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Population Pharmacokinetic (PK) and Pharmacodynamic (PD) Modeling of Ticarcillin-Clavulanate in Pediatric CF Patients

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lipocalin (NGAL) that specifically scavenge bacterial siderophores, therefore preventing bacteria from establishing infection. *P. aeruginosa* produces fluorescent siderophores (pyoverdines) found to be important for biofilm development and bacterial virulence. It is not known if *P. aeruginosa* strains persisting in CF lungs evade NGAL recognition. The aim of this work is to determine if pyoverdine binds to the antibacterial protein NGAL.

Methods: The binding of siderophores, pyoverdine from *P. aeruginosa* and enterobactin from *E.coli*, to the recombinant NGAL protein was determined using the tryptophan fluorescence quenching method.

Results: We found that pyoverdine did not bind to NGAL either in apo form or when complexed with iron. We used enterobactin as a control, and as expected, enterobactin bound to NGAL causing strong tryptophan quenching. The data indicate that the major siderophore of *P. aeruginosa*, pyoverdine, evades NGAL recognition therefore dysregulating host iron-limiting innate defenses. We then investigated whether pyoverdine modulates other innate immune defenses such as respiratory burst. Upon phagocytosis of invading pathogen, respiratory burst is triggered as a defense mechanism that leads to reactive oxygen species (ROS) release which is important for the oxidative killing of invading pathogens. We observed that pyoverdine decreased ROS release when added at the peak of the respiratory burst in THP-1 cells primed with *P. aeruginosa* LPS (10 pmol/mL) and detected using the chemiluminescent probe lucigenin. The data suggest that pyoverdine dampens the respiratory burst possibly by scavenging released ROS to spare the oxidative killing of invading *P. aeruginosa*. Taken together, our data suggest that pyoverdine is a stealth siderophore and a virulence factor that helps *P. aeruginosa* evade the ROS mediated oxidative killing.

Conclusions: *P. aeruginosa* persists in the CF host by evading both the iron-limiting innate defenses and the oxidative killing by macrophages.

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HOST CELL SIGNALING INDUCED BY SEQUENTIAL ISOLATES OF *PSEUDOMONAS AERUGINOSA*

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Bacterial activation of the type I interferon (IFN) pathway has been demonstrated to be important in the clearance of mucosal pathogens. In Gram negative infections the LPS/TLR4/TRIF signaling pathway is a major inducer of the type I IFN cascade. The immunogenicity of *Pseudomonas aeruginosa* changes over the course of colonization of the CF lung due to alterations in virulence factor expression, therefore we hypothesize that alterations in LPS structure reduces bacterial activation of the type I IFN cascade. Our hypothesis was tested by stimulating (4 hours) human immune (primary dendritic cells (DC) and THP-1) cells and epithelial (BEAS-2B) with *P. aeruginosa* strains isolated from single CF patients at early (3 or 5 months) and late (14 years) stages of colonization. Analysis of the strains by random amplification of polymorphic DNA (RAPD) confirmed that early and late isolates shared a common genetic background. Activation of NFκB (IL-8) and IFN (IFNβ) signaling was measured by RT-PCR in DCs and THP-1 immune cells, or luciferase reporter and ELISA in the BEAS-2B epithelial cells. Stimulation with the early isolate induced higher levels of NFκB signaling activation in DC (9.81 and 3.81 fold of unstimulated) and THP-1 (5.26 and 2.62 fold of unstimulated) immune cells compared with late isolates, however similar induction levels were observed in BEAS-2B (6.10 and 7.89 fold of unstimulated) cells. NFκB signaling in the BEAS-2B was confirmed by ELISA analysis of IL-8 in the supernatant of cells stimulated with early and late isolates (113.9 and 215.6 pg/mL). Induction of IFN signaling by the early isolate was also higher than that induced by the late isolate in DC (10.10 and 3.86 fold to unstimulated) and THP-1 (4.98 and 1.85 fold to unstimulated) cells. Interestingly, IFN signaling induction by the early isolate was also higher in the epithelial BEAS-2B line compared to levels induced by the late isolate (14.14 and 8 fold to unstimulated). This data demonstrates that during colonization *P. aeruginosa* loses its immunostimulatory capacity in a host cell type and pathway dependent manner. To support our hypothesis that alterations to LPS are responsible for the reduction in *P. aeruginosa* immunostimulatory capacity we stimulated murine nasal epithelial cells with the different *P. aeruginosa* isolates. Unlike human TLR4, activation of murine TLR4 is unaffected by alterations in LPS structure. We observed no difference in NFκB (21.73 and 22.71 fold to unstimulated) or IFN (228.1 and 278.2 fold to unstimulated) signaling by RT-PCR in murine epithelial cells. Therefore we concluded that modifications in *P. aeruginosa* virulence factors, most likely LPS, reduce the immunogenicity of the organism resulting in reduced activation of the type I IFN pathway, a potential mechanism by which the pathogen evades clearance by the host.

