A-471 Pharmacodynamics of aztreonam against *E.coli* studied in an in vitro model of infection

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C Ramsey, KE Bowker, AR Noel, MLG Attwood, ST Tomaselli, A P MacGowan,
BCARE, Department of Microbiology, North Bristol NHS Trust, Bristol, UK.
karen.bowker@nbt.nhs.uk

Abstract

Background:
The pharmacodynamics (PD) of aztreonam (AZT) are poorly studied but are an increasingly important area of ß.lactam PD investigation. The reasons for this are a) AZT is the only ß.lactam of the monobactam class available for clinical use, b) AZT as single or combination therapy has been identified as an old antibiotic worthy of revival, c) other monobactams are presently under development for clinical use. The aim of this study was to define the size of the pharmacodynamic index (PDI) for reduction in bacterial load and risk of emergence of resistance (eor) using an in vitro PK model.

Methods: A single compartment dilutional in vitro model was used to model nine T>MIC exposures per strain tested. Three wild type *E.coli* AZT MICs 0.25, 0.25, 0.25mg/L at an inhal inoculation of 10^6 CFU/ml was used. AZT concentrations were based on a serum half-life of 2.5h. Antibacterial effect was measured by change in viable count over 48h and changes in population profile as assessed by sub-culture onto recovery media containing AZT at MICx2. MICx4, and MICx8.

Results: AZT was bactericidal against *E.coli* strains producing a -4 log drop in initial bacterial load at the highest AZT exposures (T>MIC 100%). The %T>MIC for a 24h static, -1 log and -2 log in bacterial load was 47.1 ± 3.7%, 52.3 ± 5.9% and 57.7 ± 9.1% respectively. Equivalent %T>MIC values at 48h were 64.2 ± 9.4%, 70.6 ± 10.3% and 79.2 ± 18.2%. There was no EOR over 48h with any strain.

Conclusions: The T>MIC PDI target for AZT based on a static to -1 log drop at 24h was 40-60% for *E.coli.* Such a target should be valuable for translationally modelling of AZT doses and is longer than equivalent values for Gram-negative rods in our model with carbapenems (T>MIC target 15-40%); and cephalosporins (T>MIC target 30-45%) and more similar to those for penicillins (T>MIC target 30-60%).

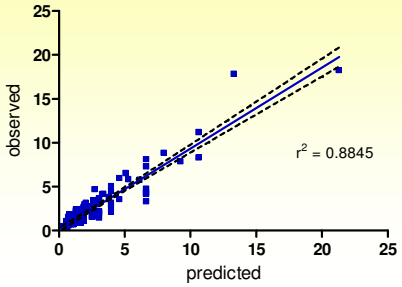
Introduction

- AZT, a monobactam ß.lactam, has been identified as an older antibiotic worthy of re-evaluation in a time of increasing antibiotic resistance.
- In addition, the monobactam pharmacore has been used for the development of new mono-bactams (BAL30072, Basilea; AiC499 AiCURIS; pyridone-conjugated monobactams; Pfizer) and also combined with ß.lactamase inhibitors (AZT-avibactam, AstraZeneca) in the hope of producing useful new therapeutics.
- AZT has proven of value in the treatment of infections due to Enterobacteriaceae and *P.aeruginosa* in both clinical trials and routine hospital practice having the unique feature of being stable to Ambler class B.metallo ß.lactamases (MBL). However, it is inactivated by ESBLs, KPC carbapenemases and stably derepressed chromosomally encoded AmpC ß.lactamases. Sadly, MBL producing isolates often carry additional ß.lactamases including ESBLs, AmpC enzymes and serine carbapenemases which inactivate AZT.
- Although detailed data on the burden of AZT resistance is lacking a recent global surveillance programme indicated 78% of Enterobacteriaceae had MIC of ≤8mg/L; 58% of *P.aeruginosa* had MICs of ≤8mg/L and 78% ≤16mg/L. In contrast only 4% of *A.baumannii* had MICs of ≤8mg/L (AAC, 2015, 59, 4239).
- Basic pharmacodynamic data on AZT is patchy; AZT shows non-concentration dependent killing against *E.coli* in the range MICx4-64 and is reported to have a post antibiotic effect (PAE) of up to 1.5h against Enterobacteriaceae but no PAE for Pseudomonas spp.
- Animal and in vitro experiments on the combination of AZT plus avibactam indicated cidal antibacterial effects of AZT and fT>MIC exposures of greater than 38% (AAC, 2013, 57, 3299) and static to -4 log drop of Enterobacteriaceae associated with fT>MIC exposures of 50-60% (JAC doi 10:1093/jac/dkv 132).
- The aim of this study was to determine the fT>MIC exposures of AZT required for static and cidal effects against *E.coli* using an in vitro model of infection.

