AN OBSERVATIONAL PHARMACOKINETIC STUDY OF CEFTOLOZANE-TAZOBACTAM IN INTENSIVE CARE UNIT IN PATIENTS WITH AND WITHOUT CRRT

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

APRIL 1, 2019
I. Population pharmacokinetics of unbound ceftolozane and tazobactam in critically ill patients without renal dysfunction

MATERIALS AND METHODS

Study design and setting. This prospective observational pharmacokinetic study was conducted at a quaternary referral intensive care unit (ICU) of the Royal Brisbane and Women’s Hospital (RBWH), Australia. Human research ethics committees of RBWH (HREC/16/QRBW/211) and the University of Queensland (No. 2016001368) granted ethical clearance.

Patients. ICU patients, aged ≥ 18 years, were enrolled if diagnosed with systemic infection known or suspected to be caused by a bacteria susceptible to ceftolozane/tazobactam. Patients were excluded if they had: renal dysfunction that necessitates the use of renal replacement therapy, known or suspected allergy to cephalosporins, received piperacillin/tazobactam in the preceding 7-days or if they were pregnant. Informed consent was obtained from each patient or their legally authorized representative.

Ceftolozane/tazobactam administration. At the discretion of the treating physician, the study participants received either 1.5 g or 3.0 g ceftolozane/tazobactam (2:1 ratio) administered every eight hours via intravenous infusion over 1 hour. The attending clinicians determined the duration of therapy based on the patients’ clinical scenario.

Sample collection. Blood samples (3 mL each) were collected in heparinized vacutainers from an established arterial line. The sampling times were as follows: first sample just prior to administration of the dose, second and third samples at 15 and 45 minutes, respectively, after
commencement of drug infusion, fourth sample at the end of line flushing (15 to 20 min) following the 1 h drug infusion, and then at 2, 3, 4, 5, 6, and 7h after the start of infusion and a final sample just before the second dose. The actual time of collection for individual samples was recorded and used for analysis. Blood samples were spun (3000 rpm for 10 minutes) immediately after collection to separate plasma, an aliquot of which was stored in -80 °C freezer until assayed by a validated chromatographic method.

Clinical data. An electronic case report form developed in REDCap web platform was used to collect clinical data including: - patient demographics; physical examination including vital signs; ICU and hospital admission and discharge dates and times; Acute Physiology and Chronic Health Evaluation II [APACHE II] score; Sequential Organ Failure Assessment [SOFA] score at ICU admission; presence of shock on days of sampling, presence of mechanical ventilation; renal function markers (serum creatinine concentrations, urinary creatinine clearance): hepatic function markers (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, international normalised ratio, bilirubin); medication list on days of sampling; antibiotic data including type, dose, dosing interval, duration of infusion, other antibiotics administered on day of sampling; and infection data (organisms isolated and sample type, minimum inhibitory concentration if available).

Ceftolozane/tazobactam assay. Unbound concentrations of ceftolozane and tazobactam in plasma were measured by a UHPLC-MS/MS method on a Shimadzu Nexera2 UHPLC system coupled to a Shimadzu 8050 triple quadrupole mass spectrometer (Kyoto, Japan). The unbound fraction of plasma was isolated by ultracentrifugation using Centrifree devices (Millipore, Tullagreen, Ireland). Sample (10 µL) was spiked with Phosphate Buffered Saline (pH 7.4) and internal standard (sulbactam & L-cefazolin) and acetonitrile. The stationary phase was C18
Ultra IBD, 100 x 2.1 mm, 3 μm column (Restek, Bellefonte, USA) operated at room temperature. Mobile phase A was 0.1% formic acid (v/v) in 10 mM ammonium formate, and mobile phase B was 100% acetonitrile with 0.1% formic acid (v/v). The mobile phase was delivered with gradient from 15% to 50% B at a flow rate of 0.3 mL/min for 5 min run-time and produced a backpressure of approximately 2800 psi. Ceftolozane was monitored by positive mode electrospray at MRM s of 667.00→199.15. Labelled cefazolin was monitored in positive mode at 457.85→326.05. Tazobactam and sulbactam were monitored by negative mode electrospray at MRM s 299.20→138.00 and 232.20→140.00, respectively. The assay method was validated using the FDA criteria for bioanalysis (1).

**Population pharmacokinetic modelling.** Population pharmacokinetic model was developed in R® using Pmetrics version 1.5.2. Unbound ceftolozane and tazobactam concentration-time data were modelled using non-parametric adaptive grid (NPAG) analysis in Pmetrics. Initially, one and two compartment structural base models were tested with either a multiplicative or additive error model. In addition assay error was modelled as a linear function of observations ([obs]) as Error= C0 + C1*[obs], where the coefficients C0 and C1 were optimized interactively. Covariate models were developed by progressively testing each plausible covariate relationship with primary model parameters following the standard forward-addition and backward-deletion approach.

