Intrapulmonary Pharmacokinetics of Cefepime and Enmetazobactam in Healthy Volunteers: Towards New Treatments for Nosocomial Pneumonia

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ABSTRACT

Cefepime-enmetazobactam is a novel ß-lactam- ß-lactamase inhibitor combination with broad spectrum antimicrobial activity against a range of multi-drug resistant Enterobacteriaceae. This agent is being developed for a range of serious hospital infections. An understanding of the extent of partitioning of both ß-lactam- ß-lactamase inhibitor into the human lung is required to better understand the potential role of cefepime-enmetazobactam for the treatment of nosocomial pneumonia. A total of 20 healthy volunteers were used to study the intrapulmonary pharmacokinetics of a regimen of cefepime-enmetazobactam 2g/1g q8h i.v. Each volunteer contributed multiple plasma samples and a single epithelial lining fluid (ELF) sample obtained by bronchoalveolar lavage. Concentrations of cefepime and enmetazobactam were quantified using LC-MS/MS. The pharmacokinetic data was modelled using a population methodology and Monte Carlo simulations were performed to assess the attainment of pharmacodynamic targets defined in preclinical models. The concentration-time profiles of both agents in plasma and ELF were similar. The mean ± standard deviation percentage partitioning of total drug concentrations of cefepime and enmetazobactam between plasma and ELF was 60.59 ± 28.62 and 53.03 ± 21.05 %, respectively. Using pharmacodynamic targets of cefepime >MIC and free enmetazobactam concentrations >2 mg/L in ELF of 20% of the dosing interval, a regimen of cefepime-enmetazobactam 2 grams/0.5 grams q8h i.v. infused over 2 hours resulted in a probability of target attainment of ≥90% for Enterobacteriaceae with cefepime-enmetazobactam MICs ≤8 mg/L. This result provides a rationale to further consider cefepime-enmetazobactam for the treatment of nosocomial pneumonia caused by multidrug resistant Enterobacteriaceae.

INTRODUCTION

Cefepime-enmetazobactam is a new ß-lactam-ß-lactamase inhibitor combination with broad-spectrum activity against multi-drug resistant Enterobacteriaceae. Enmetazobactam has potent activity against extended spectrum ß-lactamases (ESBLs) (1) and cefepime is stable against hydrolysis by OXA-48 and AmpC ß-lactamases (2). Together, this combination has demonstrated potent activity against Enterobacteriaceae expressing ESBLs, OXA-48, and/or AmpC (1, 3). Carbapenems are frequently used as the agent of choice for the treatment of ESBL producing Enterobacteriaceae and have recently been demonstrated to be superior to piperacillin-tazobactam in terms of 28-day mortality in patients with bacteremia (4). ESBL-producing Enterobacteriaceae account for 31% of culture positive cases of nosocomial pneumonia in a recent clinical study (5). The carbapenems are agents of last resort and are therefore a critically important resource for healthcare systems throughout the world. New agents that can be used as carbapenem-sparing strategies are urgently required (6).

Cefepime-enmetazobactam has recently completed a pivotal trial in patients with complicated urinary tract infection (cUTI; https://clinicaltrials.gov/ct2/show/NCT03687255; accessed 18th June 2020). In this ALLIUM Phase III trial, cefepime-enmetazobactam demonstrated superiority over piperacillin-tazobactam at the primary efficacy endpoint defined as clinical cure and microbiological eradication at test-of-cure in the mMITT population (7).

Nosocomial pneumonia is a common and frequently lethal disease with a crude mortality rate of 25-50%. The 28-day mortality in a recent Phase III clinical trial comparing ceftolozane-tazobactam and meropenem was 24 and 25.3%, respectively (5). The clinical

response after the completion of therapy is approximately 50% (5). Suboptimal clinical outcomes are driven by underlying critical illness, relatively more resistant invasive pathogens, greater overall pharmacokinetic variability and high variability of partitioning of drug to the effect site (8). Furthermore, for some agents, such as daptomycin, there may be idiosyncrasies of activity in the lung that render those agents less effective for the treatment of pneumonia (9). Hence, specific preclinical and clinical studies are required to establish the efficacy and regimen that is likely to be effective for patients (8).

Assessment of drug partitioning into epithelial lining fluid of the human lung along with a compelling PK-PD rationale is a critical step for developing new antibiotics for pneumonia (10). This was the basis for the initial approval of ceftazidime-avibactam for the treatment hospital-acquired pneumonia (HAP) including ventilator associated pneumonia (VAP) prior to completion of Phase III trial for this indication (11). Meropenem-vaborbactam was approved by EMA for use in HAP including VAP based on a statistically powered Phase III trial patients with cUTI including pyelonephritis and a smaller open-label trial which included patients with HAP/VAP (https://www.ema.europa.eu/en/documents/product-information/vaborem-epar-product-information_en.pdf; accessed 25th June 2020).

Preclinical PK-PD studies in the thigh and lung model have been recently published for cefepime-enmetazobactam (12, 13). In the latter, pharmacodynamic targets in plasma and ELF that resulted in various orders of logarithmic killing in the lung were determined (13). The primary purpose of this study was to estimate the extent of partitioning of cefepime-enmetazobactam into ELF in healthy volunteers to help identify a regimen for nosocomial pneumonia.

RESULTS

Demographics and Volunteer Details

The demographics of the 20 volunteers enrolled in this study are summarized in Table 1. One volunteer (female, 23 years, 60.6 kg, BMI 20.7 kg/m^2) could not tolerate bronchoscopy and was therefore excluded from the study. A total of 19 volunteers with complete plasma PK and ELF samples were available for analysis and the development of a population PK model. However, all 20 volunteers were included for reporting of safety.

Safety of Cefepime-Enmetazobactam

Cefepime-enmetazobactam was well tolerated. There were no serious adverse events (SAE). None of the treatment emergent adverse events (TEAEs) led to discontinuation of study drugs. All adverse events spontaneously resolved without sequelae.