Materials and methods

- Three wild type *E.coli* (ampicillin susceptible) with AZT MICs 0.25mg/L were employed: SMH56646; SMH 56645; SMH 56727.100% Muller-Hinton Broth was used for all experiments.
- A dilutional in vitro PK model using polypropylene bottles and Mueller Hinton broth was used to simulate a range of aztreonam concentrations fT>MIC 0-100%. The initial inoculum was 10^6CFU/ml in all experiments, and a simulated AZT of 2.5h was used throughout. Nine fT>MIC exposures experiments were conducted with each strain
- AZT concentrations were confirmed by HPLC.
- Changes in bacterial load were assessed by measurement of viable count up to 48hr and risk of emergence of resistance by changes in population profile assessed at 0hr, 24hr and 48hr.
- The relationship between fT>MIC and viable count was assessed using a Boltzman Emax equation (Graph Pad Prism™).

Figure 1: Observed v predicted aztreonam concentrations (mg/L)



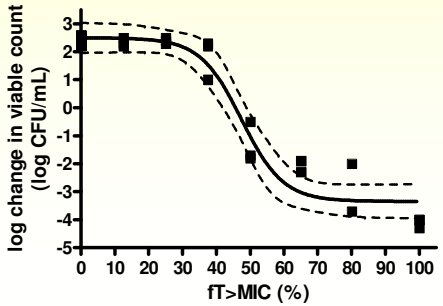
Results

- The target and achieved aztreonam are shown on Figure 1.
- The fT>MIC ratios for 24h and 48h bacteriostatic, -1 log, -2 log and -3 log drop in bacterial load are shown on Table 1.
- Figure 1 shows the relationship between 24h %fT>MIC and change in *E.coli* viable count.
- There were no changes in population profiles (EoR) to aztreonam at 24h or 48h.

Table 1: %fT>MIC aztreonam exposures associated with antibacterial effects at 24h and 48h

	24h				48h			
Strain	Static effect	-1 log drop	-2 log drop	-3 log drop	Static effect	-1 log drop	-2 log drop	-3 log drop
56645	42.9	48.7	55.3	66.1	58.8	62.1	66.3	79.3
56646	50.1	59.5	67.2	>100	75.1	82.1	92.1	>100
56727	48.3	49	50	>100	58.8	67.6	>100	>100
Mean	47.1	52.3	57.7	>100	64.2	70.6	79.2	>100
SD	3.7	5.9	9.1		9.4	10.3		

Figure 2: Relationship of %fT>MIC for aztreonam to antibacterial effect against *E.coli*



Conclusions

- The fT>MIC pharmacodynamic index target for translational modelling for aztreonam should be 50-60% - equivalent to a -2 log drop in bacterial load after 24h. Such targets are recommended in European Medicines Agency Draft Guidance.
- Equivalent fT>MIC for Gram negative rods in our in vitro model for carbapenems were shorter at 15-40% as were cephalosporins at %fT>MIC 30-40% but similar to penicillin fT>MIC 30-60%.
- No changes in population profiles were observed over 48h; though exposure times were short.