ID week 2018
Poster 1398

β-lactam Probability of Target Attainment (PTA) and Penetration into Epithelial Lining Fluid (ELF) based on Multiple Bronchoalveolar Lavage (BAL) Sampling Time Points in a Swine Pneumonia Model

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ABSTRACT (revised)

Background: Defining ELF concentrations is desired for antibiotics developed for pneumonia. For ethical reasons, BAL sampling in humans is routinely done at a single time point, thereby creating ambiguity in the precise ELF profile. It is unknown if additional sampling of the ELF would lead to more accurate exposure estimates. The swine pneumonia model was used to characterize the robust ELF profiles (5-BAL) of two β-lactams for comparison with models employing 1-BAL and 2-BAL sampling time points only.

Methods: 16 ventilated swine were infected with *Pseudomonas aeruginosa* to establish pneumonia and then treated for 72 hours with ceftolozane/tazobactam (C/T) 50mg/kg q8h (n=7) or piperacillin/tazobactam (TZP) 200mg/kg q8h (n=8). Plasma and BAL concentrations were measured in each swine at 1, 2, 4, 6, and 8 hours after the first dose. Urea correction was used to calculate ELF values. Ceftolozane and piperacillin plasma and ELF data were fitted to a two-compartment model using the nonparametric adaptive grid program in Pmetrics. Hypothetical models were refitted after randomly selecting either 1-BAL or 2-BAL sampling time points from each swine. A 5,000 subject Monte Carlo simulation was performed for each model to define PTA (60% free time above the MIC) and ELF penetration [area under the curve in ELF (AUC_{ELF}) *vs.* free AUC_{plasma}]. The KS-test was used to analyze distribution differences, reporting maximum vertical deviation (D) as percent difference; D<20% was defined as negligible.

Results: 29 C/T and 34 TZP plasma samples and 29 and 33 BAL samples were available for the full model, respectively; 1-BAL and 2-BAL sampling models used 7 - 8 and 15- 16 BAL samples, respectively. All models adequately fitted the data. C/T PTA at 4 mg/L was 93.7, 92.9, and 95.3%, for the full, 1-BAL and 2-BAL models. TZP PTA at 16 mg/L was 55.8, 46.8, and 46.7%, respectively. C/T median [interquartile range] penetration differences were negligible between the full (94% [62 – 134]) and 1-BAL (82% [60 – 136], D=10%) or 2-BAL models (97% [62 – 144], D=9%). TZP penetration differences were also minimal between the full (32% [9 – 67]) and 1-BAL (17% [5 – 49], D=17%) or 2-BAL models (27% [9 – 44], D=14%).

Conclusions: These data suggest that antibiotic ELF models constructed from a sparse BAL time point result in similar exposure estimates to full ELF profiles.

BACKGROUND

- Describing the disposition of antimicrobial agents at the site of infection is crucial to guide optimal dosing for investigational agents (1).
- For antibiotics developed to treat patients with pneumonia, concentrations are routinely determined in the epithelial lining fluid (ELF) through collection of bronchoalveolar lavage (BAL) samples (1).
- Due to ethical and logistical reasons, bronchoscopy is frequently performed only once in healthy volunteers or patients. Pooled data at each sampling time point are then averaged to estimate the pharmacokinetic profile in ELF over the dosing interval and to conduct pharmacodynamic (Monte Carlo simulation) analyses (2).
- It is currently unknown if the sparse sampling methodologies used in humans results in comparative penetration and pharmacodynamics exposure attainment to full ELF profiles.

