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PHARMACOLOGY

Activity of Aerosolized Levofloxacin against *Burkholderia cepacia* in a Mouse Model of Chronic Lung Infection

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ABSTRACT *Burkholderia cepacia* complex is an opportunistic pathogen capable of causing chronic pulmonary infections. These studies were conducted to demonstrate the activity of aerosolized levofloxacin in a chronic mouse lung infection model caused by *B. cepacia* isolates from patients with cystic fibrosis. Treatment with aerosolized levofloxacin for 4 days produced at least 1 log CFU of bacterial killing against all strains tested, suggesting possible utility in the treatment of lung infections caused by *B. cepacia* isolates.

KEYWORDS aerosolized levofloxacin, mouse lung infection, *B. cepacia*

Burkholderia cepacia complex is an opportunistic pathogen that is capable of causing various degrees of respiratory infection in patients with and without cystic fibrosis (1–3). These pulmonary infections often result in asymptomatic carrier, chronic infection, or "cepacia symptoms" (4–6). Although infections with *B. cepacia* complex are uncommon, infections in patients with cystic fibrosis can lead to a rapid decline in pulmonary function and, in some cases, morbidity (7, 8). In addition, therapeutic options for these infections are very limited due to the high level of resistance to multiple antibiotics, including tobramycin, aztreonam, imipenem, amikacin, trimethoprim-sulfamethoxazole, piperacillin-tazobactam, ceftazidime, and ciprofloxacin (4, 9–12).

Levofloxacin has potent activity against key cystic fibrosis pathogens, including *Pseudomonas aeruginosa* and *B. cepacia* complex, with no loss of *in vitro* activity in sputum (13). Furthermore, aerosol delivery of levofloxacin achieves high local concentrations at the site of infection, enhances bacterial killing, and reduces the potential for development of resistance (14).

Previous studies have shown that aerosolized levofloxacin administered once or twice daily produced more than 1 log of bacterial killing compared to that seen in untreated controls at the start of treatment and prevented mortality in acute lethal and chronic lung infection models caused by *P. aeruginosa* isolates (15). In the present study, we assessed the *in vivo* activity of aerosolized levofloxacin against five *B. cepacia* complex strains in a mouse chronic lung infection model.

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Antimicrobial susceptibility was determined by a broth microdilution assay according to CLSI reference methods (16). As shown in Table 1, levofloxacin MICs for these strains ranged between 0.25 and 8 mg/liter, and levofloxacin was more active against these isolates than aztreonam, tobramycin, or amikacin.

For *in vivo* studies, female BALB/c mice (6 to 8 weeks of age) were obtained from Envigo Laboratories (Livermore, CA) and were provided food and water *ad libitum* in accordance with National Institutes of Health guidelines for the care and use of laboratory animals (17). All studies using animals were performed under protocols approved by an Institutional Animal Care and Use Committee. Mice were rendered transiently neutropenic by administration of 150 mg/kg of cyclophosphamide (Bax-

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TABLE 1 MICs for the strains used in these studies

Strain	MIC (mg/liter) of:			
	Levofloxacin	Tobramycin	Aztreonam	Amikacin
B. cepacia ATCC 25416	1	16	64	16
B. cenocepacia (genomovar III) BC1020	8	>128	128	>128
B. cenocepacia (genomovar III) BC1021	8	>128	128	>128
B. multivorans (genomovar II) BC1013	0.25	16	4	16
B. multivorans (genomovar II) BC1014	1	32	64	64
B. multivorans (genomovar II) BC1012	4	32	64	64

ter, Deerfield, IL) intraperitoneally on days 4 and 1 before infection. Bacteria were grown overnight at 37°C for 20 h under constant aeration (300 rpm) in Mueller-Hinton broth (MHB). The infecting inoculum was prepared by removal of an aliquot from the overnight culture and subculturing into fresh MHB. The subculture was incubated at 37°C under constant aeration for 3 h to reach an absorbance of 0.30 to 0.35 at 600 nm (~108 CFU/ml). The bacterial suspensions were diluted into Hanks balanced salt solution with 0.1% (vol/vol) gelatin (Sigma) suspension to yield ~106 CFU/ml.