Model evaluation and selection was based on assessment of diagnostic plots and statistics. Diagnostic plots included observed versus population or individual predicted concentrations, and normalised prediction distribution errors (NPDE) vs time or observation plots. Statistics included regression coefficient of observed vs predicted concentrations, bias (defined as the mean weighted error of predicted minus observed concentrations, Σ(predicted-observed/standard deviation)/N), imprecision (defined as the bias-adjusted, mean weighted
squared error of predicted minus observed concentration, i.e., $\Sigma[(\text{predicted-observed})^2/(\text{standard deviation})^2]/N - \Sigma(\text{predicted-observed})/\text{standard deviations}/N$, where $N$ is the number of observations/predictions) and objective functions including log-likelihood ratio (LLR) test for the nested models, Akaike information criterion (AIC), and Bayesian information criterion (BIC). The LLR chi-squared test was used for statistical comparison of nested models ($p < 0.5$ considered as significant).

**Dosing simulations.** Using the final covariate model, Monte Carlo dosing simulations ($n=1000$) were performed to determine the probability of target attainment (PTA) during the first 24 h and at steady state from 48 – 72 h post commencement of treatment. Simulated dosing regimens of ceftolozane/tazobactam (2:1 ratio) included 1.5g intermittent infusion (over 1 h) every eight hours (q8h), 1.5 g extended infusion (over 4 h) q8h, 1.5 g loading dose over 1 h plus 4.5 g continuous infusion over 24 h, 3g intermittent infusion (over 1 h) q8h, 3g extended infusion (over 4 h) q8h, and 3g loading dose over 1 h plus 9g continuous infusion over 24 h.

The primary pharmacokinetic (PK)/pharmacodynamic (PD) dosing target used for determination of PTA was $40\% f_{T > \text{MIC}}$. This is based on pre-clinical studies that showed 32.2 $\% f_{T > \text{MIC}}$ exposure achieves a 1-log kill (2), and 40 to 50$\% f_{T > \text{MIC}}$ is likely to achieve 1 to 2-log kill (3). In addition, we determined PTA for a higher exposure of 60$\% f_{T > \text{MIC}}$, which is generally consider optimal for cephalosporins (4) and a more aggressive exposure of 100$\% f_{T > \text{MIC}}$ advocated as a prudent target for severely ill patient populations (5).

The cumulative fractional response or fractional target attainment (FTA) was estimated for *Pseudomonas aeruginosa* EUCAST MIC distribution for both empiric and directed therapy using the equation 1.

$$FTA = \sum_{i=0.125}^{n} PTA_i \times F_i \quad \text{(Equation 1)}$$
where \( i \) is MIC category ranging from 0.125 to \( n \); \( n \) is 64 mg/L for empiric therapy and the EUCAST clinical breakpoint of 4 mg/L for directed therapy; \( PTA_i \), PTAs for MIC category \( i \); \( F_i \), the fraction of the bacterial population at each MIC category.

II. A population pharmacokinetic model-guided evaluation of ceftolozane/tazobactam dosing in critically ill patients undergoing continuous venovenous hemodiafiltration

Methods

Study design and setting: This was a prospective observational population pharmacokinetic study of ceftolozane/tazobactam in critically ill patients undergoing CRRT. The study was conducted at the University of Queensland Centre for Clinical Research. Patients were recruited from the Royal Brisbane and Women’s Hospital (RBWH) quaternary referral intensive care unit (ICU; RBWH (HREC/16/QRBW/211) and the University of Queensland (No. 2016001368) human research ethics committees provided ethical clearance.

Patients: Adult patients (≥18 years) admitted to RBWH ICU, who were prescribed CRRT were enrolled if diagnosed with systemic infection known or suspected to be caused by an organism susceptible to ceftolozane/tazobactam. Patients were excluded if pregnant or had a documented or suspected allergy to penicillins and cephalosporin. Each study participant or his or her next of kin provided informed consent prior to enrolment.

Ceftolozane/tazobactam dosing: Per protocol, all patients received 1.5 g ceftolozane/tazobactam (2:1 ratio) administered 8-hourly via intravenous infusion over 1 h. Any alternative initial dosing or dose adaptation deemed necessary by the attending clinicians was allowed.