OBJECTIVES

1. Describe the influence of collecting sparse BAL samples from each subject in the population pharmacokinetic profile.
2. To compare the ELF penetration ratios and the PTA achieved by different BAL sampling approaches

METHODS

Swine pneumonia model

- The severe *Pseudomonas aeruginosa* swine model previously described (3), was used for this study. Fifteen Large White-Landrace female pigs were induced, orotracheally intubated, and mechanically ventilated for 76 hours. Animals were challenged with a clinical *P. aeruginosa*, susceptible to ceftolozane/tazobactam (MIC 4 μg/mL) and resistant to piperacillin/tazobactam (MIC 64 μg/mL).
- Following pneumonia diagnosis at 24h, animals were randomized into two treatment groups: IV 50 mg/kg of ceftolozane/tazobactam q8h (n=7) and IV 200 mg/kg of piperacillin/tazobactam q8h (n=8).

Blood and BAL sampling

- Blood and BAL samples were collected for determination of ceftolozane and piperacillin concentrations before the first antibiotic dose and at 1, 2, 4, 6, and 8h after in each swine.

Antibiotic determination, protein binding and urea correction

- Ceftolozane (4) and piperacillin (5) concentrations in plasma were determined by high-performance liquid chromatography technique at Center for Anti-Infective Research and Development (Hartford Hospital, Hartford, CT). Ceftolozane and piperacillin BAL concentrations were determined by high-performance liquid chromatography-tandem mass spectrometry method at Pure Honey Technologies (Billerica, Massachusetts, USA).
- Protein binding was assessed in duplicate at 1 and 2h after first dose of administration. Unbound fraction was calculated as % free drug= $C_{unbound}/C_{total}$*100. Bound drug in plasma was considered negligible for both ceftolozane and piperacillin.
- ELF concentrations (C_{ELF}) were determined using urea concentration as an endogenous marker as follows: $C_{ELF} = C_{BAL} * urea_{plasma}/urea_{BAL}$.

Population pharmacokinetic analyses

- Ceftolozane and piperacillin population pharmacokinetic models were developed using the non-parametric adaptive grid (NPAG) program in Pmetrics for R (Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles, CA).
- Full (5 plasma and 4-5 ELF time points per swine) ceftolozane and piperacillin available concentration data were each fitted to a 2-compartment model (**Robust model**). A multiplicative error model was used for weighting concentrations of both drugs. The best fit model was discriminated based on the lowest Akaike's information criterion.
- The model was reconstructed using all plasma concentration and only a single random BAL sample from each pig (**1-BAL model**). The model was reconstructed again using all plasma concentration and only two random BAL samples from each pig (**2-BAL model**).
- Robust profiles were considered the actual plasma and ELF profile for each swine, while the 1-BAL and 2-BAL profiles were considered experimental approximations for comparison.

Monte Carlo simulations

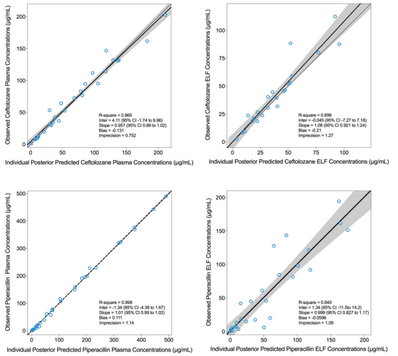
- A 5,000 subject Monte Carlo simulation was conducted for each model (robust, 1-BAL, and 2-BAL) using the Pmetrics package. A semi-parametric method was utilized where the non-parametric support points served as the mean of one multi-variate normal distribution in a multi-modal, multi-variate joint distribution.
- The penetration into ELF was determined for each simulation by the ratio of the area under the curve in plasma and ELF (AUC_{ELF}/AUC_{plasma}), calculated using the trapezoidal rule.
- For ceftolozane and piperacillin, the pharmacodynamics target was the simulated subjects who achieved at least 60% free time above the MIC (*f*T>MIC) (6). PTA was calculated at increasing MICs in doubling dilutions between 0.03 and 256μg/mL.

Statistical analyses

- The Kolmogorov-Smirnov test (KS test) was used to analyze cumulative distribution differences between resulting MCS populations. Maximum vertical deviations (D, %) between distributions were defined, considering D<20% as negligible.

RESULTS

Figure 1. Observed vs. predicted individual ceftolozane and piperacillin plasma and ELF concentrations from the final robust models.



