

POSTER F1-846

ABSTRACT

Objectives: To assess the pharmacokinetic (PK) profile of ACHN-490 in nonclinical species and use of these data to predict pharmacokinetics in humans.

Methods: Male CD-1 mice, Sprague Dawley rats, and beagle dogs were administered ACHN-490 intravenously. Blood, urine, and kidney samples were taken over 24 h and analyzed for ACHN-490 levels using LC-MS/MS. PK parameters were determined by noncompartmental analysis using WinNonLin. Plasma protein binding was determined using equilibrium dialysis. *In vitro* stability was determined using commercially available plasma, microsomes, and hepatocytes.

Results: The PK profile of ACHN-490 after intravenous dosing was similar in mice, rats, and dogs. In rats, C_{max} and exposure (area under the concentration-time curve [AUC]) were dose-linear to 75 mg/kg. ACHN-490 was renally cleared rapidly in both rats and dogs ($t_{1/2}$ ~1 h). ACHN-490 distributed to rat and dog kidneys. The volume of distribution closely matched extracellular fluid volumes.^{1,2} ACHN-490 had low plasma protein binding (<20%). ACHN-490 was stable when exposed to plasma, liver microsomes, and hepatocytes and did not inhibit the 5 major human CYPs *in vitro*.

Conclusions: ACHN-490 is metabolically stable and unlikely to show drug-drug interactions. The relatively rapid initial half-life of ACHN-490 observed in preclinical species predicts a relatively rapid initial half-life in humans (~1 h). The PK profile of ACHN-490 supports the use of high doses with short infusion times, administered once-daily to achieve high C_{max} and AUC, which could improve efficacy and safety.

INTRODUCTION

ACHN-490 is the first neoglycoside, or next-generation aminoglycoside (AG),² in clinical development. ACHN-490 contains structural modifications that confer bactericidal activity in the presence of nearly all AG-modifying enzymes (AMEs) that cause AG resistance. ACHN-490 is active against most resistant Enterobacteriaceae and many resistant strains of *Staphylococcus aureus*.

ABSORPTION

In vitro and *in vivo* models have demonstrated that AG bactericidal activity is concentration-dependent; higher concentrations of free drug cause faster killing of exposed bacteria. In the present study, we examine the pharmacokinetic (PK) profile of ACHN-490 in animals (mice, rats, and dogs) as a rationale for testing ACHN-490 in human subjects.

METHODS

- In vivo* studies were performed in CD-1 mice, Sprague Dawley rats, and non-naïve beagle dogs.
- The PK profile was assessed as a single dose intravenous (IV) bolus study in mice (10 mg/mL), rats (1, 10, or 75 mg/mL), and dogs (10 mg/mL). ACHN-490 plasma concentrations were analyzed by LC-MS/MS.
- The *in vitro* binding of ACHN-490 to mouse, rat, rabbit, dog, cynomolgus monkey, and human plasma proteins was evaluated using an equilibrium dialysis method.

PHARMACOKINETICS OF THE NOVEL NEOGLYCOSIDE ACHN-490 IN MOUSE, RAT, AND DOG

Robert T. Cass, Jenny V. McKinnell, Bo Xie, Dane E. Karr, Donald E. Schmidt Jr.
Achaogen Inc., 7000 Shoreline Court, Suite 371, South San Francisco, CA 94080

- Kidney distribution: rats (10, 30, and 100 mg/kg subcutaneously [SC] once daily) and dogs (3, 10, and 30 mg/kg IV infusion once daily) were treated with ACHN-490 for 14 days and kidney samples were obtained at day 15. Gentamicin was used as a comparator.³
- Metabolism: liver microsomes (0.2 or 1 ug/mL) or hepatocytes (25 ug/mL) were added to ACHN-490. The amount of ACHN-490 was quantitated by LC-MS/MS and compared to $t = 0$ at 10, 15, 30, and 60 minutes for the microsomal mixtures, and 30, 60, and 120 minutes for the hepatocyte mixtures.
- In vitro* cytochrome P450 (CYP) inhibition: ACHN-490 at 25 μ M was incubated in the presence of recombinant human CYP isoforms 1A2, 2C9, 2C19, 2D6, and 3A4, and probe substrates. Fluorescence was measured in the presence and absence of ACHN-490 and the percent inhibition of fluorescence in the presence of ACHN-490 was calculated.
- In vitro* plasma stability: ACHN-490 (50 μ g/mL) was incubated for 2 h at 37°C in animal or pooled human plasma. The concentration of ACHN-490 remaining in each sample was measured by LC-MS/MS and compared to $t = 0$.
- Urine excretion: urine was collected for 24 h as part of 2 single-dose PK studies after an IV bolus administration in male rats and dogs (3 animals per study). ACHN-490 was analyzed by LC-MS/MS.
- PK parameters of ACHN-490 in human subjects (70 kg) were predicted by allometric scaling studies from animal data. Gentamicin was used as a comparator.