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VORICONAZOLE PHARMACOKINETIC PARAMETERS IN PATIENTS WITH CYSTIC FIBROSIS

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Purpose: The use of voriconazole has become more prevalent over the years to treat cystic fibrosis (CF) patients with allergic bronchopulmonary aspergillosis (ABPA) failing itraconazole therapy or with invasive fungal lung disease. However, voriconazole pharmacokinetic data are lacking in CF patients, and little is known on the optimal dosage of voriconazole in pediatric patients. A small group of CF patients have undergone voriconazole therapeutic drug monitoring over the past 6 years at our institution. The objective of this study is to retrospectively assess whether standard doses of intravenous and oral voriconazole yield therapeutic concentrations in CF patients.

Methods: Electronic medical records and laboratory data were used to identify CF patients who underwent voriconazole therapeutic drug monitoring since January 1, 2004. Patients younger than 2 years of age and who did not have a definitive diagnosis of CF were excluded from this study. The following data were collected: patient age, gender, ethnicity, results from fungal cultures, liver function tests, IgE levels, indication for voriconazole use, dose of voriconazole administered, duration of voriconazole therapy, and serum level of voriconazole. Concomitant use of medications known to be contraindicated with voriconazole use, reported adverse events associated with use of voriconazole, and overall clinical outcome of patients were also evaluated in this study. Doses of voriconazole used in CF patients included in this study were compared to standard doses of voriconazole. Voriconazole therapeutic levels were also compared to current therapeutic goals in treating ABPA and other invasive fungal lung infections.

Results: The study included 15 patients (mean age 22.3 ± 9.6, range 4-36, years) receiving 21 treatment courses. The most common indication for voriconazole use was for treatment of positive respiratory fungal cultures in post-lung transplant patients (11). Other indications included prophylaxis for fungal infections in post-lung transplant patients (2), treatment of positive respiratory fungal cultures in non-transplant CF patients (3), empiric coverage of respiratory fungal infections in non-transplant CF patients (3), and treatment of ABPA (2). The mean voriconazole dose used was 207.9 ± 76.7 mg (4.8 ± 2.3 mg/kg), and the mean serum voriconazole level obtained was 1.9 ± 1.7 mg/L. The mean voriconazole dose used in this population was near the standard flat voriconazole dose in adult patients but at a higher mg/kg than in an average adult population. Only 57% of patients in this study achieved a therapeutic serum voriconazole level of ≥ 1 mg/L. Two patients had LFT increases ≥ 3 times the upper normal limit. Three patients were on medications contraindicated with concurrent voriconazole therapy; however, no toxicity was observed.

Conclusions: Serum voriconazole concentrations were unpredictable in our CF population. Patients with CF may require higher than standard voriconazole doses to yield therapeutic voriconazole levels, and therapeutic drug monitoring may be a useful tool for individualized dosing in CF patients.