Ceftolozane and piperacillin robust models

- For ceftolozane, 29 plasma and 29 BAL samples were collected from 7 swine. For piperacillin, 34 plasma and 33 BAL samples were collected from 8 swine.
- Ceftolozane and piperacillin concentration data fitted the two-compartment model well as noted by adequate correlation between observed and individual-predicted concentrations in plasma and ELF (Figure 1).

Random BAL selected models

- For ceftolozane, the 1-BAL and the 2- BAL models consisted of 29 plasma samples and 7 or 15 ELF concentrations, respectively. For piperacillin, the 1-BAL and the 2- BAL models consisted of 34 plasma samples and 8 or 16 ELF concentrations, respectively.
- The fit of the 1-BAL and 2-BAL models were similar to the robust models.
- Final pharmacokinetic parameter estimates from the robust, 1-BAL and 2-BAL pharmacokinetic models are provided in Table 1.

Table 1. Final population pharmacokinetics parameter estimates (Mean ± SD [Median]) for different sampling approaches for 15 pigs infected with *P. aeruginosa* and treated with ceftolozane (n=7) or piperacillin (n=8).

	Robust	1- BAL	2 – BAL
Ceftolozane			
CL_0 (L/h)	4.41 ± 1.61 [4.27]	4.25 ± 1.40 [3.98]	4.27 ± 1.66 [3.99]
V_1 (L)	8.91 ± 1.84 [9.55]	8.71 ± 1.89 [8.33]	8.91 ± 1.81 [9.47]
K_{12} (h^{-1})	0.18 ± 0.16 [0.15]	0.30 ± 0.25 [0.18]	0.21 ± 0.17 [0.15]
K_{21} (h^{-1})	0.56 ± 0.23 [0.43]	0.90 ± 0.77 [0.55]	0.49 ± 0.28 [0.45]
V_{ELF} (L)	2.65 ± 1.51 [2.45]	3.80 ± 1.93 [3.90]	3.26 ± 1.63 [2.78]
Piperacillin			
CL_0 (L/h)	8.97 ± 2.83 [7.97]	8.69 ± 4.01 [8.12]	9.06 ± 2.79 [8.13]
V_1 (L)	12.05 ± 4.19 [11.63]	12.39 ± 2.47 [11.63]	11.74 ± 4.37 [11.71]
K_{12} (h^{-1})	0.22 ± 0.24 [0.16]	0.19 ± 0.17 [0.15]	0.33 ± 0.34 [0.23]
K_{21} (h^{-1})	1.17 ± 0.68 [1.08]	0.42 ± 0.46 [0.22]	1.64 ± 0.70 [1.53]
V_{ELF} (L)	10.15 ± 16.33 [2.42]	26.23 ± 29.51 [16.05]	11.91 ± 17.73 [3.09]

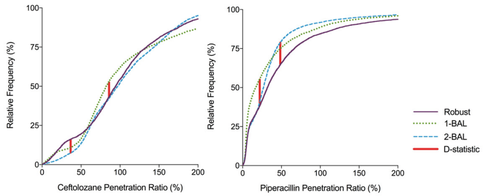
CL_0, clearance; V_1, volume of distribution of the central compartment; K_{12}, transfer rate from the central compartment to the ELF compartment; K_{21}, transfer rate from the ELF compartment to the central compartment; V_{ELF}, volume of distribution of ELF compartment.

Table 2. Estimation of penetration (Median [IQR]) of ceftolozane and piperacillin components into ELF using Monte Carlo simulation of different BAL sampling strategies.

