Correlating survival of *Galleria mellonella* with PKPD modelling of ciprofloxacin and meropenem.

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1 INTRODUCTION

Characterising direct relationships between dosing regimens of antimicrobial agents and successful outcomes in *in vivo* models is a challenge that can result in prolonged screening phases with financial and ethical considerations. Linking treatment outcome of an agent in an invertebrate infection model with its in vitro PK/PD relationship enables a targeted approach to dosing in *in vivo* models by identifying early the pharmacokinetic drivers of bacterial kill in lead candidate anti-infectives.

Greater wax moth (*Galleria mellonella*) larvae are an established model of infection for virulence testing and host-pathogen interactions, due to their ability to thrive at 37°C and an immune response with direct similarities to the innate immune system of mammals. They are also ideal for large-scale experiments owing to their small size, being without regulatory burden and not requiring specialised equipment. TruLarvTM larvae (Biosystems Technology) used in these experiments are research grade larvae bred free of hormones and antibiotics that are specified pathogen free.

Readouts of *G. mellonella* infection models typically involve survival of the larvae alone, however the relationship between larval pharmacokinetics, CFU response and survival is not well characterised. By understanding better how survival response is linked to pharmacokinetic exposure, a more targeted approach to dosing can used when entering in vivo models. The magnitude of distinct PK parameters in a dosing regimen can be quantified, helping identify a suitable dosing regimen, which when linked with the susceptibility of the organism can drive treatment success.

2 MATERIALS AND METHODS

G. Mellonella infection

G. mellonella were infected with a lethal dose of *P. aeruginosa* before treatment with increasing doses of either meropenem or ciprofloxacin at 2 hours post infection (hpi). Larvae were incubated at 37°C. Survival was monitored over 8h and CFU taken from satellite groups at 2, 4, 6, and 8 hpi (ciprofloxacin) and 2,4, and 6 hpi (meropenem).

Pharmacokinetics

In a separate experiment uninfected *G. mellonella* larvae were dosed with the same titration of ciprofloxacin or meropenem. At 2, 4, 6, 8 and 24 hpi haemolymph was sampled from each larva and stored at -20°C. Antimicrobial concentrations were determined by microbiological PK determination by spotting 10 µL haemolymph onto blank antimicrobial discs overlaid on agar plates spread with a highly susceptible *E. coli* strain. A standard curve was produced using blank haemolymph containing known antimicrobial concentrations.

PK/PD Model

Concentrations determined in the microbiological PK assay were modelled in a one-compartment PKPD model. The starting concentration was held for 1h before antimicrobial elimination was modelled by instillation of fresh broth and elimination of waste by peristalsis. At 2, 4, 6, 8 and 24h CFU were taken, washed and enumerated by standard methods.

4 CONCLUSIONS

Both ciprofloxacin and meropenem at 8-16µg/mL prevented death in *G. mellonella* at 8hpi (Fig. 1). AUCCFU analysis of infected *G. mellonella* revealed a dose-dependant response with ciprofloxacin treatment, as was expected (Fig.2). Meropenem treatment resulted in a basal AUCCFU level being achieved at a dose of 16µg/mL, with further increases in concentration not resulting in further response.

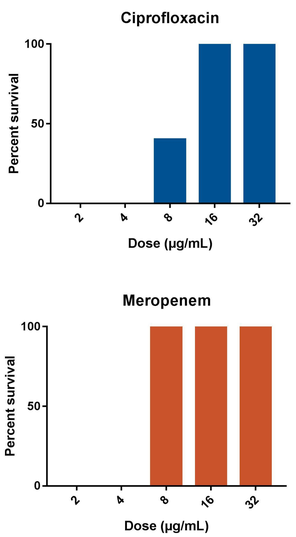
PK analysis of haemolymph (Fig. 3) sampled from treated *G. mellonella* larvae revealed a first order elimination rate dependent on drug concentration. Concentrations were static between 0 and 1h post treatment..

A ciprofloxacin AUC/MIC of >30 resulted in a 3Log reduction in AUCCFU (dosing regimens 32 and 16µg/mL) correlating with 100% survival at 8hpi in *G. mellonella*.

