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Pharmacokinetics/pharmacodynamics of Epetraborole, a Novel Bacterial Leucyl-tRNA Synthetase Inhibitor, and High Intracellular Penetration in the Intracellular Hollow Fiber System Model of *Mycobacterium avium* Complex Lung Disease

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ABSTRACT

Background: Epetraborole (EBO) is a boron-containing, oral inhibitor of bacterial leucyl-tRNA synthetase, an essential enzyme in protein synthesis; EBO demonstrates potent activity against nontuberculous mycobacteria. Dose-effect studies in the intracellular hollow fiber system model of *Mycobacterium avium* complex (MAC) lung disease, or HFS-MAC, have demonstrated that EBO is highly bactericidal. In this study we used dose fractionation in the HFS-MAC system to find the best pharmacokinetic/ pharmacodynamic (PK/PD) index that was associated with EBO microbial kill and acquired microbial resistance (AMR).

Methods:. A dose-fractionation design was used to dose 30 HFS-MAC units. ED_{20}, ED_{50} and ED_{80} doses were administered as one of 4 dose schedules of twice daily, once daily, twice weekly, or once weekly at a half-life of 10.4h. Total MAC burden and EBO-resistant MAC burden were determined every 7 days by culturing HFS-MAC contents on Middlebrook 7H10 agar. For drug concentrations, the central compartment of each HFS-MAC unit was sampled throughout the 28 days, and after the final dose, intracellular (IC) PK in the MAC-infected monocytes were established. EBO PK/PD index parameters including 0-168h area under the concentrations-time curve ($AUC_{0\text{-}168}$), peak concentration, or % of time concentration persists above the intracellular MIC ($\%T_{MIC}$), which was 0.5 mg/L, were modeled versus total MAC burden using the inhibitory sigmoid maximal effect (E_{max}) model. The PK/PD index was chosen using Akaike Information Criteria (AIC). For AMR, EBO PK/PD index exposure value versus EBO-resistant MAC burden were modeled using a quadratic function, and the best model was chosen using AIC.

Results: The measured EBO concentrations demonstrated PKs shown in Figure 1. The median EBO IC versus extracellular (IC/EC) elimination rate constants were $0.018h^{-1}$ versus $0.061h^{-1}$ ($p< 0.0001$), which means slower IC EBO elimination, leading to IC/EC to AUC ratio of 11. Based on AIC scores in Table 1, the PK/PD driver for microbial kill was unequivocally AUC on each sampling day. For AMR the PK/PD driver was also AUC.

Conclusions: The PK/PD index best associated with EBO microbial kill and AMR was AUC.

INTRODUCTION

- *Mycobacterium avium* complex (MAC) accounts for approximately 80% of all pulmonary NTMs.
- Standard of care (SOC) of a macrolide (clarithromycin or azithromycin), a rifamycin, and ethambutol, is associated with sustained sputum conversion rates of only 64% at 6 months[1].
- With SOC, 70% of patients develop treatment related adverse event; 30% discontinue therapy.
- Epetraborole (EBO) is a boron-based bacterial leucyl-tRNA synthetase inhibitor[2].
- In murine pulmonary MAC, EBO had a significantly greater $\log_{10}$ kill than clarithromycin, and in several strains matched the three-drug SOC (**Poster No. 1704**).
- In the intracellular hollow fiber system model of MAC (HFS-MAC) dose-response studies EBO matched the three-drug SOC for the first 14 days (**Poster No. 1697**).
- In pulmonary MAC in patients, immunohistochemistry has revealed intracellular MAC in monocyte-lineage cells in alveoli and infected multinucleated giant cells in necrotic lesions.
- EBO penetration was approximately 500 to 600% into human alveolar macrophages compared to plasma levels in healthy volunteers[3], which means it could have a PK advantage as an anti- MAC drug in people.