One day after the last dose of cyclophosphamide, mice were anesthetized by isoflurane administration (5% isoflurane in oxygen running at 4 liters/min) and then infected by intratracheal instillation of 0.05 ml of inoculum using a curved oral gavage tip attached to a 1-ml syringe. Treatments started 72 h postinfection and were administered once or twice daily for 4 days. Levofloxacin and saline were aerosolized using a microspray aerosol device (MicroSprayer model IA-C; Penn-Century, Philadelphia, PA) attached to a high-pressure syringe (FMJ-250; Penn-Century). For aerosol administration, each mouse was anesthetized with isoflurane (5% isoflurane in oxygen running at 4 liters/min) and positioned securely at a 45° to 50° angle by the upper teeth, the microspray aerosol tip was inserted into the bifurcation, and 50 μl of bacterial suspension was administered. An untreated group of mice ($n = 4$) was sacrificed before the initiation of treatment to determine baseline bacterial counts (72 h postinfection). The control and treated animals ($n = 4$ to 8) were sacrificed 12 to 16 h after the last dose by carbon dioxide asphyxiation. The lungs were

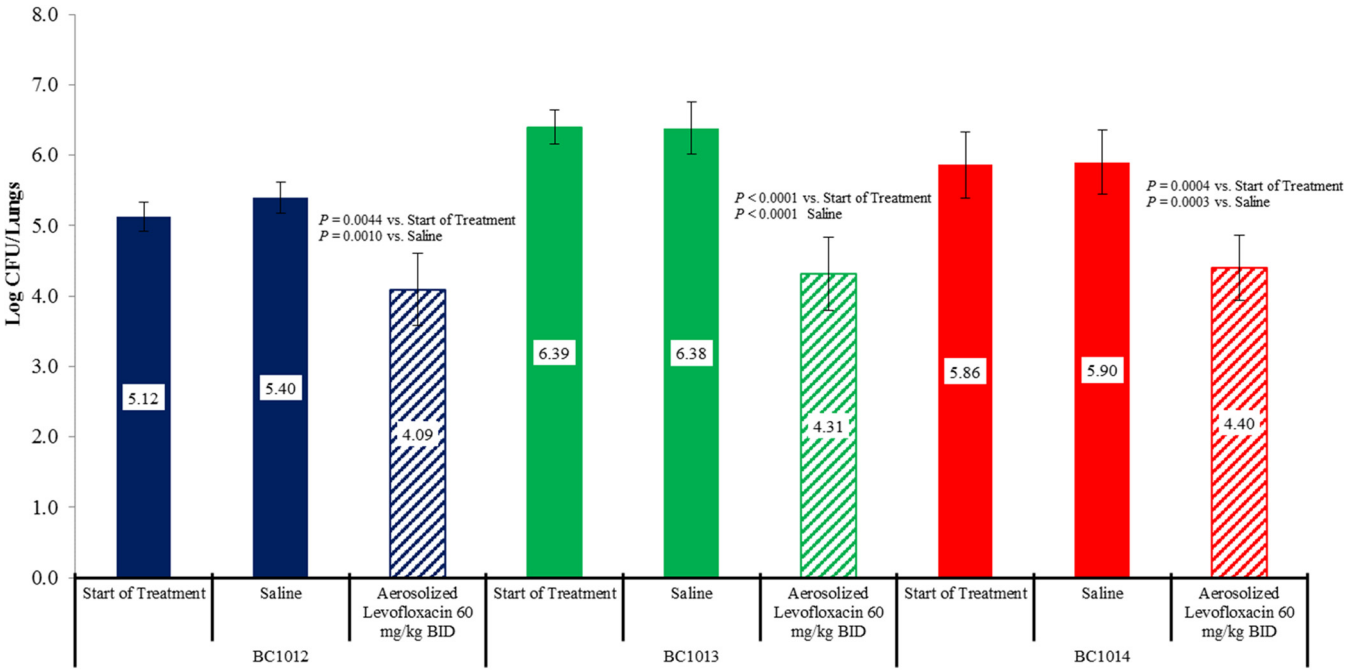

FIG 1 Activity of aerosolized levofloxacin administered twice daily for 4 consecutive days against three *B. multivorans* strains in a mouse chronic lung infection model.

