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Pharmacodynamic and pharmacokinetic profiling of delafloxacin in a murine lung model against community-acquired respiratory tract pathogens

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ABSTRACT

Increasing antimicrobial resistance in community-acquired pneumonia (CAP) pathogens has contributed to infection-related morbidity and mortality. Delafloxacin is a novel fluoroquinolone with broad-spectrum activity against Gram-positive and -negative organisms, including *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA). This study aimed to define the pharmacodynamic profile of delafloxacin against CAP pathogens using a neutropenic murine lung infection model. Five *S. pneumoniae*, 2 methicillin-susceptible *S. aureus* (MSSA), 2 MRSA and 2 *Klebsiella pneumoniae* isolates were studied. Delafloxacin doses varied from 0.5 mg/kg/day to 640 mg/kg/day and were given as once-daily to every 3 h regimens over the 24-h treatment period. Efficacy was measured as the change in $\log_{10}$ CFU at 24 h compared with 0-h controls. Plasma and bronchopulmonary pharmacokinetic studies were conducted. Delafloxacin demonstrated potent in vitro and in vivo activity. Delafloxacin demonstrated high penetration into the lung compartment, as epithelial lining fluid concentrations were substantially higher than free drug in plasma. The ratio of the area under the free drug concentration–time curve to the minimum inhibitory concentration of the infecting organism (*f*AUC/MIC) was the parameter that best correlated with the efficacy of the drug, and the magnitude required to achieve 1 $\log_{10}$ CFU reduction was 31.8, 24.7, 0.4 and 9.6 for *S. pneumoniae*, MRSA, MSSA and *K. pneumoniae*, respectively. The observed in vivo efficacy of delafloxacin was supported by the high pulmonary disposition of the compound. The results derived from this pre-clinical lung model support the continued investigation of delafloxacin for the treatment of community-acquired lower respiratory tract infections.

1. Introduction

When considering community-acquired pneumonia (CAP) due to a bacterial aetiology, the majority of cases are due to *Streptococcus pneumoniae*, followed by *Haemophilus influenzae* and *Staphylococcus aureus*, despite vaccination efforts against the pneumococcus [1,2]. Although *S. aureus* is responsible for a smaller percentage of CAP cases, the presence of Panton–Valentine leukocidin (PVL), a virulence factor that has been associated with community-acquired methicillin-resistant *S. aureus* (MRSA), can result in a severe necrotising form of the disease [3]. Albeit less frequent, *Klebsiella pneumoniae* has been noted as a problematic pathogen as it has been associated with a higher incidence of CAP-related mortality than *S. pneumoniae* [4,5]. As a result of the prevalence of the pneumococcus and the potential role of the atypical pathogens, current US and British CAP guidelines suggest that hospitalised CAP patients be given a fluoroquinolone or β-lactam plus a macrolide [6,7]. British guidelines also recommend adding fluoroquinolones for patients with severe CAP not responding to the combination regimen of a β-lactam plus a macrolide [7].

Owing to the continued rise of resistance among community-acquired respiratory tract organisms, new therapeutic entities are under development. One such example is delafloxacin, a novel fluoroquinolone with broad-spectrum activity against Gram-positive organisms, namely *S. pneumoniae* and MRSA, as well as Gram-negative organisms such as *K. pneumoniae* [8]. A phase 2 study in patients with acute bacterial exacerbation of chronic bronchitis (ABECB) showed that delafloxacin had similar clinical and bacteriological outcomes for *S. pneumoniae* ($n = 46$ patients) and *S. aureus* ($n = 19$ patients) compared with levofloxacin [9,10]. As a result of its spectrum and efficacy in the ABECB population, delafloxacin is currently being investigated in a phase 3 clinical trial of CAP [11].

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Whilst it is well recognised that the ratio of the area under the free drug concentration–time curve to the minimum inhibitory concentration of the infecting organism (*f*AUC/MIC) is the pharmacokinetic/pharmacodynamic (PK/PD) parameter that best predicts the efficacy of the clinically approved fluoroquinolones, data regarding the PK/PD profile of delafloxacin are not currently available [12]. This study aimed to define the magnitude of delafloxacin exposure required to produce sustained in vivo kill against potential CAP pathogens.

2. Materials and methods

2.1. Antimicrobial test agents

Delafloxacin lyophilised Captisol®-containing formulation (Melinta Therapeutics, Inc., Lincolnshire, IL; lot 12DEL1) was used throughout these experiments. For in vitro studies, delafloxacin was first dissolved in sterile water with dropwise addition of 0.1 M NaOH until completely dissolved, followed by dilution in Mueller–Hinton II broth (Becton, Dickinson & Co., Sparks, MD) to the final concentrations used. For in vivo studies, delafloxacin vials (300 mg/20 mL vials) were used. Delafloxacin powder was reconstituted with sterile 5% dextrose in water according to the procedure provided by the manufacturer to achieve a concentration of 25 mg/mL. Delafloxacin was administered by subcutaneous injections of 0.2 mL. For in vitro MIC studies, azithromycin, ceftriaxone and levofloxacin analytical standard powders (Sigma-Aldrich, St Louis, MO) were utilised.