CRRT procedures: The standard protocol for CRRT at the Royal Brisbane and Women Hospital was followed. The general CRRT modality at RBWH was CVVHDF using the Prismaflex® (Gambro, Lund, Sweden) hemodiafiltration machine with an AN69 ST150 or
ST100 (Gambro, Lund, Sweden) polyacrylonitrile filters (surface areas of 1.50 m² and 0.9 m² respectively). The dialysis and replacement fluid were either Hemofiltration Solution (HF1) (Gambro) or lactate-free Hemosol BO (Gambro). Replacement fluid was administered both pre- and post-filter, or pre-filter only. The blood flow rates were 100 to 200 mL/min. The dialysate flow rates were 1000 to 1500 mL/h. Replacement fluid rates were adjusted to each patient’s specific requirements.

Sample collection: Blood samples were collected pre and post filter during a dosing interval in lithium-heparin blood collection tubes. Pre-filter sampling times were, just before the dose, during ceftolozane/tazobactam infusion at 15 min and 45 min, 15 minutes after end of 1 h infusion, at 2 h, 3 h, 4 h, 5 h, 6 h, and 7 h post commencement of infusion, and at 8 h just before the next dose. Post-filter samples were collected at 45 min, 2 h and 6 h after the start of ceftolozane/tazobactam infusion. Ultrafiltrate samples from the effluent line were collected at 1 h, 2 h, 4 h, 6 h and 8 h post commencement of ceftolozane/tazobactam infusion. In addition, the ultrafiltrate volume in the effluent bag was measured at each of these time points with ultrafiltrate samples taken from the bag for drug concentration measurement.

Ceftolozane and tazobactam assay

Unbound concentrations of ceftolozane and tazobactam in plasma and renal replacement therapy effluent were measured by a UHPLC-MS/MS method on a Shimadzu Nexera2 UHPLC system coupled to a Shimadzu 8050 triple quadrupole mass spectrometer (Kyoto, Japan). The unbound fraction of plasma was isolated by ultracentrifugation using Centrifree devices (Millipore, Tullagreen, Ireland). Sample (10 µL) was spiked with Phosphate Buffered Saline (pH 7.4) and internal standard (sulbactam & L-cefazolin) and acetonitrile. The stationary phase was C18 Ultra IBD, 100 x 2.1 mm, 3 µm column (Restek, Bellefonte, USA) operated at room temperature. Mobile phase A was 0.1% formic acid (v/v) in 10 mM ammonium formate, and
The mobile phase B was 100% acetonitrile with 0.1% formic acid (v/v). The mobile phase was delivered with gradient from 15% to 50% B at a flow rate of 0.3 mL/min for 5 min run-time and produced a backpressure of approximately 2800 psi. Ceftolozane was monitored by positive mode electrospray at MRM of 667.00→199.15. Labelled cefazolin was monitored in positive mode at 457.85→326.05. Tazobactam and sulbactam were monitored by negative mode electrospray at MRM of 299.20→138.00 and 232.20→140.00, respectively. The calibration range for ceftolozane was 1 to 100 mg/L and for tazobactam was 0.5 to 100 mg/L. For ceftolozane at total concentrations of 160, 20 and 3 mg/L, the precision of the unbound analysis was 6.3, 6.2 and 8.2% with unbound fractions of 90%, 99% and 101%. For tazobactam at total concentrations of 80, 10 and 1.5 mg/L, the precision of unbound analysis was 6.2, 7.5 and 8.1% with unbound fractions of 89, 91 and 92%. The assay method was validated using the FDA criteria for bioanalysis (1).

**Pharmacokinetic analysis**

Initially non-compartmental analysis was performed to set the initial boundaries for relevant model parameters during subsequent population pharmacokinetic modelling. The extraction ratio (ER), sieving coefficient (SC), and extracorporeal clearance by the CVVHDF machine (\(CL_{CVVHDF}\)) were determined using the equations below based on observed concentrations:

\[
(\text{Extraction Ratio}) = \frac{\text{Concentration in postfilter blood sample}}{\text{Concentration in prefilter blood sample}}
\]

Equation 1

\[
(\text{Sieving Coefficient}) = \frac{\text{Effluent drug Concentration}}{\left[\left(\frac{\text{Prefilter plasma concentration}}{\text{Prefilter plasma concentration}}\right) + \left(\frac{\text{Postfilter plasma concentration}}{\text{Postfilter plasma concentration}}\right)\right]/2}
\]

Equation 2

\[
CL_{CVVHDF} = \frac{A_{CVVHDF}}{AUC_{0-8}}
\]

Equation 3
Where $A_{CVVHDF}$ is the total amount of ceftolozane or tazobactam recovered in the ultrafiltrate and $AUC_{0-8}$ is the area under the ultrafiltrate concentration-time curve determined by the linear trapezoidal rule.