METHODS

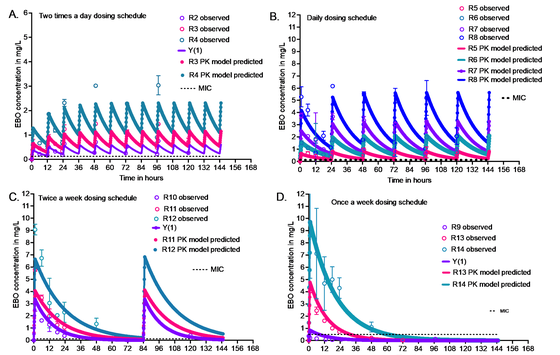
THP-1 monocytes were infected with MAC (ATCC 700898), after which extracellular MAC was washed off, and 20 mL of infected cells added to the peripheral compartment of each HFS-MAC. EBO treatment was started 24hrs later, as shown in Table 1. Doses were chosen based on results of dose-effect studies (Poster No. 1697) and maximizing Fischer information (and Shannon entropy). PKs were confirmed by repetitive sampling of the central compartment; intracellular PKs were established by simultaneously sampling the infected monocytes in the peripheral compartment, followed by measurement of cell volume and cell count. The peripheral compartment was sampled to quantify intracellular bacterial burden, by counting both total and EBO-resistant colonies on agar containing 16 mg/L of EBO.

Table 1. EBO dose regimens

Regimen ID	Dose Regimen	Actual C_{max} (mg/L)	Actual $AUC_{0\text{-}168}$ (mg.h/L)
R1	None	0	0
R2	EC_{20}/ Twice Daily	0.713	63.46
R3	EC_{50}/ Twice Daily	2.84	126.23
R4	EC_{80}/ Twice Daily	5.65	256.40
R5	EC_{20}/ Daily	1.38	72.25
R6	EC_{50}/ Daily	1.61	186.25
R7	EC_{80}/ Daily	10.4	284.62
R8	EC_{90}/ Daily	10.9	501.16
R9	1/2EC_{20}/ Weekly	1.36	26.46
R10	EC_{20}/ Biweekly	0.32	96.19
R11	EC_{50}/ Biweekly	3.82	160.5
R12	EC_{80}/ Biweekly	7.83	305.05
R13	EC_{20}/Weekly	5.23	67.6
R14	EC_{50}/ Weekly	12.3	199.8

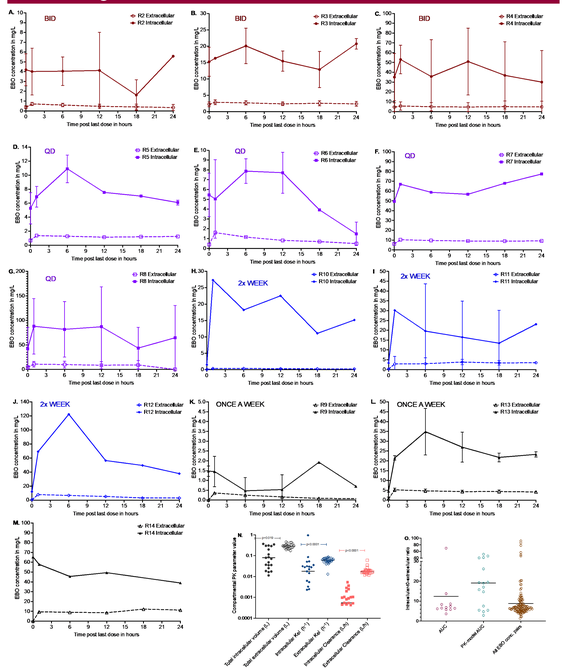
RESULTS

Figure 1. EBO concentrations and PKs measured in HFS-MAC



- Symbols are mean concentrations and line graphs are ADAPT PK model derived
- Dose fractionation design was successful based on PKs from the 4 dose schedules

Figure 2. EBO Intracellular vs Extracellular PKs



- Results are from day 28, at steady state
- Symbols are mean concentrations and line graphs are point-to-point naïve pooling
- Repetitive sampling in the HFS-MAC allowed measurement of intracellular PKs simultaneously with peripheral compartment
- Intracellular concentrations were always higher than extracellular
- Both high drug penetration and slower elimination inside infected cells contributed to the high intracellular EBO concentrations

Figure 3. Inhibitory sigmoid E_{max} (microbial kill)