A total of 59 adverse events were reported in 18 (90%) volunteers. Of these 59 adverse events, 57 events were reported in 18 (90%) volunteers were TEAEs, and 20 reported in 8 (40%) volunteers were TEAEs that were judged to be causally related to the study drug. None of the TEAEs were of severe intensity. A total of 54 TEAEs were mild. A total of 3 TEAEs reported in 2 (10%) volunteers (1 case of increased blood pressure reported by one volunteer, 1 case of hypotension and 1 case of presyncope reported by another volunteer) were rated as moderate. None of these 3 events was a drug related-TEAE.

The 20 drug-related-TEAEs were: cannula site pain (n=2), increased alanine aminotransferase (n=2), dizziness (n=2), nausea (n=2), thrombophlebitis (n=2), headache (n=2; i.e., 2 occurrences in one volunteer), palpitations (n=2; 2 occurrences in one volunteer),

diarrhoea (n=1), discoloured stools (n=1), dissociation (n=1), elevated creatinine kinase (n=1), rash (n=1), and urine odour abnormal (n=1).

Pharmacokinetics of Cefepime and Enmetazobactam

The plasma and ELF pharmacokinetics are shown in Figure 1. The shape of the ELF PK profile was comparable to the shape of the plasma concentration time profile for both cefepime and enmetazobactam. Concentrations of both cefepime and enmetazobactam were detectable in plasma for 24 hours after the last administration of drug (i.e., in the window 64-88 hours post study initiation). There was no sampling of ELF after 72 hours (i.e., 8 hours after the final administration of drug).

Population Pharmacokinetic Modeling

The fit of a three-compartmental population PK model (representing central, peripheral and ELF compartments) to the total drug concentration-time data was acceptable for both drugs in plasma and ELF. The observed-predicted plots after the Bayesian step and using the median parameter estimates are shown in Figure 2. For each drug and output, a linear regression of observed-predicted values had an intercept and slope that approximated 0 and 1, respectively. The coefficient of determination for plasma and ELF was an r^2 of >97% for both drugs, and outputs there were acceptable measures of bias and imprecision.

Measures of central tendency for each parameter and their estimated dispersions are summarized in Table 2. The full covariance matrix is supplied in supplementary data (Table S1). The $AUC_{64\text{-}88}$ (i.e., AUC_{ss} determined on day 5 of dosing) in both plasma and ELF was

142 calculated from the Bayesian posterior estimates from each volunteer, which were estimated
143 in Pmetrics using the trapezoidal rule. The mean ± standard deviation percentage
144 partitioning of both cefepime and enmetazobactam between plasma and ELF (i.e., $AUC_{64\text{-}88}$
145 plasma: $AUC_{64\text{-}88}$ ELF) was 60.59 ± 28.62 and 53.03 ± 21.05 %, respectively. These estimates
146 were based on measured total drug concentrations in plasma and ELF.

147

148 *Assessment of Model Performance*

149 A visual predictive check showed the majority of observations were contained within
150 the 5^{th} and 95^{th} centiles of the simulated population that was constructed based on the
151 healthy volunteers receiving the same regimen as had been used for the volunteers
152 (i.e., cefepime/ enmetazobactam of 2g/1g q8h i.v. infused over a 2-hour period), suggesting
153 that the simulation recapitulated the starting population (Figure 3). The full covariance
154 matrix was used for the Monte Carlo simulations to account for any potential covariance
155 between the PK of cefepime and enmetazobactam.

156

157 *Relationship Between Drug Exposure in Plasma and ELF*

158 An assessment for the extent of correlation between measures of drug exposure for
159 cefepime and enmetazobactam in plasma and ELF was performed (Figure 4). Plasma AUC
160 does not correlate in a statistically significant manner with ELF AUC in human volunteers. This
161 could be due to the relatively low number of observations. In contrast, however, the
162 estimates for plasma exposure for cefepime and enmetazobactam were strongly correlated
163 (r=0.642, p<0.01, n=19) and this relationship was even stronger for ELF (r=0.916, p<0.001,
164 n=19), suggesting that the two test items may have similar pharmacokinetic properties.

165

166 *Probability of Target Attainment*

167 A Monte Carlo simulation was performed using the regimen that has been recently
168 studied in a Phase III clinical trial for patients with cUTI and that is proposed for cefepime for
169 use in nosocomial pneumonia (i.e., cefepime/ enmetazobactam 2g/0.5 g q8h i.v. infused over
170 a 2-hour period). The ELF pharmacodynamic targets from a murine model of pneumonia
171 were used for these calculations that induced a ≥2 log drop (13). These were 20% *f*T>MIC for
172 cefepime in ELF, and 20% *f*T>2 mg/L in ELF for enmetazobactam. The joint probability of
173 target attainment in ELF was near 100% for isolates with an MIC ≤ 4 mg/L. The was a
174 probability of joint target attainment (PTA) of 94.4% and 78.1% for an MIC of 8 mg/L and 16
175 mg/L, respectively. Using a 90% joint PTA as an endpoint provided a pharmacodynamic
176 rationale for setting breakpoint of susceptible 8 mg/L and resistant >8 mg/L.

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DISCUSSION

This study provides one of the key pieces underpinning evidence for the potential role of cefepime-enmetazobactam for patients with nosocomial pneumonia. There is a strong preclinical rationale from neutropenic murine models of thigh infection and pneumonia for the clinical efficacy of cefepime-enmetazobactam. The EMA has indicated that new β-lactamase inhibitors, when combined with approved β-lactam antibiotics, can be potentially approved for use in nosocomial pneumonia based on demonstrated clinical efficacy, a PK-PD rationale and evidence of adequate partitioning into the epithelial lining fluid in volunteers. The current study addresses the latter.