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POPULATION PHARMACOKINETIC (PK) AND PHARMACODYNAMIC (PD) MODELING OF TICARCILLIN-CLAVULANATE IN PEDIATRIC CF PATIENTS

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Background: The Intermountain CF Pediatric Center utilizes ticarcillin-clavulanate 400 mg/kg/day div q 6 hours, (max 24 grams/day). This

dosing strategy is higher than the FDA approved package labeling. We simulated in model the microbiological efficacy of this dosing regimen. The primary objective of this study was to use PK/PD models to estimate the likelihood of achieving bacteriostatic and bactericidal effects against a range of *P. aeruginosa* minimum inhibitory concentration (MIC) values using a higher than FDA approved dose of ticarcillin-clavulanate.

Methods: This was a population-based PK/PD modeling study of pediatric CF patients admitted from 01/01/05 to 12/1/09 who received the dosing regimen for ≥7 days. Population PK and PD models were used to estimate PK and PD parameters. Serum ticarcilin concentration levels were modeled using published PK parameters. A 10,000 patient Monte Carlo simulation was performed to estimate the target time in which free drug concentrations exceeded the MIC of the infecting organism. The two PK/PD MIC breakpoints used included ≥30% for bacteriostasis, and ≥50% for bactericidal effects of ticarcillin-clavulanate at higher than FDA approved doses.

Results: A total of 127 patients met inclusion criteria. Predicted intermittent ticarcillin peak concentrations were 288±93.4 mg/L. The model predicted the probability of target attainment (PTA) of MICs for *P. aeruginosa* with a near-maximal bactericidal PK-PD MIC breakpoint of 16 mcg/mL, and a bacteriostasis PK-PD MIC breakpoint of 32 mcg/mL. (See Table.)

Conclusions: In our simulation, higher than FDA approved doses of ticarcillin-clavulanate are effective in achieving bactericidal effects among pseudomonal isolates with MICs below 16 mcg/mL. Bacteriostatic and bactericidal effects were not frequently achieved among *P. aeruginosa* isolates with MICs above 32 mcg/mL.

Probability of target attainment (%) for ticarcillin at 30% and 50% of the time in which free drug concentrations exceed the MIC

Minimum inhibitory concentration (mcg/mL)	Ticarcillin 30% T>MIC	Ticarcillin 50% T>MIC
0.015	100.0%	100.0%
0.03	100.0%	100.0%
0.06	100.0%	100.0%
0.125	100.0%	99.9%
0.25	100.0%	99.9%
0.5	100.0%	99.9%
1	100.0%	99.8%
2	100.0%	99.8%
4	100.0%	99.7%
8	99.9%	99.1%
16	99.9%	96.0%
32	98.9%	74.3%
64	77.3%	17.1%
128	6.3%	0.3%

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LUNG FUNCTION IMPROVEMENT WITH AZTREONAM FOR INHALATION SOLUTION (AZLI) IN THE SINGLE-ARM EXTENSION OF A RANDOMIZED TRIAL OF INHALED ANTIPSEUDOMONAL ANTIBIOTICS IN PATIENTS WITH CYSTIC FIBROSIS (CF) AND *PSEUDOMONAS AERUGINOSA* INFECTION

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Objectives: In a 6 month randomized, active comparator trial of three 28-day on/off courses of AZLI (Cayston®, Gilead Sciences) vs. tobramycin inhalation solution (TIS) in 268 CF patients (pts) with chronic *Pseudomonas aeruginosa* (PA) infection, AZLI was superior to TIS at 28 days and over 3 treatment courses in lung function (FEV1) improvements. A single-arm extension phase (EXT) of this study evaluated the safety and efficacy of AZLI for 3 additional 28-day on/off treatment cycles (ClinicalTrials.gov NCT00757237).

Methods: CF pts ≥6 yrs old with chronic PA infection, baseline FEV1 ≤75% predicted, and stable pulmonary disease were enrolled in the randomized, active comparator phase of the trial (RAND). EXT was open to European pts completing ≥1 course of AZLI or TIS during RAND. Pts were off antibiotics for 14-28 days prior to starting RAND. All pts in EXT received AZLI 75 mg TID via the Pari Investigational eFlow® Nebulizer System for 3 treatment courses (28 days on/28 days off). Mean % changes from RAND baseline in FEV1 % predicted at the end of each of the 3 EXT courses were calculated using a mixed-model repeated measures analysis.

