Abstract # 3254

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# Efficacy of ACHN-490 in a Murine Urinary Tract Infection Model with *Escherichia coli*

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## Abstract

**Background:** ACHN-490 is a next-generation aminoglycoside (AG), in clinical development with activity against multidrug resistant Gram-negative and select Gram-positive pathogens. ACHN-490 shows broad-spectrum bactericidal activity *in vitro*, and its potency is unaffected by all clinically relevant AG-modifying enzymes that confer resistance to legacy AGs. In the present study, ACHN-490 was compared to gentamicin (GEN) and levofloxacin (LVX) in a murine urinary tract infection (UTI) model.
**Methods:** Female C3H/HeJ mice were placed on 5% glucose water 6 days prior to infection. Anesthetized animals were transurethrally infected with a strain of *E. coli* that is uropathogenic, but antibiotic susceptible (ACHN-490, GEN and LVX MICs of 0.5, 0.5, and 0.06 µg/mL respectively), at 8.8 $\log_{10}$ colony-forming units (CFU) per animal. Beginning 4 days after infection, ACHN-490, GEN, and LVX were dosed subcutaneously twice daily for 3 consecutive days. Kidneys, bladders, and urine were collected for CFU counts 18 hours after the final dose. Efficacy was determined by comparing the mean CFU of treated groups to untreated controls.
**Results:** The mean $\log_{10}$ CFU counts in the kidneys, bladders, and urine of 7-day untreated controls were 7.4, 7.3, and 7.4, respectively. ACHN-490 doses ranging from 0.125 to 8 mg/kg decreased mean $\log_{10}$ CFU kidney counts by 2.4 to 4.5 compared to the levels in the 7-day controls. The same dose range of ACHN-490 also decreased bladder counts by 0.4 to 4.1 $\log_{10}$ CFU, and urine counts by 0.2 to 4.5 $\log_{10}$ CFU. Against this susceptible strain, GEN effected a similar dose response; GEN doses ranging from 0.125 to 2 mg/kg decreased mean $\log_{10}$ CFU kidney counts by 3.1 to 3.8, and bladder counts by 2.1 to 2.5 $\log_{10}$ CFU. Urine counts from GEN-treated animals were lowered by 0.8 to 3.8 $\log_{10}$ CFU. LVX also effected a similar response against this susceptible strain; LVX doses of 0.125 and 0.5 mg/kg maximally decreased $\log_{10}$ CFU counts in kidneys, bladders, and urine by 3.7, 2.9, and 0.54, respectively.
**Conclusion:** Administration of ACHN-490 effectively reduced CFU counts in the kidneys, bladders, and urine of animals with an ascending UTI infection, suggesting that ACHN-490 has potential as an option for the clinical treatment of UTIs.

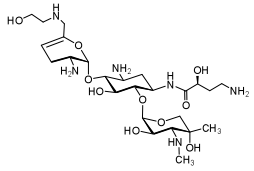
## Introduction

Urinary tract infections (UTIs) rank among the most prevalent of human-associated infectious diseases and significantly impact the health of many individuals throughout the world. Uropathogenic *Escherichia coli* (UPEC) has been identified as the most common etiological agent associated with UTIs. With an increasing incidence and prevalence of antibiotic resistance among UPEC isolates, there is a continual need for new antibiotics that are capable of treating UTIs. One such clinical candidate, ACHN-490 Injection, is currently undergoing Phase II trials for complicated UTIs and acute pyelonephritis. ACHN-490 is a next generation aminoglycoside (AG) that is designed to overcome all clinically relevant AG-modifying enzymes that confer resistance to legacy AGs. Additionally, ACHN-490 has displayed broad-spectrum activity against MDR Enterobacteriaceae, *Pseudomonas aeruginosa*, and MRSA. This creates a potential for ACHN-490 to be a useful frontline therapy for the treatment of infections caused by MDR bacterial pathogens. These results, combined with the fact that ACHN-490 Injection produced high $C_{max}$ and AUC values in human Phase I trials, suggest that ACHN-490 Injection has the potential to be clinically utilized as a treatment option for UTIs caused by UPEC and other agents.
The current study was undertaken to evaluate the efficacy of ACHN-490 and two comparators against a susceptible UPEC isolate in an ascending, mouse urinary tract infection model.