Both cefepime and enmetazobactam partition into epithelial lining fluid in a similar way as estimated according to total drug AUC_{plasma}: AUC_{ELF}, and a visual inspection of the concentration-time profile of both agents. This significantly simplifies the selection of candidate regimens for pneumonia, especially for agents that exhibit time-dependent pharmacodynamics where both agents must be present at the effect site to derive efficacy—there is nothing to be gained by having high concentrations of the ß-lactamase inhibitor when there is no cefepime to protect. Similarly, if cefepime is not protected by a ß-lactamase inhibitor it is susceptible to hydrolysis by ß-lactamases. The schedule of drug administration used in this study and the Monte Carlo simulations suggest the proposed regimen for pneumonia (i.e., cefepime/ enmetazobactam 2g/0.5g i.v. q8h infused over 2-hours) achieves drug exposure targets that result in orders of logarithmic killing in well-characterized murine models of pneumonia across a wide range of MICs.

The importance of considering the full covariance matrix by fitting the PKs from both agents simultaneously is highlighted by the extraordinarily high degree of correlation

between AUC in plasma and ELF for both agents. The use of a full covariance matrix enables the pharmacokinetic extremes to be captured and the implications for attainment of desired drug exposures explored. Covariance that results in either concordant or discordant drug exposure may be missed if the PKs are considered as independent events—they clearly are not. The underlying biological reason for the correlation is uncertain, but perhaps suggests that both agents are actively distributed into the ELF. Whether this is true requires further study. There is still little information on the active processes that may be responsible for movement of drug from plasma to ELF and even less on the impact of infection and inflammation on these mechanisms.

The risks of misidentification of an optimal regimen of cefepime-enmetazobactam for nosocomial pneumonia is relatively low but deserve further discussion. First, the preclinical murine targets that have been used are based on those that results in ≥2 logs of kill relative to stasis in the mouse. This exceeds the 1-log kill targets achieved in experiments that determined the targets for ceftazidime-avibactam and ceftolozane-tazobactam (14, 15). These preclinical murine targets were determined using murine PK with an underlying assumption that the conversion of pharmacodynamic index corrects for discordant PK profiles in mice and humans. At the extremes of pharmacokinetics this assumption may break down. Secondly, this study used healthy volunteers rather than patients. Partitioning of cefepime into the lung of critically ill patients has been previously described (16, 17). The point estimates for the PK parameters may be different from patients and the patterns of drug partitioning may also be different (18). Almost certainly there will be less variability. We did not artificially inflate the variance in the simulations, but this is possible. Higher CV% for clearance and volume in patients compared with volunteers generally results in a proportional change in the CV% of drug exposure and generally costs 1-2 MIC dilutions in

coverage. Hence the pharmacodynamic breakpoint (i.e., the MIC at which the probability of target attainment is >90%) may fall from 8 mg/L to 4 mg/L. Thirdly, there was no assessment in this study or that considered the emergence of resistance, which may be an issue in pneumonia where the bacterial burden typically exceeds the mutational frequency of resistance. Finally, the dosages used in the healthy volunteer study and those proposed for use in nosocomial pneumonia are different. An assumption has been made that the pharmacokinetics in ELF is linear, whilst the linearity has been confirmed for pharmacokinetics in plasma. Despite these limitations, this study provides a solid pharmacodynamic rationale to consider the use of cefepime-enmetazobactam 2g/0.5g q8h i.v. for nosocomial pneumonia.

METHODS

Volunteers

This study was approved by the North West-Greater Manchester Centre Research Ethics Committee (17/NW/0171). A total of 20 healthy volunteers were enrolled at the Royal Liverpool Hospital Clinical Research Unit, which is a Medicines and Healthcare Regulatory Agency (MHRA) accredited Phase I unit.

Volunteers from the safety analysis set were males (n=9, 45%) and females (n=11, 55%) aged between 19 and 64 years, with a mean (SD) age of 32.8 (15.2) years (Table 1). Among them, 12 (60%) were never-smokers or had never used nicotine containing products and 2 volunteers (10%) never drank alcoholised beverages. Volunteers had a body mass index (BMI) that ranged between 21 and 32 kg/m² (median: 25.3 kg/m²). They had a prior history of skin or cutaneous disorders (50%), surgical or medical history (40%), psychiatric disorders (35%), infections or infestations (30%). The most frequently reported prior medications belonged to the following Anatomical Therapeutic Chemical (ATC) classes: sex hormones and modulators of the genital system (25%), analgesics (20%), other gynaecological drugs (15%), and vitamins (10%).

One volunteer could not tolerate bronchoscopy and was removed from the study. Two cohorts were used with an interim analysis performed after n= 10 volunteers to ensure sampling times for plasma and ELF were appropriate.

Drugs

Cefepime (Bristol-Myers Squibb, München) powder was stored at room temperature and was reconstituted with 20 mL saline in a 2 g-containing vial. Further dilutions were made in saline. Enmetazobactam powder was stored at -20°C and was reconstituted with 5 mL saline in a 500 mg-containing vial. Further dilutions were made in saline. All volunteers received 2 grams of cefepime infused IV over 2 hours and 1 gram of enmetazobactam infused over 2 hours. Dose formulations were stored at 4°C for the length of the study (no longer than 24 hours). The regimen that was chosen for the current study occurred when there was debate about the most appropriate dose of enmetazobactam for serious infections (i.e., 0.5 g q8h versus 1 g q8h i.v.). Ultimately, a lower dose (i.e., 0.5 g q8h i.v.) was chosen for the Phase III study

Pharmacokinetic Sampling

Both cefepime and enmetazobactam were administered q8h i.v. A single fixed regimen of 2 g cefepime and 1 g enmetazobactam was simultaneously administered on a q8h schedule by 2-hour infusion with sampling after the 9th dosage—i.e., from 64 hours post study initiation and administration of the first dose. Plasma samples were obtained at 65, 66, 66.5, 67, 68, 70, 72, 76, 80, and 88 hours post dosing (i.e., 1, 2, 2.5, 3, 4, 6, 8, 16- and 24-hours post dose) in each volunteer. A single bronchoalveolar lavage fluid (BAL) supernatant sample was obtained per volunteer at 66, 68, 70, or 72 hours (i.e., 2, 4, 6, 8 hours post dose) post study initiation. The dilution of ELF was corrected using the ratio of urea concentrations in plasma and the lavage fluid. The PK sampling period lasted from the time of last episode of drug administration to the end of study, which was 64-88 hours, respectively.