Subsequently, a non-parametric population pharmacokinetic analysis was performed in R® using the Pmetrics® user interface to describe the unbound concentration-time profiles from pre-filter plasma, post-filter plasma and CVVHDF ultrafiltrate samples simultaneously. Three and four compartment models with first order CVVHDF and residual non-CVVHDF clearance were tested. CVVDHF clearance was from the compartment representing pre-filter samples. Residual clearance was tested on both compartments representing post- and pre-filter samples. All between compartment distributions were modelled as linear processes. Error models were based on standard deviation (SD) of observations [obs] available in Pmetrics as additive (Error= $[SD^2 + \lambda^2]^{0.5}$) and multiplicative (Error=SD*$\gamma$) models, where $\lambda$ and $\gamma$ represent process noise. In addition assay error was modelled with a first-degree polynomial function (Error= C0 + C1*[obs]). Plausible clinical covariates were tested on residual non-CVVHDF clearance, inter-compartmental clearances and volumes of pre and post filter compartments. Available covariates considered for analysis include sex, height, weight, body max index, body surface area, albumin concentration, serum creatinine, Sequential Organ Failure Assessment (SOFA) score, Acute Physiology and Chronic Health Evaluation (APACHE) II score, dialysate flow rate, transmembrane pressure, filter type, and blood flow rate.

Models were evaluated by the combination of diagnostic goodness of fit plots and statistics. Diagnostic plots included scatter plots of observed-versus-predicted concentrations, visual predictive check plots and normalised prediction distribution error (NPDE) versus time and output plots. Statistical evaluation of observed-versus-predicted concentrations was based on regression coefficient $r^2$, bias and imprecision. In Pmetrics, bias is defined as the mean
weighted error of predicted minus observed concentrations, \( \Sigma \frac{(predicted-observed)}{(standard\ deviation)}/N \), and imprecision is defined as the bias-adjusted, mean weighted squared error of predicted minus observed concentration, i.e., \( \Sigma \frac{(predicted-observed)^2}{(standard\ deviation)^2}/N \) - \( \Sigma (predicted-observed)/standard\ deviations/N \), where N is the number of observations/predictions. In addition, statistical model evaluation was performed based on objective function values including log-likelihood ratio (LLR), Akaike information criterion (AIC), and Bayesian information criterion (BIC). The LLR chi-squared test within Pmetrics was used for statistical comparison of nested models (p < 0.05 considered as significant).

The final model was used to perform Monte Carlo dosing simulations (n=1000) and assess the probability of target attainment (PTA) and extent of accumulation for selected dosing regimens. Simulated regimens included 0.75 g, 1.5 g and 3.0 g ceftolozane/tazobactam (2:1 ratio) administered by 1 h intermittent infusion every eight hours (q8h), by 4 h extended infusion q8h and by continuous infusion (CI) of the total daily dose following a single loading dose (LD) given over 1 h. Additional dosing regimens simulated included, a front loaded intermittent regimen of 1.5g q8h for 24h followed by 0.75g q8h, and a single 3.0g LD followed by 0.75g q8h. For ceftolozane, the primary target for PTA assessment was 40% \( fT_{>MIC} \), which is considered adequate for 1to 2 log kill (2, 3). Secondary targets studied include 60 and 100 % \( fT_{>MIC} \). For tazobactam, on the other hand, we assessed against previously suggested targets of 20 % \( fT_{>1mg/L} \) (20% of the time above minimum effective concentration of 1mg/L) (6), and 50 % \( fT_{>2mg/L} \) (7). In addition, given in vitro susceptibility of beta-lactam/tazobactam combination is tested fixing tazobactam concentration at 4 mg/L (8), we assessed attainment of 100% \( fT_{>4mg/L} \). Pre-filter patient plasma exposure was used for all PTA assessments.

Fractional target attainment (FTA) was estimated for ceftolozane, using *Pseudomonas aeruginosa* EUCAST MIC distribution (accessed August 2019), for both empiric and directed
therapy. An FTA value of ≥ 85% considered optimal. The following equation 4 was used for FTA calculation.

\[
FTA = \sum_{i=0.125}^{n} PTA_i \times F_i \quad \text{(Equation 4)}
\]

Where \(i\) is MIC category ranging from 0.125 to \(n\); \(n\) is 64 mg/L for empiric therapy and the EUCAST clinical breakpoint of 4 mg/L for directed therapy; \(PTA_i\), PTA at each MIC category; \(F_i\), the fraction of the bacterial population at each MIC category.
REFERENCES