## Methods and Materials

**Minimum inhibitory concentrations (MICs):** MICs were determined for ACHN-490, gentamicin, and levofloxacin against the UPEC strain, ATCC 700336, by using the microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI).
**Mouse UTI model**
**Bacterial inoculum**: The infecting inoculum for ATCC 700336 was generated from overnight (18 - 20 hours) cultures incubated at 37°C on tryptic soy agar (TSA). Bacterial agar growth was suspended to 1.0 x $10^{10}$ colony-forming units (CFU)/mL in tryptic soy broth (TSB), and the inoculum was held at room temperature until it was used for infection.
**Infection:** The UTI model was done in accordance with the protocol approved by the UNTHSC-Institutional Animal Care and Use Committee. Female, C3H/HeJ mice (The Jackson Laboratory) weighing 18 to 20 grams were used, and diuresis was induced by placing the mice on 5% (w/v) glucose-water 6 days prior to infection and then maintaining them on the glucose-water throughout the length of the study. On the day of infection, anesthetized mice (ketamine at 40 mg/kg, xylazine at 6 mg/kg) were infected by placing 2 to 3 cm of polyethylene tubing (0.61 mm, o.d.) transurethrally into each mouse and injecting 0.05 mL of the inoculum (8.8 $\log_{10}$ CFU) into the urinary tract's lumen.
**Antibiotic treatment & CFU determination:** ACHN-490, gentamicin, and levofloxacin were subcutaneously dosed, 2 times per day for 3 days beginning 4 days after infection. Urine, bladders, and kidneys were collected from each mouse 18 hours after the final antibiotic dose. Bladders and kidney pairs were placed into 2 mL of sterile 1 x PBS, homogenized, and plated for CFU counts.

## Panel 1: Chemical structure of ACHN-490

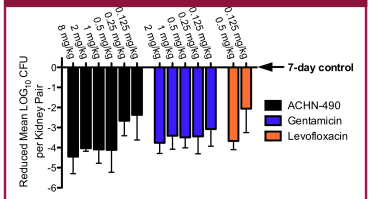


## Panel 2: MIC Values against UPEC strain, ATCC 700336

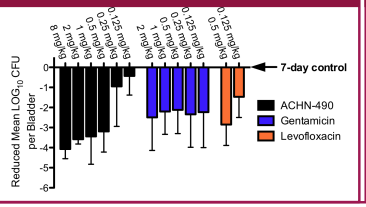
| Compounds | MIC values |
|---|---|
| ACHN-490 | 0.5 µg/mL |
| Gentamicin | 0.5 µg/mL |
| Levofloxacin | 0.06 µg/mL |

- MIC values determined by the microdilution method according to CLSI guidelines.
- *E. coli* reference strain ATCC25922 was included as a quality control for each MIC test (data not shown).

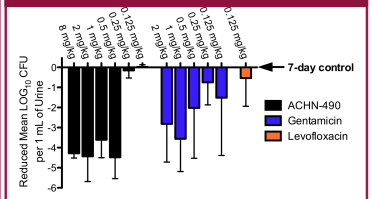
## Panel 3: Mean $LOG_{10}$ CFU Reduction in Kidneys - Antibiotic Treated vs. 7-Day Controls