Blood samples (approximately 1 mL) were collected from all individual test volunteers for quantitation of cefepime or enmetazobactam plasma concentrations and subsequent population PK analysis. Whole blood was collected by venipuncture into heparinized syringes. Whole blood was then placed into Eppendorf tubes, centrifuged and the plasma supernatant was removed. Plasma was stored at -80°C until bioanalysis (cefepime or enmetazobactam plasma concentration analysis) was performed. Both drugs were demonstrated to be stable in plasma stored at -80°C for at least 6-months.

Bronchoscopy

Bronchoscopy with bronchoalveolar lavage was performed once for each volunteer and was planned at one of 4 predefined time-points within the 9th dosing interval (time 64-72 hours post study initiation). The target times were 2 hours, 4 hours, 6 hours, and 8 hours after the final dosage at 64 hours post treatment initiation. Five volunteers were studied at each BAL time point (although one volunteer could not tolerate bronchoscopy).

The exact time point corresponding to saline installation and aspiration was recorded. Volunteers fasted for a minimum of 4 hours prior to bronchoscopy. Midazolam (i.v.) was used to achieve the appropriate level of sedation to enable bronchoscopy. Lignocaine spray and/or jelly was applied to the oropharynx and nasal passageway, respectively. Further anaesthesia of the bronchi and vocal cords and was achieved with 1% and 2% lignocaine, respectively.

Four aliquots of 50 mL of warmed sterile normal saline (0.9% w/v) were instilled into the right middle lobe. After each aliquot, gentle suction was used to aspirate dwelled fluid and placed on ice. All BAL aspirates were pooled, and the total volume recorded. The pooled

304 sample was centrifuged at 400 x g for 5 minutes and the supernatant removed. Two 3 mL
305 aliquots of supernatant were placed in separate tubes for bioanalysis of cefepime and
306 enmetazobactam along with estimation of urea concentrations. All samples were frozen and
307 stored at -70°C. Measured ELF concentrations of cefepime and enmetazobactam were
308 corrected for dilution induced by BAL using the ratio of urea concentrations in plasma and
309 BAL. This dilution factor was used to "correct" the measured concentrations of cefepime and
310 enmetazobactam.

311 *Measurement of Cefepime and Enmetazobactam by LC-MS/MS in Human Plasma*

312 Cefepime was extracted from 25 µL of human plasma by protein precipitation using
313 acetonitrile containing $^{13}C^{2}H_3$-cefepime as isotopic labelled internal standard and the MRM
314 transition values for cefepime and the internal standard were m/z 481→125 and m/z
315 485→125, respectively. Enmetazobactam was extracted from 20 µL of human plasma by
316 protein precipitation using acetonitrile containing an isotopically labelled internal standard
317 ([$^{2}H_3$]- enmetazobactam), the MRM transition values were m/z 315→84 for enmetazobactam
318 and m/z 318→87 for the internal standard.

319 Concentrations of cefepime and enmetazobactam in human plasma were measured
320 using a Waters UPLC system coupled with an API4000 in tandem mass spectrometry mode
321 (LC-MS/MS). The chromatography was performed for cefepime using gradient elution on a
322 BETASIL Phenyl-Hexyl (50*2.1 mm, 3.0 µm; Thermo) and for enmetazobactam, isocratic
323 elution was achieved using an Atlantis HILIC column (50*2.1, 3 µm; Waters). The dynamic
324 range for cefepime and enmetazobactam was 0.5-500 mg/L and 0.05-50 mg/L, respectively.
325 The coefficient of determination for a linear regression of the standard curve was >0.99 for

both analytes. The inter-run precision was 8.8% and 3.5% for cefepime and enmetazobactam, respectively.

Measurement of Cefepime and Enmetazobactam by LC-MS/MS in Human ELF

Concentrations of cefepime and enmetazobactam in human BAL were measured using a Waters I-Class UPLC system coupled with a Xevo TQ-S in tandem mass spectrometry mode (LC-MS/MS). For both analytes, the chromatography was performed in isocratic elution on a BEH HILIC (50*2.1 mm, 1.7 µm; Waters) and PBS containing 1% BSA was used as a surrogate matrix for the preparation of calibration standard and quality control samples. The compounds were extracted from 20 µL of samples by protein crash using acetonitrile containing the respective labelled internal standard. The MRM transition values were m/z 481→125 and m/z 485→125 for cefepime and its internal standard ($[^{13}C^{2}H_3]$-cefepime), respectively, and m/z 315→84 for enmetazobactam and m/z 318→87 for its internal standard ($[^{2}H_3]$-enmetazobactam). The dynamic range for both agents was 0.01-10 mg/L. The coefficient of determination for a linear regression of the standard curve was >0.99 for both analytes. The inter-run precision was 1.2% and 5.9% for cefepime and enmetazobactam, respectively.

Measurement of Urea by LC-MS/MS

Urea concentrations were measured in human plasma and human epithelial lining fluid following modification of a previously described method (19). A Waters I-Class UPLC system coupled with a Xevo TQ-S in tandem mass spectrometry mode (LC-MS/MS) was used. In both matrices, the chromatography was performed in isocratic elution on a BEH HSS T3

(50*2.1 mm, 1.8 µm; Waters) and calibration curve and quality control samples were prepared in the respective matrix. A protein crash was achieved with acetonitrile followed by a derivatization with camphanic chloride. [$^{13}C^{15}N_2$]-urea was used as the internal standard. The MRM transition values were m/z 241→109 and m/z 244→109 for urea and the internal standard, respectively. The dynamic range was 5-5000 mg/L and 0.5-50 for human plasma µg/mL and BAL, respectively. The coefficient of determination for a linear regression of the standard curve was >0.99 in both matrices. The inter-run precision was 2.5% and 4.8% in plasma and ELF, respectively.

