Method for Generating a Pharmacokinetic/Pharmacodynamic (PK/PD) Breakpoint from Murine Infection Studies with Multiple Strains

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Abstract 101

Background

Plazomicin is an aminoglycoside designed to overcome the vast bulk of aminoglycoside modifying enzymes, the most common aminoglycoside resistance mechanism in Enterobacteriaceae.

Plazomicin is FDA approved for cUTI, including pyelonephritis. An additional study in patients with serious bacterial infections due to CRE has been conducted.

Use of murine thigh and murine pneumonia models is an accepted approach to generate Probability of Target Attainment (PTA) data. When multiple isolate evaluations give differing targets, the method of rationally dealing with this situation is unclear.

Two of the most difficult decisions in clinical infectious diseases are: 1) what is the "correct" dose of an antibiotic for a specific patient and infection 2) what represents a "susceptible" organism.

Susceptibility breakpoints are often derived from Probability of Target Attainment Analyses, which employ exposure targets from pre-clinical models.

Materials & Methods

Organisms for Study Eight *K. pneumoniae*, *E. coli* and Enterobacter strains were evaluated in dose-range studies for plazomicin in the neutropenic murine thigh infection model. The strains were selected and supplied by Achaogen, Inc. Some of the isolates were resistant to one or more carbapenem antibiotics and/or to the legacy aminoglycoside gentamicin, tobramycin or amikacin

In vitro Susceptibility Testing The *in vitro* susceptibilities were performed using the broth microdilution method described by CLSI.

Neutropenic Murine Thigh Infection Model

All animal experimentation was approved by the University of Florida Institutional Animal Care and Use Committee.

Female, 16 to 20 g BALB/c mice (Charles River [NCI colony], Frederick, MD) were rendered transiently neutropenic with 150 mg/kg of cyclophosphamide given intraperitoneally (i.p.) 4 days prior to infection plus 100 mg/kg given i.p. 1 day before infection.
Neutropenic mice were inoculated with one of eight *K. pneumoniae, E. cloacae* or *E. coli* strains at 10^5 CFU to each posterior thigh muscle. A cohort was sacrificed at hour 2 for documentation of bacterial burden. Therapy was initiated with multiple doses of plazomicin in a humanized fashion. Twenty-four hours after start of therapy, animals were sacrificed and thigh muscles were quantitatively cultured.

Mathematical Analysis Inhibitory Sigmoid-Emax Analysis: A 4 parameter system (E_{con}; E_{max}; E_{50}; H) was employed to link exposure to response. Fit of the model to the data was accomplished with the Estimation Module of the ADAPT V package of programs. A maximum likelihood estimator was employed. Stasis was defined as the AUC/MIC Ratio exposure which was required to keep the bacterial burden at the size measured when therapy was initiated.
Monte Carlo simulation was also performed with the Simulation Module of ADAPT V. A Log-Normal distribution was assumed. There were 5000 iterates.

Objective

Develop a method to identify a robust PK/PD susceptibility breakpoint when multiple organisms with different MICs were studied

Results

Table 1: Activity of plazomicin and comparator antibiotics against 8 clinical isolates of Enterobacteriaceae and an ATCC isolate (AKPN001, ATCC 43816)

		Mode MIC (µg/mL)					
Strain Code	**Resistance Phenotype**	**Plazomicin**	**Amikacin**	**Gentamicin**	**Tobramycin**	**Meropenem**	**Ceftazidime**
AKPN001[1]	none	0.5	1	0.25	0.25	0.03	0.25
AECO1176	aac(6)-Ib[2]	1	32	1	>8	≤ 0.015	2
AECO1179	aac(3)-IIa, aac6-Ib	2	32	>32	>8	0.03	>32
AKPN1171	Not reported	4	8	4	4	0.03	0.5
AKPN1169	KPC-3[3]	2	32	>32	>8	>32	>32
AEAE1034	Not reported	1	4	0.5	1	0.06	>32
AECO1173	aac(3)-IIa	0.25	1	>32	8	≤ 0.015	0.25
AECO1180	aac(3)-IIa	4	8	>32	>8	0.03	>32
AKPN1170	aac(6)-Ib, KPC-2	2	32	4	>8	8	>32

1. Reference strain only
2. Resistance phenotypes that begin with "aac" are aminoglycoside modifying enzymes as mechanisms of resistance.
3. KPC: *Klebsiella pneumoniae* carbapenemase.
4. KPN = *Klebsiella pneumonia*; ECO = *E. coli*; EAE = *Enterobacter aerogenes*.

