Supporting Information

In Vivo Pharmacokinetic and Pharmacodynamic Profiles of Antofloxacin against *Klebsiella pneumoniae* in a Neutropenic Murine Lung Infection Model

Yu-Feng Zhou[a,b], Meng-Ting Tao[a,b], Wei Huo[a,b], Xiao-Ping Liao[a,b], Jian Sun[a,b] and Ya-Hong Liu[a,b]*

[a] National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, South China Agricultural University, Guangzhou, China;

[b] Guangdong Provincial Key Laboratory of Veterinary Pharmaceutics Development and Safety Evaluation, South China Agricultural University, Guangzhou, China;

* **Corresponding author:**

Ya-Hong Liu, PH.D., Laboratory of Veterinary Pharmacology, College of Veterinary Medicine, South China Agricultural University, Guangzhou, P. R. China. E-mail: lyh@scau.edu.cn. Tel: +86-020-85280006; Fax: +86-020-85280006

Supplementary Materials & Methods

Sample processing and LC-MS/MS method

A 0.1 mL aliquot of plasma sample or BAL fluid was transferred into a capped centrifuge tube, and then mixed with 0.1 mL of acetonitrile. After vortexing (1 min) and centrifuging (12000 × g, 10 min), the supernatant was filtered through a 0.22 μm nylon syringe filter and collected into a sample vial for concentration determination. The drug concentration analyses were performed using a HPLC-ESI-MS/MS system (Agilent 1200 HPLC system; Agilent Technologies, Santa Clara, CA, USA; API 4000 triple quadrupole mass spectrometer; Applied Biosystems, Carlsbad, CA, USA) equipped with a short column (Waters Symmetry C18, 2.1×50 mm, 3.5 μm). The injection volume was 5 μL, and column temperature should be maintained at 30 °C. The mobile phase consisted of (A) acetonitrile and (B) 0.1% formic acid in water containing 2 mM ammonium acetate using a gradient elution with a flow rate of 200 μL/min: 0-0.5 min (5% A), 0.5-3.5 min (5-85% A), 3.5-4.0 min (85% A), 4.0-4.5 min (85-5% A), 4.5-11 min (5% A). The total run time was 11 min. The mass conditions were as follows: ionspray voltage, 5000 V; curtain gas, 20 psi; nebulizer gas, 55 psi; collision gas, 20 psi; source temperature, 600 °C. Ion transitions of m/z 377.2→333.2 and 377.2→276.1 were chosen for multiple reaction monitoring (MRM) experiments in positive mode. The correlation coefficients (r) were above 0.999 in the linear range of 0.01-0.5 μg/mL. All samples that had concentrations above 0.5μg/mL were diluted proportionally prior to extraction with acetonitrile. The limit of quantification (LOQ) and detection (LOD) were 0.01 and 0.005μg/mL, respectively. Mean extraction

recoveries from the five replicate assays were 88.9 ± 7.96%, 91.2 ± 7.05% and 96.7 ± 5.71% at spiked drug concentrations of 0.02, 0.2, and 2 μg/mL, respectively. The intraday coefficients of variation for replicate control samples (n = 5) within these concentration ranges varied from 2.2 to 8.7%, and the interday coefficients of variation ranged from 5.9 to 9.7%.

Supplementary Table

Table S1. Protein binding of antofloxacin in murine plasma at three spiked levels [a]

Spiked levels (mg/L)	Binding (%) [b]
0.05	18.6 ± 2.38
0.5	20.2 ± 0.72
5	22.3 ± 1.02

[a] Values are means ± standard deviation; n = 3.

[b] The plasma protein binding of antofloxacin is 20.3% for mice determined in the present study, which was similar to the values of 17.5% for humans (1, 2).

References

1. **Yu X, Wang G, Chen S, Wei G, Shang Y, Dong L, Schon T, Moradigaravand D, Parkhill J, Peacock SJ, Koser CU, Huang H.** 2016. Wild-Type and Non-Wild-Type *Mycobacterium tuberculosis* MIC Distributions for the Novel Fluoroquinolone Antofloxacin Compared with Those for Ofloxacin, Levofloxacin, and Moxifloxacin. Antimicrob Agents Chemother **60:**5232-5237.
2. **Liu L, Pan X, Liu HY, Liu XD, Yang HW, Xie L, Cheng JL, Fan HW, Xiao DW.** 2011. Modulation of pharmacokinetics of theophylline by antofloxacin, a novel 8-amino-fluoroquinolone, in humans. Acta Pharmacol Sin **32:**1285-1293.