Population PK Modelling

The PK data from cefepime and enmetazobactam in plasma and ELF were co-modeled in Pmetrics (20) to identify any potential covariance for the PKs of the two agents. There was no implicit assumption of a PK interaction, but the co-modelling enabled possible covariances between the agents to be captured and be available for subsequent Monte Carlo simulation. Total drug concentrations were modelled without correction for protein binding. The estimated protein binding for enmetazobactam is 0% in human and mouse plasma (21). Similarly, the estimated protein binding for cefepime is 20 and 0% in human and mouse plasma, respectively (22, 23). For measurements beneath the limit of quantification in plasma a value half-way between zero and the lower limit of quantification were used (i.e., 0.5 and 0.025 mg/L for cefepime and enmetazobactam, respectively).

The following structural model was fitted to the total drug concentrations for both cefepime and enmetazobactam in plasma and ELF:

For cefepime:

$$XP(1)=R(1)-(SCLcef/Vcef)*X(1)-K12*X(1)+K21*X(2)-K13*X(1)+K31*X(3) \quad \text{Equation 1}$$

$$XP(2)=K12*X(1)-K21*X(2) \quad \text{Equation 2}$$

$$XP(3)=K13*X(1)-K31*X(3) \quad \text{Equation 3}$$

For enmetazobactam:

$$XP(4)=R(2)-(SCLenm/Venm)*X(4)-K45*X(4)+K54*X(5)-K46*X(4)+K64*X(6) \quad \text{Equation 4}$$

$$XP(5)=K45*X(4)-K54*X(5) \quad \text{Equation 5}$$

$$XP(6)=K46*X(4)-K64*X(6) \quad \text{Equation 6}$$

Equation 1, 2 and 3 describe the rate of change of the mass of cefepime in the central, peripheral and ELF compartments, respectively. Similarly, Equation 4, 5, and 6 describe the rate of change of the mass of enmetazobactam in the central, peripheral and ELF compartments, respectively. R(1) and R(2) is the infusion of cefepime and enmetazobactam into the bloodstream (central compartment), respectively. SCLcef and SCLenm is the first-order clearance of cefepime and enmetazobactam from the central compartment, respectively; Vcef and Venm is the volume of the central compartment for cefepime and enmetazobactam, respectively; K with the appropriate subscript represent the first order intercompartmental rate constants. XP(1), XP(2) and XP(3) represent the rate of change of cefepime (mass; mg) in compartments 1, 2 and 3, which represent the central, peripheral and ELF compartments, respectively. XP(4), XP(5) and XP(6) represent the rate of change of

enmetazobactam (mass; mg) in compartments 4, 5 and 6 , which represent the central, peripheral and ELF compartments, respectively.

There were 4 output equations to describe the concentrations in plasma and ELF of cefepime (equations 1 and 2, respectively) and for enmetazobactam in plasma and ELF (equations 3 and 4 respectively).

Output Equations

$$Y(1) = X(1)/Vcef \quad \text{Equation 7}$$

$$Y(2) = X(3)/Vcef\_elf \quad \text{Equation 8}$$

$$Y(3) = X(4)/Venm \quad \text{Equation 9}$$

$$Y(4) = X(6)/Venm\_elf \quad \text{Equation 10}$$

The output equations contained two additional parameters that were estimated that were not contained within the ordinary differential equations. Vcef_elf and Venm_elf are the volume of the ELF compartment for cefepime and enmetazobactam, respectively. The observed data were weighted by the estimated assay variance in plasma and ELF for both cefepime and enmetazobactam. Given the complexity of the base structural model, the number of primary parameters to be estimated and the relatively small sample size, covariate building was not attempted.

Bridging and Monte Carlo Simulation

Monte Carlo simulations were performed with Pmetrics (20). The full covariance matrix (Supplementary Table 1) was used for both cefepime and enmetazobactam to enable PK parameters that may co-vary to do so. The candidate clinical regimen for nosocomial pneumonia that was explored in the simulations was cefepime/ enmetazobactam 2g/ 0.5g. A total of 1000 simulated patients were generated. Assessments for target attainment were performed between 64 and 72 hours post start of therapy. Targets for success were set for cefepime and enmetazobactam using free drug at *f*T>MIC in ELF of 20% and at *f*T>2 mg/L in ELF of 20%, respectively, which was based on a recently published murine model of pneumonia that defined dual pharmacodynamic targets in ELF (13). This drug exposure results in ≥2 log decline in bacterial burden in the murine lung relative to stasis (13). Measured drug in ELF was assumed to be 100% free (i.e., there was no protein binding). The requirement to simultaneously achieve both targets to define success was required because enmetazobactam has no intrinsic activity and no antibacterial activity in the absence of cefepime. The rate of success was assessed across a range of MICs (0.125-16 mg/L). The distribution of those MICs for cefepime-enmetazobactam against 102 ESBL-producing *Klebsiella pneumoniae* obtained from a study of Morrissey et al (3) was used.

ACKNOWLEDGMENTS

This work was funded by Allecra Therapeutics. The authors thank the NIHR North West Coast Local Clinical Research Network. Shampa Das holds or has recently held research grants with Spero Therapeutics, Antabio, Allecra, Bugworks, and NAEJA-RGM and award from the European Commission. Shampa Das has received personal fees in her capacity as a consultant CARBX, Spero Therapeutics, Wellcome Trust and Centauri Therapeutics. William Hope holds or has recently held research grants with F2G, Astellas Pharma, Spero Therapeutics, Antabio, Allecra Therapeutics, Bugworks, and NAEJA-RGM. He holds awards from the Medical Research Council, National Institute of Health Research, FDA and the European Commission. William Hope has received personal fees in his capacity as a consultant for F2G, Amplyx, Spero Therapeutics, Pfizer and BLC/TAZ.