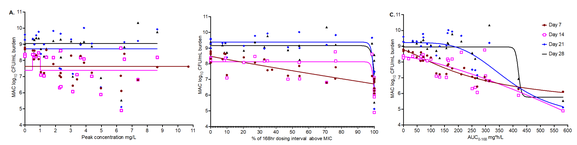


Table 2. Goodness of fit for PK/PD index versus MAC CFU/mL

PK/PD parameter	Akaike Information Criteria Scores			
	Day 7	Day 14	Day 21	Day 28
$AUC_{0\text{-}168}$	-60.21	-9.674	-5.26	-9.63
Peak	-0.47	17.90	21.79	15.11
$\%T_{MIC}$ [intracellular MIC=0.5 mg/L]	-38.3	9.88	-5.27	-6.86
	r^2			
$AUC_{0\text{-}168}$	0.89	0.60	0.67	0.61
Peak	0	0	0	0
$\%T_{MIC}$ [intracellular MIC=0.5 mg/L]	0.42	0.16	0.16	0.11

- The PK/PD parameter linked to microbial kill was, unequivocally, AUC

Figure 4. EBO-resistant CFU/mL vs PK/PD parameter: Quadratic function based on antibiotic resistance arrow of time model[1]

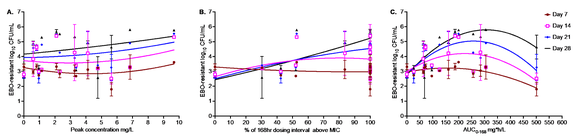


Table 3. Goodness of fit for PK/PD index versus EBO-Resistant CFU/mL: Quadratic Function

PK/PD parameter	Akaike Information Criteria Scores			
	Day 7	Day 14	Day 21	Day 28
$AUC_{0\text{-}168}$	-45.64	3.543	-10.73	-4.341
Peak	-30.86	10.68	8.404	16.61
$\%T_{MIC}$ [intracellular MIC=0.5 mg/L]	-32.86	8.95	0.21	6.28
	r^2			
$AUC_{0\text{-}168}$	0.50	0.27	0.57	0.62
Peak	0.11	0.03	0.05	0.06
$\%T_{MIC}$ [intracellular MIC=0.5 mg/L]	0.18	0.09	0.33	0.40

- The PK/PD parameter linked to EBO resistance development was AUC but the r^2 was relatively low.
- Therefore, we reanalyzed using % of EBO resistant CFU/mL to total CFU/mL

Figure 5. EBO-resistant % of total vs PK/PD parameter: Quadratic function based on antibiotic resistance arrow of time model[5]

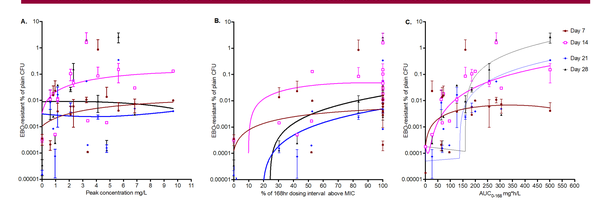


Table 5. EBO-resistant % of total vs PK/PD parameter: Quadratic function based on antibiotic resistance arrow of time model[1]

PK/PD Parameter	Akaike Information Criteria Scores			
	Day 7	Day 14	Day 21	Day 28
$AUC_{0\text{-}168}$	-247.8	154.7	-198.7	-106.3
Peak	-239.5	-133.0	-197.3	-149.8
$\%T_{MIC}$ [intracellular MIC=0.5 mg/L]	-235.6	-133.8	-208.8	-157.6
	r^2			
$AUC_{0\text{-}168}$	0.50	0.90	0.99	0.98
Peak	0.27	0.27	0.01	0.01
$\%T_{MIC}$ [intracellular MIC=0.5 mg/L]	0.11	0.15	0.48	0.36

- The PK/PD parameter linked to % EBO-resistant CFUs, based on AIC criteria, was AUC until day 7 when it appeared to change to $\%T_{MIC}$

CONCLUSIONS

- **EBO achieved high concentrations inside MAC-infected monocytes, with an AUC penetration ratio of 11. Given the 50% ELF concentrations[3], it means AUCs in infected monocytes in lung lesions will be about 5-fold those in plasma.**
- **EBO microbial kill of intracellular MAC is AUC driven.**
- **EBO resistance in HFS-MAC is driven by AUC early during therapy but seems to switch to $\%T_{MIC}$ by end of therapy when the non-susceptible subpopulation starts to replace the susceptible population.**
- **In the HFS-MAC dose fractionation study, the average EBO EC_{50} value of the dosing time of 7 to 28 days was an $AUC_{0\text{-}168h}$ of 283 mg*h/L, with a corresponding $AUC_{0\text{-}24h}$ of 40.44 mg*h/L. This falls within 95% CI of values from the dose-response study (Poster No. 1697).**

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