	Ceftolozane			Piperacillin		
	Robust	1-BAL	2-BAL	Robust	1-BAL	2-BAL
Plasma *f*AUC_{0-8h} (mg*h/L)	377.2 [324.4 – 465.3]	365.0 [321.1 – 434.3]	381.2 [327.4– 464.0]	770.8 [593.3 – 906.6]	733.1 [517.4 – 870.4]	788.0 [620.2 – 937.5]
ELF AUC_{0-8h} (mg*h/L)	379.0 [217.6 – 493.1]	306.7 [228.7 – 477.7]	409.7 [238.1 – 514.6]	275.3 [55.0 – 578.2]	117.1 [31.8 – 444.4]	219.7 [57.7 – 401.3]
Penetration ratio (%)	94.3 [61.8 – 134.0]	82.2 [59.7 – 136.2]	97.0 [62.3 – 143.7]	31.8 [8.8 – 66.6]	16.9 [5.2 – 48.6]	26.9 [9.1 – 43.6]

*f*AUC, free area under the curve; Penetration ratio, AUC_{ELF}/ $fAUC_{plasma}$; IQR, interquartile range [25th – 75th percentiles]

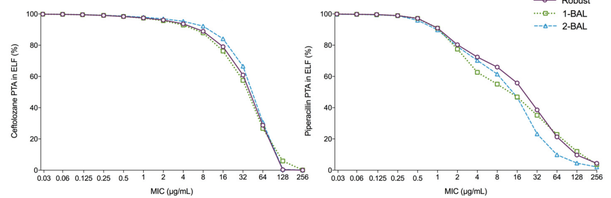
Figure 2. Cumulative distribution of penetration ration ($AUC_{ELF}/fAUC_{plasma}$) of ceftolozane and piperacillin of Monte Carlo simulation results from different BAL sampling approaches.



ELF penetration and PTA

- The results from the 5,000-subject Monte Carlo simulation based on all models are displayed in Table 2. Despite the effect of outliers on greatest variability, the median values for *f*AUC and penetration ratios were similar between BAL sampling strategies.
- The maximum vertical deviations (D statistic, %) of 10.2% and 17.1% were found between Robust and 1-BAL approaches in ceftolozane and piperacillin models, respectively (Figure 2).
- For ceftolozane simulations, PTA to achieve 60% *f*T>MIC at susceptibility breakpoint 4 μg/mL was 93.7, 92.9, and 95.3%, for the robust, 1-BAL and 2-BAL models, respectively (Figure 3).
- For piperacillin simulations, PTA to achieve 60% *f*T>MIC at susceptibility breakpoint 16 mg/L was 55.8, 46.8, and 46.7%, respectively (Figure 3).

Figure 3. Probability of the ceftolozane and piperacillin components of ceftolozane/tazobactam (50 mg/kg q8h 60-min infusion) and piperacillin/tazobactam (200 mg/kg q8h 60-min infusion) achieving 60% *f*T>MIC in ELF between Monte Carlo simulation results from different BAL sampling approaches



DISCUSSION and CONCLUSIONS

- Population pharmacokinetic models were successfully constructed for ceftolozane and piperacillin using robust, 1-BAL, and 2-BAL models. Related drug models resulted in similar pharmacokinetic parameter estimates.
- Although minor differences in ELF AUC and penetration for piperacillin were observed for the 1-BAL model, no changes to interpretation of the penetration ratio were made when the 5,000-subject simulations were run.
- Consistent PTA in ELF were displayed across all MICs for both drugs.
- These data suggest that ELF models constructed from sparse BAL time point data, including single sampling strategies, result in similar exposure estimates to robustly sampled BAL profiles.

ACKNOWLEDGEMENTS

This study was supported by a grant from Merck & Co., Kenilworth, NJ USA. AT and GLB were the principal investigators for this research recipient. AM is the recipient of a Long-Term Research Fellowship (LTRF 2017- 01-00073) from the European Respiratory Society (ERS) and Spanish Society of Pneumology and Thoracic Surgery (SEPAR). We acknowledge Christina Sutherland from CAIRD and Pure Honey Technologies (Billerica, MA, USA) for their technical assistance with the MS-HPLC assays and all other individuals in the Division of Animal Experimentation at Hospital Clinic of Barcelona for their valuable assistance with sampling procedures and preparation.

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