## Panel 4: Mean $LOG_{10}$ CFU Reduction in Bladders - Antibiotic Treated vs. 7-Day Controls




## Panel 5: Mean $LOG_{10}$ CFU Reduction in Urine – Antibiotic Treated vs. 7-day Controls




- Mean $\log_{10}$ CFU reductions were determined as the difference between the mean CFU counts of untreated 7-day controls vs. antibiotic treated groups.
- Urine samples were collected from animals just prior to euthanasia, while kidneys and bladders were taken from euthanized animals, placed into sterile 1 x PBS, and homogenized. The homogenates (kidneys and bladder) and urine collected from each animal were serial diluted, plated onto TSA + charcoal, and incubated 37°C for 18 hours.
- Indicated doses (x-axis) represent the amount subcutaneously administered twice daily for 3 consecutive days, starting 4 days after infection.
- Urine samples could not be obtained for the animals dosed with Levofloxacin at 0.5 mg/kg
- Error bars represent the SD of the reduced mean CFUs for each group. Due to unscheduled deaths and sampling issues, total kidney (pairs) and bladder samples for each group ranged from 5 to 8, while total urine samples ranged from 0 to 7 for each group.

## Panel 6: Group Mean $Log_{10}$ CFU Counts (± SD) in Mouse UTI Study with ACHN-490, Gentamicin and Levofloxacin

| | 4-Day Controls | 7-Day Controls | ACHN-490 (mg/kg) | | | | | | Gentamicin (mg/kg) | | | | | Levofloxacin (mg/kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.125 | 0.25 | 0.5 | 1 | 2 | 8 | 0.125 | 0.25 | 0.5 | 1 | 2 | 0.125 | 0.5 |
| Kidneys | 6.7±0.7 | 7.4±0.4 | 5.1±1.3 | 4.8±0.8 | 3.3±1.1 | 3.4±0.7 | 3.4±0.2 | 3.0±0.9 | 4.4±0.9 | 4.0±0.9 | 3.9±0.5 | 4.0±0.7 | 3.7±0.5 | 5.4±1.2 | 3.8±0.4 |
| Bladders | 5.8±1.3 | 7.3±0.5 | 6.9±1.0 | 6.3±2.0 | 4.1±1.0 | 3.9±1.4 | 3.7±0.2 | 3.2±0.5 | 5.1±1.8 | 4.9±1.6 | 5.2±1.2 | 5.1±1.1 | 4.8±1.7 | 5.8±1.0 | 4.4±1.0 |
| Urine | 7.5±0.6 | 7.4±0.0 | 7.4±0.1 | 7.2±0.4 | 2.9±1.1 | 3.8±0.9 | 2.9±1.3 | 3.1±0.2 | 5.9±2.9 | 6.6±1.1 | 5.4±2.5 | 3.8±1.6 | 4.6±1.9 | 6.8±1.4 | NA |

## Summary and Conclusions

- MIC values for ACHN-490, gentamicin, and levofloxacin indicate that the uropathogenic *E. coli* (UPEC) strain used, ATCC 700336, was susceptible to all 3 antibiotics.
- The results from the 4-day infection controls indicated that stable and consistent CFU counts were achieved in the kidneys (6.7 $\log_{10}$), bladders (5.8 $\log_{10}$), and urine (7.5 $\log_{10}$) of inoculated animals.
- ACHN-490 administration resulted in 2.4 – 4.5 mean $\log_{10}$ CFU reduction in the kidneys as compared to 7-day controls. Gentamicin counts were reduced by 3.7 – 4.4 $\log_{10}$ over a similar dose range, while levofloxacin at 0.125 mg/kg or 0.5 mg/kg had mean $\log_{10}$ reductions of 2.1 and 3.7, respectively.
- As compared to 7-day controls, study associated dose ranges for ACHN-490, gentamicin and levofloxacin resulted in $\log_{10}$ CFU reductions of 0.4 – 4.1, 2.1 – 2.5 and 1.5 – 2.9 in the bladder and 0.2 – 4.5, 0.8 – 3.6 and 0.5 in the urine, respectively.
- The results from the current study demonstrate the efficacy of ACHN-490 in a murine model of a urinary tract infection with a uropathogenic *E. coli* and indicate that further testing of ACHN-490 against drug-resistant UPEC could be warranted in order to provide additional non-clinical data that supports the potential use of ACHN-490 in treating UTIs caused by resistant isolates.

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