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Demographic	Mean	Standard Deviation
Sex	Male 45%	-
Age	32.8	15.16
Weight	77.18	11.60
Height (cm)	173.85	8.31
Body Mass Index	25.49	3.32

Table 1. Demographic details of the 20 volunteers included in this study.

549

550 Table 2.

551

Parameter[a](Units)	Mean	Median	SD
SCLcef (L/h)	7.969	7.116	2.190
Vcef (L)	5.414	5.065	2.692
K_{12} (h^{-1})	9.645	8.856	6.001
K_{21} (h^{-1})	7.305	5.105	6.498
K_{13} (h^{-1})	12.444	9.364	6.049
K_{31} (h^{-1})	15.732	15.277	7.577
SCLenm (L/h)	7.822	7.670	1.335
Venm (L)	4.422	4.101	2.949
K_{45} (h^{-1})	11.577	10.765	8.429
K_{54} (h^{-1})	7.058	4.028	8.118
K_{46} (h^{-1})	16.349	15.958	8.350
K_{64} (h^{-1})	14.684	16.862	8.673
Vcef_elf (L)	9.915	6.469	7.441
Venm_elf (L)	10.148	7.537	6.301

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553 [a]Parameter: SCLcef (liters/h) is the first-order clearance of cefepime from the central

554 compartment; Vcef (liters) is the volume of the central compartment for cefepime; K12, K21,

555 K13, K31 are the first-order intercompartmental rate constants, and Vcef_elf is the volume of

556 the epithelial lining fluid for cefepime. Similarly, SCLenm is the first-order clearance of

557 enmetazobactam from the central compartment; Venm is the volume of the central

558 compartment for enmetazobactam; K45, K54, K46, K64 are the first-order

559 intercompartmental rate constants, and Venm_elf is the volume of the epithelial lining fluid

560 for enmetazobactam.

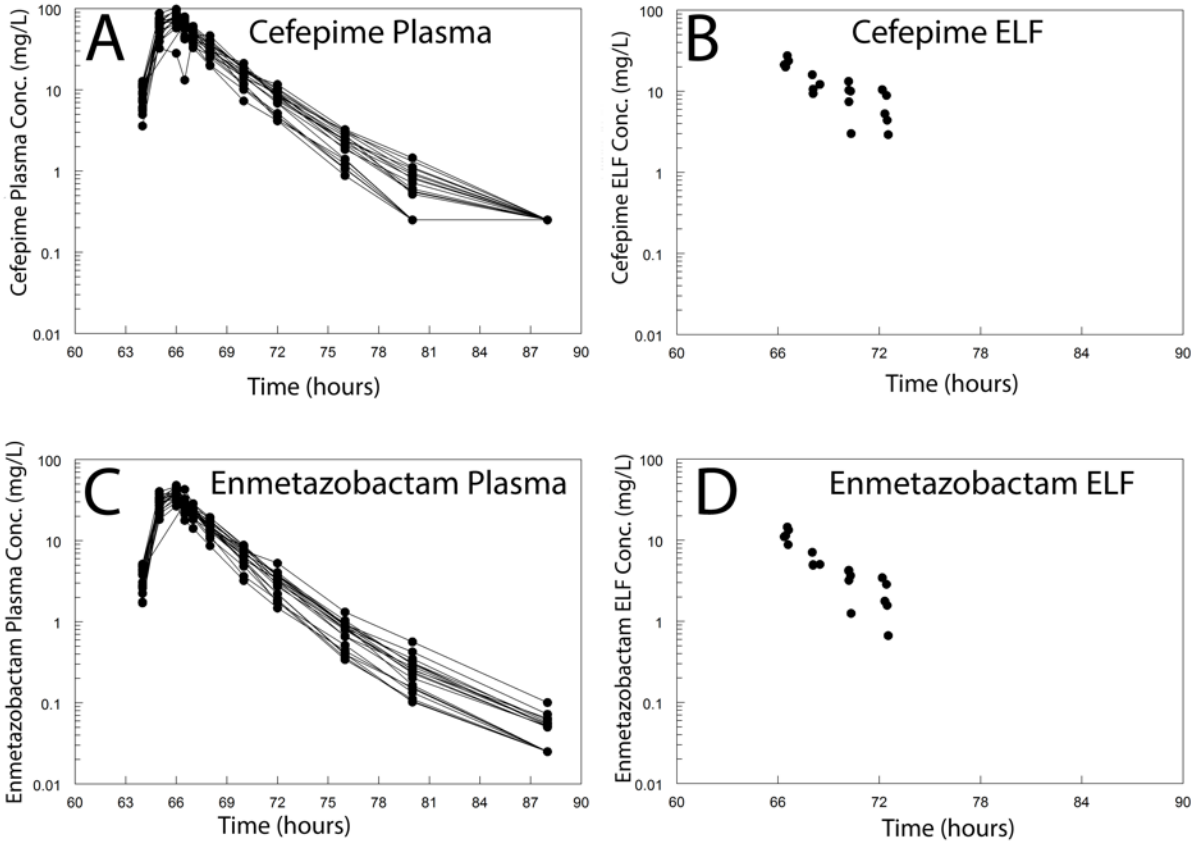


Figure 1. Raw pharmacokinetic data from the 19 volunteers for cefepime and enmetazobactam. Each solid black circle is a datapoint from plasma or ELF. Each volunteer contributes multiple plasma points that are connected by a solid black line and a single ELF estimate.