2.2. Bacterial isolates

Five *S. pneumoniae*, two methicillin-susceptible *S. aureus* (MSSA), two MRSA and two *K. pneumoniae* isolates from the Center for Anti-Infective Research and Development (CAIRD, Hartford, CT) culture collection were studied. The MICs of delafloxacin, azithromycin, ceftriaxone and levofloxacin were determined in triplicate by broth microdilution in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [13], and modal MICs were reported. Isolates were stored in skim milk (Becton, Dickinson & Co.) at –80 °C and were subcultured twice onto trypticase soy agar with 5% sheep blood (TSA II™; Becton, Dickinson & CO.) within 48 h prior to use.

2.3. Neutropenic murine lung infection model

Specific pathogen-free, female ICR mice weighing 20–22 g and BALB/c mice aged 7–8 weeks upon delivery were obtained from Envigo RMS, Inc. (Indianapolis, IN). The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital (Hartford, CT). Due to variability in bacterial growth of different bacterial families between mouse species [14–16], ICR mice were used in studies with *S. pneumoniae* and *K. pneumoniae*, whilst BALB/c mice were used in studies with *S. aureus*. Mice were rendered transiently neutropenic by intraperitoneal injection (0.2 mL in normal saline) of cyclophosphamide (Sigma-Aldrich) at doses of 150 mg/kg 4 days and 100 mg/kg 1 day prior to infection in *S. pneumoniae* studies and at 250 mg/kg 4 days and 100 mg/kg 1 day prior to infection in *K. pneumoniae* and *S. aureus* experiments. A total of six mice per treatment regimen and control group were infected with one of the test isolates. Mice were lightly anaesthetised using isoflurane (2.5% v/v in 100% oxygen carrier). Bacterial inocula were prepared as a suspension in 5% dextrose/0.9% sodium chloride for *S. pneumoniae* and in 3% hog gastric mucin in 0.9% sodium chloride for *S. aureus* and *K. pneumoniae* to ensure the establishment of infection in these murine species.

Pneumonia was induced by instillation of 0.05 mL of the prepared inoculum into the oral cavity of the anaesthetised animal whilst blocking the nares and holding the mouse in a vertical position. Bacterial suspensions were prepared at an inoculum size of 10^8–10^9 CFU/mL for *S. pneumoniae* and 10^7 CFU/mL for *S. aureus* and *K. pneumoniae*. After allowing full recovery from anaesthesia in an oxygen-enriched chamber, mice were randomised to control (sham treatment) or treatment groups.

2.4. Pharmacokinetic (PK) studies

2.4.1. Plasma pharmacokinetics

Neutropenic ICR female mice were infected with *K. pneumoniae* as described above. At 2 h post-infection, a group of 48 ICR mice were administered delafloxacin via the subcutaneous route at single doses of 0.5, 20 or 80 mg/kg. Next, terminal blood samples from CO_2-asphyxiated mice ($n = 6$ mice per time point) were collected via cardiac puncture and were placed in K_2 EDTA Microtainer® tubes (BD, Franklin Lakes, NJ). Blood samples were collected at eight time points (0.25, 0.5, 1, 1.5, 2, 4, 6 and 8 h post-dose). Plasma was separated by centrifugation, was transferred to microfuge tubes (USA Scientific, Ocala, FL) and was stored at –80 °C until shipped for analysis.

To confirm similar pharmacokinetics in BALB/c mice, a single-dose PK study, using the same methodology (although mice were infected with *S. aureus*), was conducted using a single dose of 20 mg/kg.

2.4.2. Pulmonary distribution

To evaluate the pulmonary disposition of delafloxacin, lavage fluid was collected via bronchoalveolar lavage (BAL) subsequent to the terminal blood collection, during studies noted above, at 1, 2, 4 and 6 h post-dose. BAL was performed by inserting a catheter in the trachea and instilling four aliquots of 0.4 mL of normal saline followed by immediate removal of this fluid. The four aliquots were combined into one sample vial. BAL samples were centrifuged to remove blood and cellular debris. The BAL supernatants were stored at –80 °C until shipped for analysis of delafloxacin concentration.

2.4.3. Delafloxacin concentration determinations

Plasma and BAL fluid concentrations of delafloxacin were determined by PharmOptima, LLC (Portage, MI) using a validated liquid chromatography–tandem mass spectrophotometry (LC–MS/MS) methodology. The assay was linear with a coefficient of determination (R^2) of 0.992 and 0.996 for plasma and BAL fluid concentrations of delafloxacin, respectively. The percentage coefficient of variation of the quality control samples was 6% for intraday and 7.6% for interday assays. The lower limit of detection of the assay was 10 ng/mL.

2.4.4. Urea concentration analysis

Aliquots of plasma and BAL samples were used for the determination of urea to derive drug levels in epithelial lining fluid (ELF) as described previously [17]. Urea concentrations in BAL fluid and plasma were analysed by colorimetric enzymatic assay (Teco Diagnostics, Anaheim, CA) via a spectrophotometric detection method (Cary 50 Series; Varian, Walnut Creek, CA) at CAIRD. The urea assay was linear with $R^2 \geq 0.999$ for both plasma and BAL fluid urea concentrations over the range of 0.1–2.0 mg/dL. Quality control samples of 0.15 mg/dL and 1.5 mg/dL had intraday and interday variabilities with coefficient of variation percentages of 6.5% and 9.3%, and 1.4% and 5.9%, respectively.

2.5. In vivo efficacy

Doses of delafloxacin ranging from 0.5 to 80 mg/kg were administered subcutaneously in a schedule stretching from once daily to every 3 h over the 24-h treatment period. Control animals received vehicle (5% dextrose in water) as sham treatment in the same