of drug concentration, and plasma pharmacokinetic values were calculated.

**Table 1. Pharmacokinetic Parameters in Mice Following Single IV Bolus Dose of PTK 0796**

| Dose (mg/kg) | $\lambda_z$ Half (Hr) | AUC (Hr·mg/ml) | Cmax (mg/ml) | $V_D$ (ml/kg) | Cl (ml/Hr/kg) |
|---|---|---|---|---|---|
| 10 | 5.56 | 6.23 | 2.43 | 12097 | 1506 |

**Rat.** The pharmacokinetics of PTK 0796 in Sprague Dawley Rats following an IV injection of PTK0 0796 at 5, 10, 20, or 40 mg/kg were characterized using plasma or tissue concentration vs. time data from two animals per dose per time point. Blood and tissue samples were obtained at 5 and 30 min and at 1, 2, 4, 8, 16 and 24 hr post dosing. The pharmacokinetic parameters Cmax, AUC, Elimination half-life ($\lambda_z$ Half). Volume of distribution ($V_D$) and Clearance (Cl) were estimated.

The data were analyzed using WinNonlin 4.0.1, using a noncompartmental model. An IV dose model was used for the plasma data, and an extravascular input model was used for the tissues.

**Table 2. Pharmacokinetic Parameters in Rats Following Single IV Bolus Dose of PTK 0796**

| Dose (mg/kg) | $\lambda_z$ Half (Hr) Mean, Range | AUC (Hr·mg/ml) Mean, Range | Cmax (mg/ml) Mean, Range | $V_D$ (ml/kg) Mean, Range | Cl (ml/Hr/kg) Mean, Range |
|---|---|---|---|---|---|
| 5 | 4.65<br>4.56 – 4.74 | 2.97<br>2.94 – 3.1 | 0.83<br>0.78 – 0.88 | 8328<br>8130 – 8526 | 1241<br>1188 – 1293 |
| 10 | 3.83<br>3.80 - 3.86 | 5.62<br>5.62 – 5.62 | 1.71<br>1.42 – 1.99 | 7426<br>7373 - 7479 | 1343<br>1342 – 1344 |
| 20 | 5.60<br>4.66 – 6.54 | 12.53<br>11.89 – 13.06 | 3.11<br>2.77 – 3.44 | 9539<br>8404 – 10674 | 1189<br>1130 – 1249 |
| 40 | 4.42<br>3.57 – 5.26 | 16.07<br>12.49 – 19.64 | 3.39<br>3.16 – 3.62 | 12839<br>7922 - 12839 | 1939<br>1540 - 2338 |

**The plasma $\lambda_z$ half-life was 5.6 hr in mice and ranged from 3.6 to 6.5 hr and was not dose-dependent in rats. The $AUC_{0\text{-}t}$ was almost linearly related to dose (from 5 to 20 mg/kg). The theoretical $C_o$ appeared to plateau at the 20 mg/kg dose, being very similar at 40 mg/kg.**

**Table 3. Pharmacokinetic Parameters of PTK 0796 in Kidneys Following IV Bolus Administration**

| Dose (mg/kg) | $\lambda_z$ Half (Hr) | AUC (Hr·mg/ml) | Cmax (mg/g) | Time above 1mg/g (Hr) |
|---|---|---|---|---|
| 5 | 3.24 | 31.93 | 18.04 | 7.66 |
| 10 | 3.61 | 59.02 | 26.93 | 12.75 |
| 20 | 4.25 | 69.97 | 19.52 | 17.21 |
| 40* | NC* | NC* | NC* | NC* |

*NC - Not Calculated.

**Drug concentrations in the kidney were found to be from six to twenty-fold higher than those in the plasma. $AUC_{(0\text{-}24)}$ appeared to increase slightly with increasing dose, while the half life and tissue concentrations appear similar at all doses. Dose proportionality in kidney tissue was not observed. High concentrations of PTK 0796, in the range that might be of microbiologic significance were observed for 7.7 to at least 17 hr.**

**Table 4. Pharmacokinetic Parameters of PTK 0796 in Lung Following IV Bolus Administration**

| Dose (mg/kg) | $\lambda_z$ Half (Hr) | AUC (Hr·mg/ml) | Cmax (mg/g) | Time above 1mg/g (Hr) |
|---|---|---|---|---|
| 5 | 4.07 | 18.87 | 7.38 | 6.24 |
| 10 | 3.85 | 33.74 | 20.74 | 8.54 |
| 20 | 2.56 | 63.66 | 30.42 | 11.08 |
| 40 | 4.36 | 188.01 | 72.11 | 21.78 |

**Initial tissue concentrations were found to be about ten-fold that of the plasma, and elimination half-lives ranged from 2.6 to 4.4 hr. Concentrations of 1 mg/g were maintained for 6.2 to 21.8 hr, and increased with increasing dose. These concentrations are thought to be of microbiologic significance since most susceptible bacteria are inhibited by PTK 0796 at less than 1 mg/ml. Furthermore, while presently undefined for PTK 0796, the critical pharmacodynamic parameter of the tetracycline class is related to the duration of time that concentrations exceed inhibitory concentrations. The dose proportionality of lung tissue is more apparent at all doses than is observed in kidneys and plasma.**

**Monkey.** One male and one female non-naïve Cynomolgus monkey (*Macaca fascilularis)* were provided by Charles River Laboratories Discovery and Development Services, Worcester, MA, and the in-life portion of the study was performed at that facility. The animals were fasted over night before dosing, and were restrained in chairs to which they had been previously acclimated for the duration of the experiment. Test article was made up in PlasmaLyte on the morning of the study at a concentration of 12.5 mg PTK 0796/ml. IV dosing was performed via a temporary percutaneous catheter in the saphenous vein. Dose volume was 2 ml/kg. Immediately following the IV dose, the catheters were flushed with 3 ml of saline prior to removal. The duration of dose administration was 2 min. Blood samples (1.3 ml anticoagulated with EDTA) were drawn before administration of the test article, and at 5, 15, 30 min and 1, 2, 4, 6, 8, 10, and 24 hr post dosing.

**Table 5. Pharmacokinetic Parameters of PTK 0796 in Monkeys Following a Single IV Bolus Dose**

| Dose (mg/kg) | $\lambda_z$ Half (Hr) | AUC (Hr*mg/ml) | Cmax (mg/ml) | $V_D$ (ml/kg) | Cl (ml/Hr/kg) |
|---|---|---|---|---|---|
| 25 (Male) | 11.27 | 72.83 | 79.73 | 4363 | 268.4 |
| 25 (Female) | 10.10 | 80.65 | 68.03 | 3751 | 257.4 |

**In this study, PTK 0796 was shown to have an elimination phase half-life after IV dosing of more than 10 hr. Plasma concentrations were greater than 1.0 mg/ml for 24 hr.**

## CONCLUSIONS

- In all species, after IV administration, plasma concentrations of PTK 0796 (BAY 73-6944) exhibited a broadly exponential decline with a rapid distribution phase followed by a slower elimination phase.
- The elimination half life appeared to increase in higher species.
- When administered as an IV bolus to rats, PTK 0796 (BAY 73-6944) distributed rapidly into tissues; kidneys and lung were found to have concentrations 6 to more than 20 fold that of plasma. Elimination half lives of 4 - 5 hr were observed.
- In rats, concentrations of PTK 0796 (BAY 73-6944) in tissues appeared to be of microbiologic significance for between 6 and 20 hr, depending on dose.
- The elimination half life achieved values of 10 hr in the plasma of Cynomolgus monkeys.