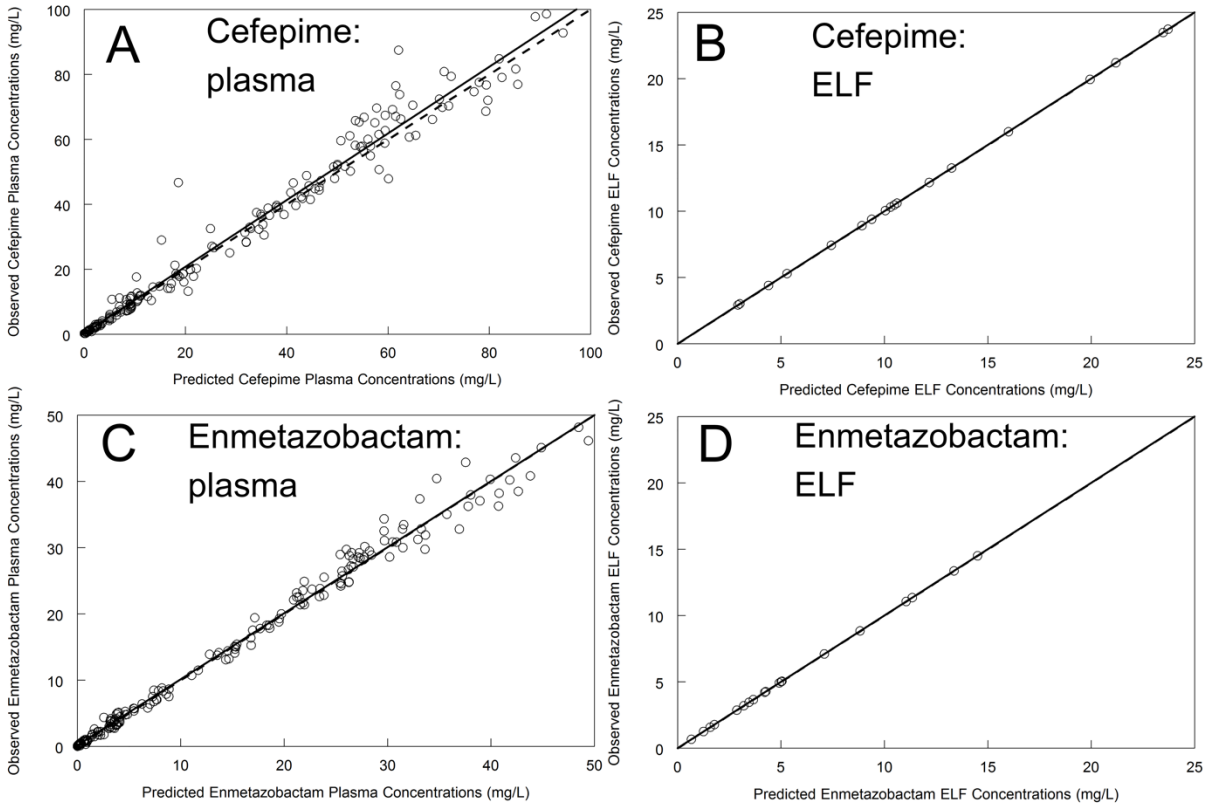


Figure 2. Observed-predicted plots after the Bayesian step for cefepime in plasma and epithelial lining fluid (ELF) in Panels A and B, respectively; and enmetazobactam in plasma and epithelial lining fluid (ELF) in Panels C and D, respectively. The mean parameter values were used to calculate the Bayesian estimates for each volunteer. The solid line is the linear regression and the broken line is the line of identity (i.e., observed=predicted). For Panel A: Observed=0.29+1.03*Predicted; r^2=0.97; for Panel B: Observed=-0.002+Predicted; r^2=1.00; for Panel C: Observed=0.156+0.997*Predicted; r^2=0.99; and for Panel D: Observed=-0.0002+Predicted; r^2=1.00.