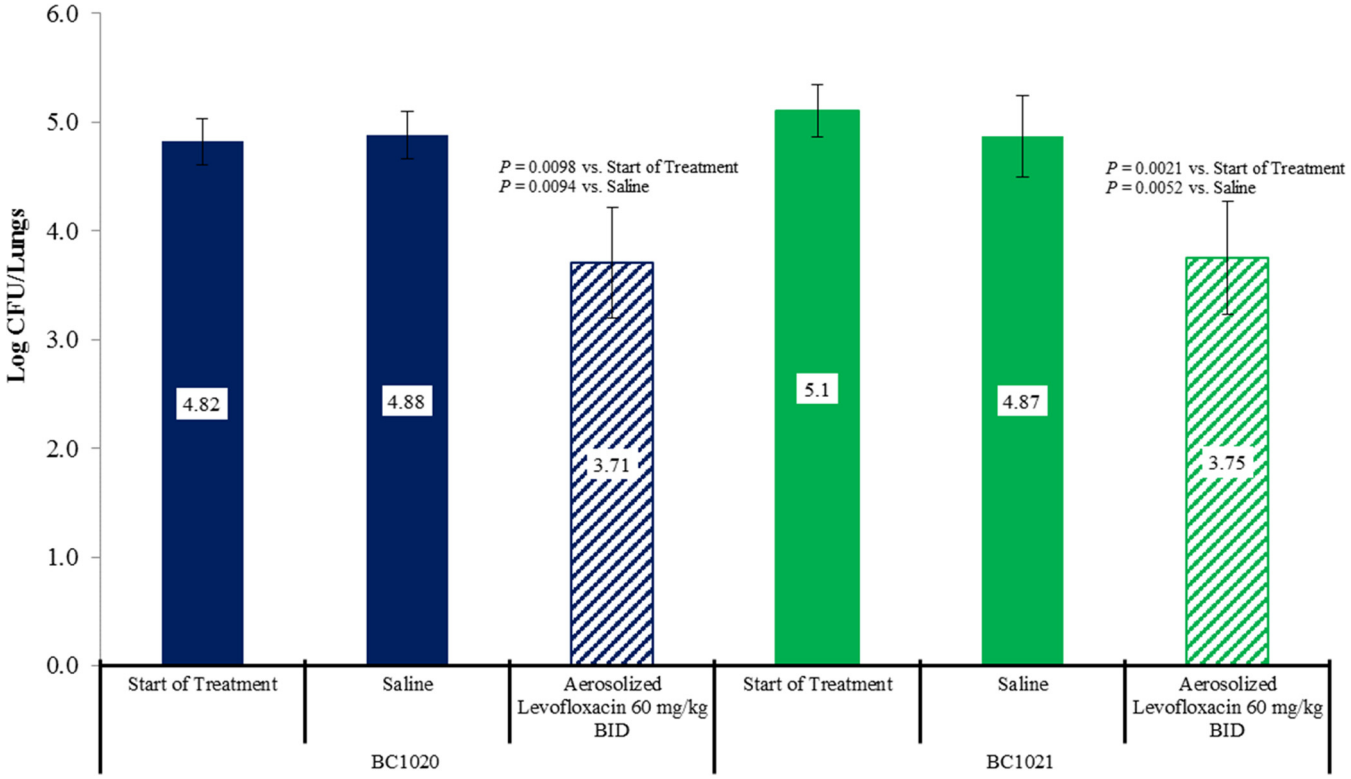

FIG 2 Activity of aerosolized levofloxacin administered twice daily for 4 consecutive days against two *B. cenocepacia* strains in a mouse chronic lung infection model.

removed aseptically and homogenized (Pro200 homogenizer; Pro Scientific, Monroe, CT) in 1 ml of sterile saline. Serial 10-fold dilutions of the homogenized lungs were plated on Mueller-Hinton agar plates. The plates were incubated overnight at 37°C, and then colonies were counted. Bacterial counts in lungs were analyzed using an unpaired *t* test (GraphPad Prism, version 6.03). A *P* value of <0.05 was considered statistically significant.

Model development experiments with all five strains used in this study established that the untreated bacterial burden in the lungs did not decline for up to 8 days under these experimental conditions (data not shown). Additionally, the *Burkholderia multivorans* isolates used in these studies were found to produce a persistent infection for up to 16 days in a neutropenic mouse chronic lung infection model (18).

Figure 1 shows that treatment with 60 mg/kg of aerosolized levofloxacin twice daily for 4 days produced 1.03-, 2.08-, and 1.46-log CFU reductions in lung bacterial counts in mice infected with *B. multivorans* strains BC1012, BC1013, and BC1014, respectively. The bacterial killing produced by aerosol levofloxacin was statistically significant for all three strains compared with that in saline-treated controls at the end of treatment and untreated controls at the start of treatment.

The lung bacterial counts for *B. cenocepacia* strains BC1020 and BC1021 are presented in Fig. 2. For strains BC1020 and BC1021, treatment with 60 mg/kg aerosolized levofloxacin twice daily produced 1.11- and 1.35-log CFU reductions in lung bacterial counts, respectively. The bacterial killing produced by aerosol levofloxacin was statistically significant for both strains compared with that in saline-treated controls at the end of treatment and untreated controls at the start of treatment and was similar to the level produced against *B. multivorans* strains despite having up to 32-fold higher MICs. Because levofloxacin was administered by inhalation, the lung levels were substantially higher than those after a systemic dose, allowing for coverage of organisms with MICs that are resistant to systemic treatment (15). Previous studies showed that aerosol administration of the same total daily dose of levofloxacin given once or twice daily