- **Plazomicin generates potent activity against these isolates**
- **Plazomicin's activity is superior to legacy aminoglycosides**

Figure 1: Humanized concentration-time profile in murine plasma for plazomicin at a dose in mice of 225 mg/kg/d. The mean human concentration-time curve and its 95% confidence intervals for plazomicin in humans at a dose of 15 mg/kg is shown. The $AUC_{0\text{-}24\ hr}$ matches well with the human data and the murine humanized data. A validation study was performed to demonstrate that the humanization did achieve the desired profile.

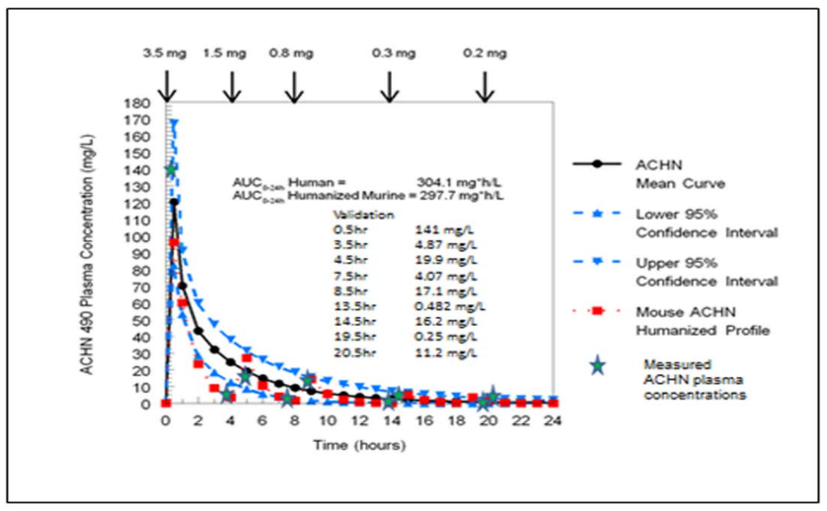
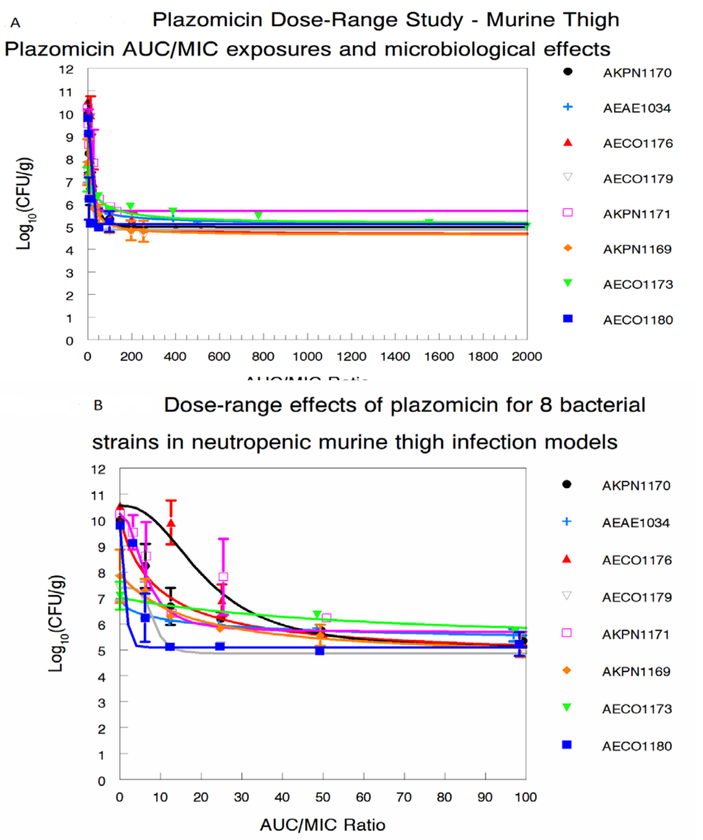


Figure 2: Microbiologic effect of differing plazomicin doses on bacterial kill for 8 isolates. The doses were transformed to AUC/MIC ratios. In Panel A, the full range of AUC/MIC ratios is shown. In panel B, only the lower AUC/MIC ratios is show to highlight the early dynamics of plazomicin.