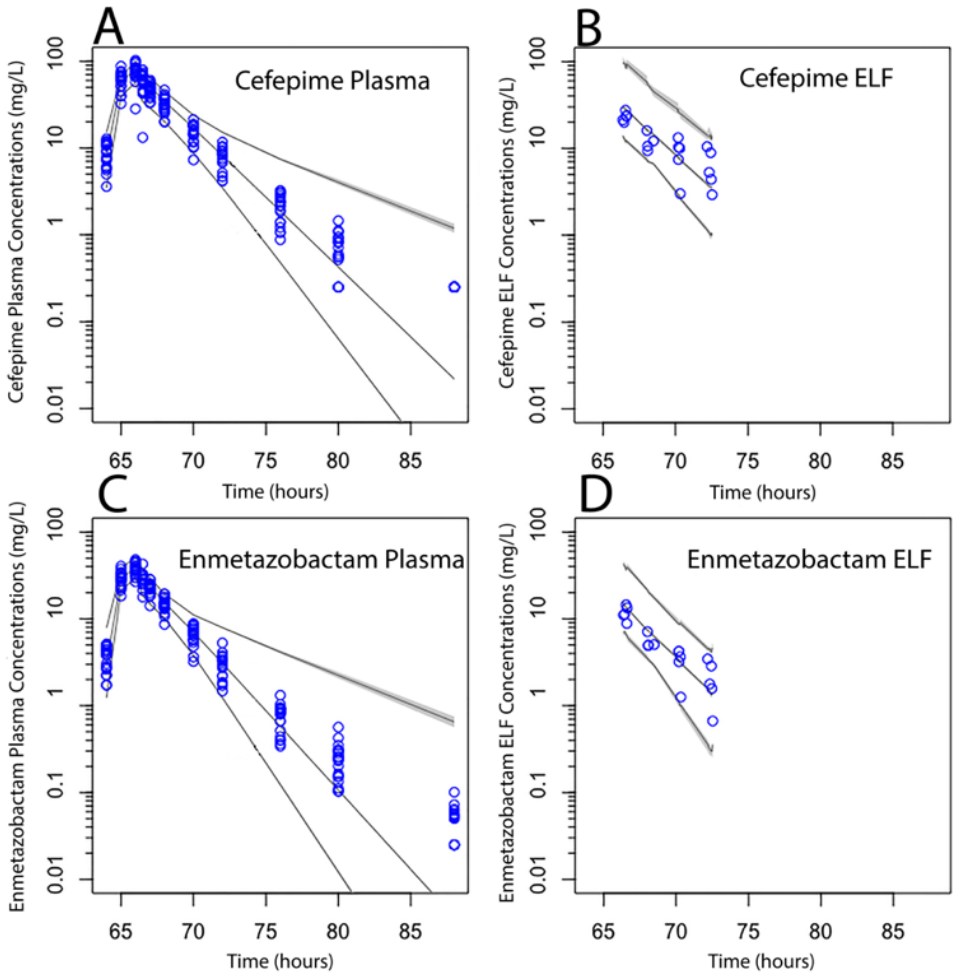
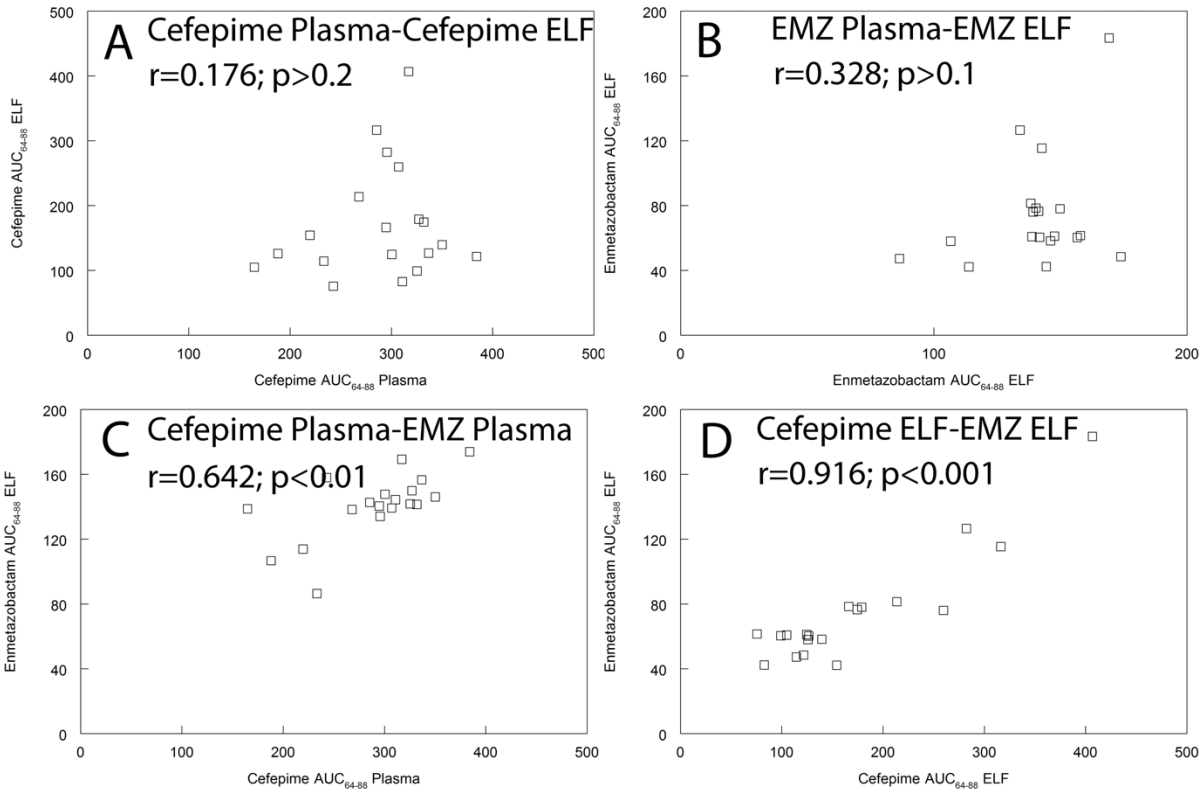


Figure 3. Visual Predictive Check of the fit of the population model to the data obtained from cefepime in plasma and ELF (Panels A and B, respectively) and enmetazobactam in plasma and ELF (Panels C and D, respectively). The open blue circles are the datapoints from plasma and ELF. The three grey lines in each plot represent the 5th, 50th and 95th centile and the shaded areas of the centiles represent the 95% confidence bound around those estimates.



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595 Figure 4. The correlation between area under the concentration-time curve in plasma and

596 epithelial lining fluid (ELF) for cefepime and enmetazobactam. Panel A and B: there is not a

597 statistically significant relationship between AUC in plasma and AUC in ELF for either

598 cefepime or enmetazobactam. In contrast, there was a strong correlation between the AUC

599 in plasma for cefepime and enmetazobactam (Panel C) and in ELF (Panel D).

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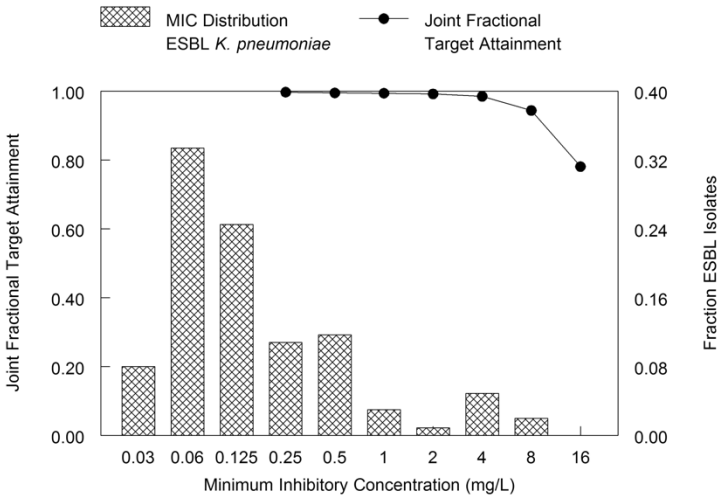

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607 Figure 5. The probability of target attainment in ELF (solid circles) plotted with the
608 distribution of minimum inhibitory concentration (MIC) values for cefepime-
609 enmetazobactam against 102 ESBL-producing *Klebsiella pneumoniae* represented by solid
610 squares. The pharmacodynamic targets used to define success were determined from a
611 preclinical murine model of pneumonia using a variety of *Klebsiella pneumoniae* strains as the
612 challenge organisms.