RESULTS

PHARMACOKINETICS

- The PK profile of a single dose of ACHN-490 administered as an IV injection was examined in mice, rats, and dogs, and the average plasma concentration of ACHN-490 at each time point for each species is presented in Figure 1.
- The ACHN-490 PK parameters calculated for mouse, rat, and dog based on noncompartmental analysis are shown in Table 1.

Figure 1: Plasma Concentration-time Curves for a Single Bolus IV Injection of ACHN-490 Administered at 10 mg/kg in Mice, Rats, and Dogs

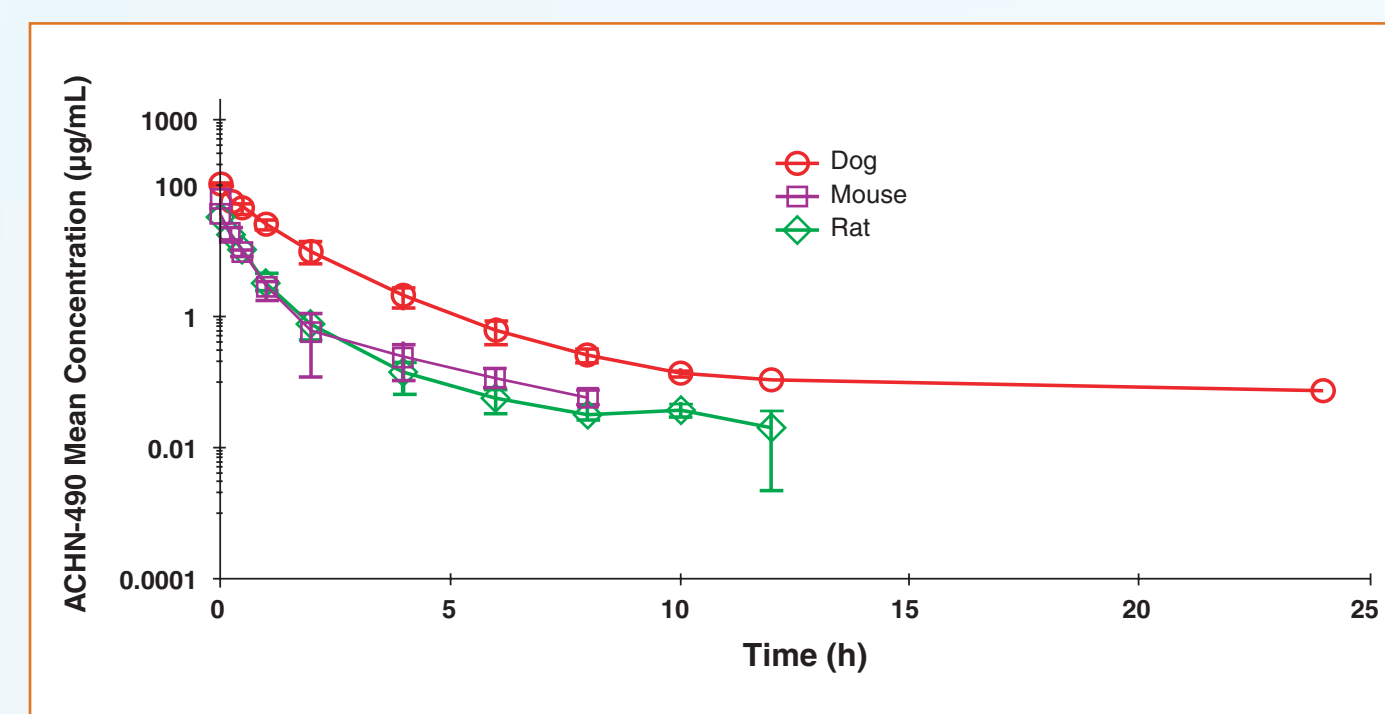


Table 1: PK Parameter Estimates for a Single Bolus IV Injection of ACHN-490 Administered at 10 mg/kg in Mice, Rats, and Dogs

