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Determination of antibiotic pharmacokinetic/pharmacodynamic relationships in a neutropenic mouse thigh infection model using a clinical *E.coli* strain.

There is an ongoing unmet medical need for new antimicrobial therapies due to the emergence of multi-drug resistant bacteria. Novel therapies in conjunction with optimized dosing strategies both play a key role in addressing this need. The efficacy of antibiotics is driven by the pharmacokinetic/pharmacodynamic (PK/PD) parameters: time above minimum inhibitory concentration (MIC), AUC/MIC and C_{max}/MIC, and determination of the dominant parameter can be used to aid human dose predication and dosing strategy. Here we developed a murine neutropenic thigh model with a clinical*E.coli*isolate (ATCC 700928) and assessed the PK/PD parameters of known antibiotics.

Female CD-1 mice (18-20 g, n = 4/group) were rendered neutropenic by intraperitoneal injection of cyclophosphamide on days -4 (150mg/kg) and -1 (100 mg/kg) prior to infection. On day $0{,}1x10^4$- $1x10^7$ colony forming units (cfu) of *E. coli*were injected into the left thigh at -2 hours (h). Once an infection was established antibiotic therapy was assessed. Meropenem, aztreonam, ciprofloxacin or tobramycin were administered subcutaneously starting at 0h and given either as a single dose or dose fractionated and given in equal aliquots and time intervals over 24 hours. At 0 and 24 h post infection, animals were euthanized by CO_2asphyxiation. The thigh was excised, homogenized and cultured for bacterial enumeration. The static dose (mg/kg/day), the dose required to maintain bacterial levels similar to that at the start of therapy, or magnitude of PK/PD parameter was calculated using the equationLog_{10}static dose or Log_{10} PK/PD parameter = [log (E / Emax-E)] / |n| + log EC_{50}, where, E is the control growth (log_{10}change in cfu per thigh in untreated controls after the 24 h period of study), E_{max} is the maximum effect, ED_{50} is the dose required to achieve 50% of E_{max}, and N is the slope of the dose effect curve.

Dose fractionation studies were performed using aztreonamor tobramycin. The static dose was calculated for each dosing regimen. When treating with aztreonam the static dose decreased as the dosing frequency increased, suggesting time above MICas the driver for efficacy (Q12H - 610 mg/kg/day, Q6H - 283 mg/kg/day and Q4H - 61 mg/kg/day). In contrast, the static dose for tobramycin remained similar despite changes in dosing frequency which, suggests AUC as the predominant PK/PD driver (Q24H - 4 mg/kg/day, Q12H - 10 mg/kg/day and Q6H - 16 mg/kg/day). The model was used to test aztreonam, meropenem, tobramycin and ciprofloxacin using a Q4H or Q24H dosing strategy. All treatments reduced bacterial load in the thigh in a dose dependent-manner, reachingstatic levels. The dose and magnitude of key PK/PD parameters required for bacterial stasis were calculated for each compound (**Table 1**).

Table 1: Calculated static dose or magnitude of PK/PD parameter required for stasis against *E.coli* (ATCC 700928). *f*, free; Q4H, dosing every 4 hours; Q24H, dosing every 24 hours.

Treatment	mg/kg/day	Key PK/PD Parameter(s)	Magnitude
Aztreonam Q4H	105	fT>MIC	46
Meropenem Q4H	103	*f*T>MIC	40
Tobramycin Q24H	12	*f*AUC/MIC,*f*C_{max}/MIC	16, 26
Ciprofloxacin Q24H	10	*f*AUC/MIC,*f*C_{max}/MIC	75, 28

Here, it is demonstrated that *E.coli* ATCC 700928 is susceptible to antimicrobial therapy in a neutropenic thigh model and can be used to assess PK/PD using a dose fractionation approach. This strain can now be used for the determination of efficacy and PK/PD relationships of novel compounds.