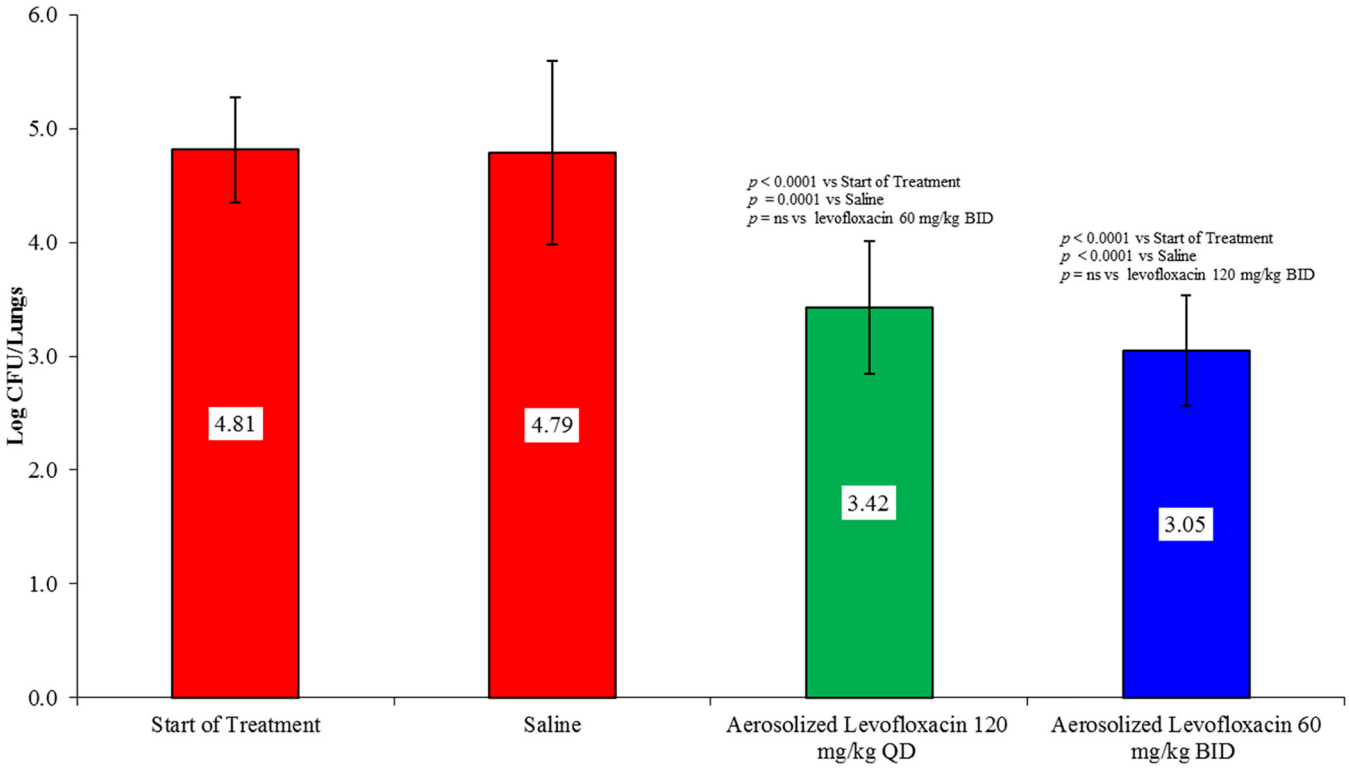

FIG 3 Comparative activity of aerosolized levofloxacin administered once (120 mg/kg) or twice (60 mg/kg) daily for 4 consecutive days in a mouse chronic lung infection model against *B. cenocepacia* strain BC1021.

against *P. aeruginosa* strains produced similar bacterial killing in mice (15). To determine whether *B. cepacia* isolates would respond the same way, we examined the activity 120 mg/kg of aerosolized levofloxacin administered as a single 120-mg/kg dose or divided into two 60-mg/kg doses administered 12 h apart in the mouse chronic lung infection model from *B. cenocepacia* strain BC1021. Figure 3 shows the activity of aerosolized levofloxacin administered at 120 mg/kg once daily or 60 mg/kg twice daily for 4 days. As observed previously with *P. aeruginosa* isolates, both regimens produced similar bacterial killing against *B. cenocepacia* strain BC1021.

In summary, aerosolized levofloxacin produced significant bacterial killing in this mouse chronic lung infection model against all five *B. cepacia* complex strains tested. Aerosol administration of the same total daily dose once or twice daily produced similar bacterial killing, as observed previously, suggesting some potential flexibility in the dosage regimen. Overall, these data suggest that aerosolized levofloxacin may be useful in the management of chronic pulmonary infections caused by *B. cepacia* complex.

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We have no conflicts of interest to declare.

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