Results: Of 174 European pts in RAND, 133 (76%) enrolled in EXT; 65 TIS-treated pts in RAND switched to AZLI in EXT (TIS/AZLI); 68 AZLI-treated pts in RAND continued AZLI in EXT (AZLI/AZLI).

Mean % changes from RAND baseline in FEV1 % predicted:

At RAND week 20 after 3 treatment courses: AZLI: +7.2% vs. TIS: -2.4%

At the end of each of the 3 AZLI treatment courses in EXT: TIS/AZLI: +3.9, +3.4 and +1.0;

AZLI/AZLI: +5.6, +3.7 and +4.2.

Body weight remained above baseline for AZLI/AZLI pts through all 6 AZLI treatment courses. TIS/AZLI pts had weight loss during RAND while on TIS, but gained and maintained weight above baseline values after switching to AZLI in EXT. No new safety issues were noted.

Conclusions: CF pts treated with AZLI demonstrated FEV1 improvements after each of 6 treatment courses. CF pts showed no FEV1 improvements after 3 courses of TIS but had favorable lung function responses after switching to AZLI in EXT. In addition, AZLI but not TIS treatment was associated with weight gain above baseline.

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BORDETELLA BRONCHISEPTICA IN A PEDIATRIC CYSTIC FIBROSIS CENTER

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Background: *Bordetella bronchiseptica (Bb)* is a gram-negative coccobacillus typically found in domestic animals such as dogs, horses and cats. It occurs as a commensal or pathogenic organism. In dogs, it causes an infectious tracheobronchitis commonly known as "kennel cough." Despite frequent contact with domestic animals, it is rarely reported in humans and typically occurs in immunocompromised hosts. The role of this organism as a pathogen or colonizer is poorly understood, in part due to a challenging and labor intensive process to isolate it. We describe isolation of *Bb* on twenty-three occasions in seven children with cystic fibrosis (CF). Our experience suggests that *Bb* may play a potential pathogenic role in CF.

Methods: A retrospective, descriptive study was conducted of our center's CF patient population between 1991 and 2011. The CF center currently cares for approximately 130 active patients per year. Patients were included if they had *Bb* isolated on one or more occasion. Patient charts were reviewed for demographics, CF diagnosis, lung function, nutritional status and exposure to domestic animals. In addition, data on previous or concurrent airway cultures, organism sensitivities, signs and symptoms of pulmonary exacerbations, hospitalizations, treatment, and response to therapy one year before and after first *Bb* isolation were reviewed.

Results: Seven patients (female N=5) between ages 1-20 with CF had *Bb* isolated in airway cultures on 23 occasions. Of these, four patients grew *Bb* once and three on multiple occasions. All patients had one copy of the ΔF508 mutation and four were homozygous ΔF508. On one occasion, *Bb* was initially identified as an organism called *CDC-IVc-2*, which is a short to medium rod that closely resembles *Bb*. Six patients experienced increased cough within one month prior to first isolation, and four had additional pulmonary symptoms. Four patients required hospitalization. All patients isolated methicillin-sensitive *Staphylococcus aureus (MSSA)* either historically or concurrently with *Bb*. None of our patients were colonized with *Pseudomonas aeruginosa* mucoid although two patients isolated *Pseudomonas aeruginosa* matte intermittently. All patients had documented contact with domestic animals. Two patients were exposed to animals with a veterinarian-diagnosed case of "kennel cough."

Conclusion: As successful targeted therapies improve the care of patients colonized with *Pseudomonas aeruginosa*, other potential pathogens have emerged. While its significance as a respiratory pathogen is unclear, our experience suggests *Bb* may act as a pathogenic organism in CF. Laboratory isolation and identification of *Bb* can be challenging, suggesting that it may be more common than previously reported. Close laboratory scrutiny of unusual isolates in culture is recommended. In addition, we suggest routine environmental history to include contact with and immunization of domestic animals that potentially carry *Bb*.