| | No./Sex | C_0 (μ g/mL) | $AUC_{0-\infty}$ ($h^* \mu$ g/mL) | $t_{1/2}$ (hr) | CL (mL/hr/kg) | V_{ss} (mL/kg) |
|--------------------|---------|---------------------|------------------------------------|----------------|---------------|------------------|
| Mouse ^a | 15/M | 88 | 19 | 1.4 | 541 | 340 |
| Rat ^a | 3/M | 38 \pm 8 | 16 \pm 3 | 0.9 \pm 0 | 648 \pm 96 | 437 \pm 35 |
| Dog ^a | 3/M | 120 \pm 14 | 83 \pm 12 | 1.2 \pm 0 | 123 \pm 19 | 164 \pm 16 |

M = males; C_0 = concentration at time zero; $AUC_{0-\infty}$ = area under the concentration-time curve from time zero to infinity; $t_{1/2}$ = half-life; CL = clearance; V_{ss} = volume of distribution at steady state
^aSingle values determined using the naive pooled data method; ^bMean values (\pm standard deviation)

- In the rat, ACHN-490 displayed dose linearity between 1, 10, and 75 mg/kg IV dose levels and proportionality within this dose range.

ABSORPTION

- For rats and mice, SC absorption of ACHN-490 at 10 mg/kg showed exposures (area under the concentration-time curve [AUC]) slightly higher, but comparable to those for IV administration.
- Maximal drug concentrations (C_{max}) for SC administration were lower than initial drug concentrations (C_0) for IV administration of ACHN-490.

DISTRIBUTION

Plasma Protein Binding

- The percentage of bound ACHN-490 was independent of concentration and not species specific (12%, 15%, 10%, 18%, 11%, and 12% in mouse, rat, rabbit, dog, cynomolgus monkey, and human plasma, respectively).

Kidney Distribution

- The distribution of ACHN-490 in rat and dog kidney after 14 days of repeat dosing is shown in Table 2.
- In rats, distribution of ACHN-490 was not dose proportional, but was gender-specific: higher in male rat kidneys than in female. Distribution in dogs was also nonlinear, but not gender-specific.
- Rats administered gentamicin 100 mg/kg/day in 3 divided doses had an average 47% higher distribution in the kidney than rats administered the daily dose as a single injection.
- For the dog groups dosed at 30 mg/kg/day, an average of 38% more gentamicin was distributed per gram of kidney than for ACHN-490. This difference in kidney distribution was statistically significant ($p < 0.05$).

Table 2: Distribution of ACHN-490 to Rat and Dog Kidney

| Species | No./Sex | Dose (mg/kg) | Regimen | ACHN-490 in Kidney Mean (μ g/g) \pm Std Dev |
|---------|---------|--------------|--------------------------------------------------|----------------------------------------------------|
| Rat | 3/M | 10 | SC once daily for 14 days | 260 \pm 86 |
| | 3/F | 10 | SC once daily for 14 days | 140 \pm 28 |
| | 3/M | 30 | SC once daily for 14 days | 580 \pm 39 |
| | 3/F | 30 | SC once daily for 14 days | 223 \pm 46 |
| | 3/M | 100 | SC once daily for 9, 10, or 13 days ^a | NA ^a |
| Dog | 3/F | 100 | SC once daily for 8 or 14 days ^a | 537 and 429 ^a |
| | 2/M | 3 | 20-minute IV infusion, once daily | 230 \pm 33 |
| | 2/F | 3 | 20-minute IV infusion, once daily | 276 \pm 42 |
| | 2/M | 10 | 20-minute IV infusion, once daily | 559 \pm 64 |
| | 2/F | 10 | 20-minute IV infusion, once daily | 641 \pm 91 |
| | 2/M | 30 | 20-minute IV infusion, once daily | 649 \pm 126 |
| | 2/F | 30 | 20-minute IV infusion, once daily | 536 \pm 52 |

M = males; F = females

^aAnimals from these groups were sacrificed prior to 14 days of dosing due to toxic effects

METABOLISM

In Vitro Metabolic Stability, CYP Inhibition, and Plasma Stability

- ACHN-490 does not appear to be metabolized by liver microsomes or hepatocytes in any species tested.
- ACHN-490 at 25 μ M did not inhibit the *in vitro* metabolism by the 5 major human CYP isoforms 1A2, 2C9, 2C19, 2D6, or 3A4.
- ACHN-490 is stable after incubating at 37°C for 60 minutes in plasma from all species tested.