Results (Cont'd)

- **The humanization scheme provided a good approximation of a human concentration-time profile**

Table 2: Plazomicin AUC/MIC Ratio to achieve stasis for 8 isolates of Enterobacteriaceae

Strain	MIC	Challenge Inoculum	Stasis Exposure AUC/MIC Ratio
AECO1176	1	10^5	33.0
AECO1179	2	10^5	7.6
AKPN1171	4	10^5	15.9
AKPN1169	2	10^5	20.2
AEAE1034	1	10^5	10.9
AECO1173	0.25	10^5	43.5
AECO1180	4	10^5	5.7
AKPN1170	2	10^5	23.6

- **There was a broad range of stasis targets (range 5.7- 43.5 AUC/MIC Ratios). This provided a Mean ± S.D. of 20.05 ± 13.05 AUC/MIC Ratio.**

Figure 3: Probability of Target Attainment plot for eight isolates. The mean PTA curve is derived from the weighted mean of the PTA plots from the 1st, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 99th percentiles of a 5000 iterate Monte Carlo simulation of targets. The mean and standard deviation of the targets were calculated from Table 2.

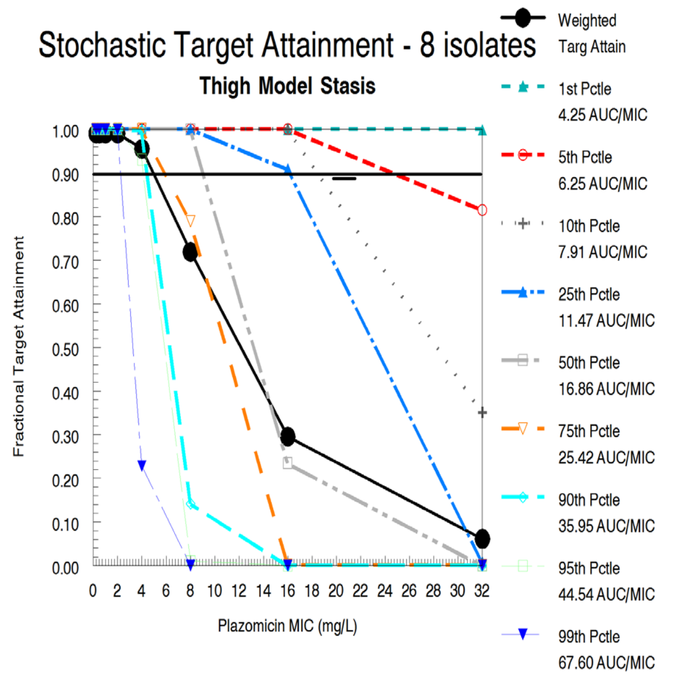


Table 3: The stasis targets (AUC/MIC Ratio) for the 1st through the 99th percentiles of the distribution as calculated from a 5000-iterate Monte Carlo Simulation. The breakpoint was the highest MIC value where 90% target attainment was achieved. The probability weighted breakpoint value was 4 mg/L.

Percentiles	1st	5th	10th	25th	50th	75th	90th	95th	99th
Targets (AUC/MIC Ratio)	4.25	6.25	7.91	11.47	16.86	25.42	35.95	44.54	67.60
Breakpoint	32	16	16	16	8	4	4	4	2

Conclusions

- **The problem of multiple targets has important implications**
- **Regulatory authorities require a rational way forward to set susceptibility breakpoints**
- **In the same way that there is true between-patient variance in PK, there is true between-strain variance in bacterial effect targets**
- **Generating a distribution of targets allows the impact of the targets on breakpoints to be directly observed**
- **Table 3 demonstrates that, depending on the percentile in the distribution, a tentative breakpoint would range from 32 mg/L (1st percentile) down to 2 mg/L (99th percentile)**
- **Using the weighted average (weighted by the percentiles of the distribution) shows that a breakpoint of 4 mg/L will provide robust (>90%) target attainment for >95% of the targets**
- **This will allow optimal utilization of this new agent by minimizing the likelihood of having the breakpoint set too high (which may increase the probability of poor outcomes) or too low (decreasing the use of plazomicin in patients who could benefit)**

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