In meropenem, a correlation was observed between the AUCCFU and the AUC/MIC in the model. While t>MIC is considered the best indicator of meropenem efficacy, some have reported that extending the half-life of meropenem results in AUC/MIC being the most predictive index. As the half-life of meropenem was recorded as being longer than in mammalian subjects pharmacokinetic considerations must be made when using invertebrate models of infection.

Modelling *G. mellonella* pharmacokinetics enables the assessment of potential novel compounds and allows identification of the pharmacodynamic driver of bacterial kill. This analysis provides some insight into potential efficacious dosing regimens for use in larger scale invertebrate and *in vivo* animal models. Caution must also be taken when translating data derived from invertebrate to mammalian models as the factors affecting the pharmacokinetics (elimination, protein binding etc.) may differ.

3 FIGURES



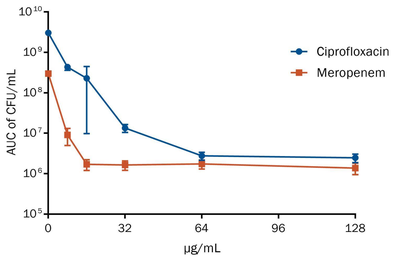


Figure 1 (left): Percentage survival of *G. mellonella* after infection with *P. aeruginosa* PAO1 and escalating dosing of ciprofloxacin and meropenem (n=5, except 2 and 4 µg/mL where n=3, hpi=8).

Figure 2 (above): AUC_{CFU24} of CFU/mL recovered from *G. mellonella* with increasing doses of ciprofloxacin and meropenem. A reduction in AUC_{CFU} is observed in a dose dependent manner with ciprofloxacin treatment to a 3-Log reduction at 64 µg/ml. Meropenem treatment resulted in basal AUC_{CFU24} at 16 µg/mL.

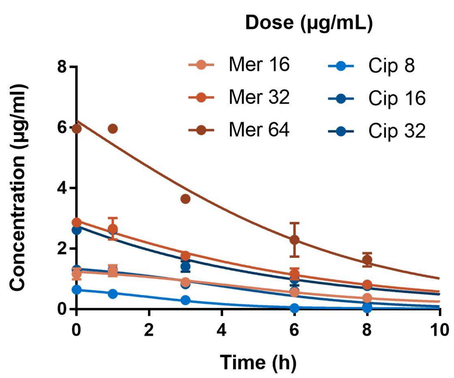


Figure 3: Meropenem and ciprofloxacin concentrations detected in the haemolymph of *G. mellonella* over time (64 to 8 µg/mL shown). Concentrations were measured by microbiological assay measured from standard curves performed with blank haemolymph from untreated *G. mellonella* (n=3 ± SD). First order elimination was observed with elimination rate dependent on starting concentration of the antimicrobial agent.

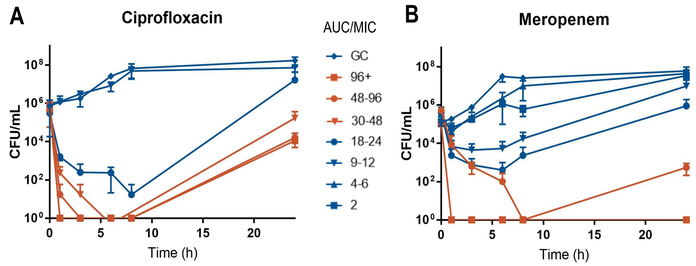


Figure 4 (above): *In* vitro CFU response against measured PK profiles in *G. mellonella* in **(A)** ciprofloxacin and **(B)** meropenem. Response curves are grouped by AUC/MIC (n=3-6 ±SEM). Significant regrowth at 24h occurred in all experiments except in meropenem with an AUC/MIC of >96.

Figure 5 (left): Plot of AUC_{CFU} against AUC/MIC of ciprofloxacin (A) and meropenem (B). A ciprofloxacin AUC/MIC of >30 was sufficient to reduce AUC_{CFU} by 3Log and rescue *G. mellonella* infected with an LD of PAO1. Response to meropenem showed better correlation with AUC/MIC than t>MIC. This may be due to the an extended half-life being detected in *G. mellonella* compared to mammalian subjects (n=3).

R^2 value A = 0.847.
R^2 value B = 0.909.