URINARY EXCRETION

- The primary route of elimination of ACHN-490 from the body appears to be excretion in the urine.
- In the single-dose rat and dog studies, the total urinary recovery of ACHN-490 collected in the first 24 h following a 10 mg/kg bolus IV dose was 59% \pm 13% and 119% \pm 22% in rats and dogs, respectively.

ESTIMATED PK PARAMETERS IN HUMAN SUBJECTS

- Experimentally derived results for gentamicin are shown in Table 3 and were used for the allometric scaling in Table 4.
- Allometric scaling predicts ACHN-490 human PK to be similar to that of gentamicin.

Table 3: Gentamicin PK Parameters after a Single, 10 mg/kg IV Dose in Mice, Rats, and Dogs

| | C_0 (μ g/mL) | $AUC_{0-\infty}$ ($h^* \mu$ g/mL) | $t_{1/2}$ (h) | CL (mL/h/kg) | V_{ss} (mL) |
|--------------------|---------------------|------------------------------------|---------------|--------------|---------------|
| Mouse ^a | 97 | 37 | 1.1 | 268 | 252 |
| Rat | 83 \pm 32 | 23 \pm 2 | 0.7 \pm 0.1 | 432 \pm 45 | 188 \pm 18 |
| Dog | 126 \pm 23 | 104 \pm 17 | 1.3 \pm 0.1 | 98 \pm 15 | 144 \pm 20 |

C_0 = concentration at time zero; $AUC_{0-\infty}$ = area under concentration-time curve from time zero to infinity; $t_{1/2}$ = half-life; CL = clearance; V_{ss} = volume of distribution at steady state
^aSingle values based on sparse sampling across multiple animals

Table 4: Predicted Human PK Parameters Based on Allometric Scaling of Animal Data for ACHN-490 and Gentamicin Administered IV at 10 mg/kg

| PK Parameter | Predicted Human PK Values for Gentamicin | Predicted Human PK Values for ACHN-490 |
|-----------------------|------------------------------------------|----------------------------------------|
| $t_{1/2}$ (h) | 0.9 | 1.1 |
| C_0 (μ g/mL) | 85 | 71 |
| AUC ($h^* \mu$ g/mL) | 122 | 113 |
| CL (mL/h) | 6105 | 6193 |
| V_1 (mL) | 7948 | 9811 |
| V_{ss} (mL) | 8260 | 10 088 |

$t_{1/2}$ = half-life; C_0 = concentration at time zero; AUC = area under the concentration-time curve; CL = clearance; V_1 = initial volume; V_{ss} = volume of distribution at steady state

7000 Shoreline Court, Suite 371
South San Francisco, CA 94080
Phone: 650-266-1120
Fax: 650-266-1130

DISCUSSION

The similarity in the nonclinical PK/absorption, distribution, metabolism, and excretion profile of ACHN-490 in rats and dogs, as well as the similar *in vitro* plasma protein binding and *in vitro* metabolism profile of ACHN-490 in animals and humans, supports the use of ACHN-490 in humans. ACHN-490 did not inhibit the human CYP isoforms 1A2, 2C9, 2C19, 2D6, or 3A4 *in vitro*. Therefore, it is unlikely to show drug-drug interactions with compounds that are CYP450 inhibitors, inducers, or substrates. ACHN-490 has a relatively low molecular weight and is highly polar so it would not be expected to be metabolized or interact with cytochromes. The half-lives and C_0 values determined for ACHN-490 in the mouse, rat, and dog are virtually identical to gentamicin. The relatively rapid initial half-life of ACHN-490 observed in the mouse, rat, and dog suggests a relatively rapid initial (approximately 1 h) half-life in humans. Thus, we predict that short infusions of ACHN-490 could be used to minimize accumulation in the kidney, while maintaining high C_{max} levels important for efficacy in human subjects.

CONCLUSIONS

- ACHN-490 is extensively renally cleared with a very low risk of drug-drug interactions resulting from cytochrome inhibition.
- The PK profile of ACHN-490 after IV administration was similar to gentamicin in mice, rats, and dogs.
- ACHN-490 plasma protein binding was similar among all species tested. Binding was low and concentration-independent.
- The comparative kidney distribution between ACHN-490 and gentamicin showed less ACHN-490 in the dog kidney after 14 days of repeat dosing than for gentamicin.

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