The Neutropenic Rat Thigh Infection Model is Ideal for Characterisation of Pharmacokinetics/Pharmacodynamics (PK/PD) of Anti-infectives Following Intravenous Infusion Administration

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Introduction and Purpose

The mouse is the workhorse for characterisation of anti-infective PK/PD with high predictive value in humans (1), but it has some limitations. The rat model offers advantages over the mouse in the following cases: a) Evaluation of Pharmacokinetics (PK)/Pharmacodynamics (PD) of anti-infectives administered as intravenous (IV) infusions similar to clinical situations; b) Obtaining simultaneous PK and PD data from the same animal.

Limited information exists on PK/PD of drugs following IV infusion dosing in the neutropenic thigh infection model in rat.

Aim: To characterise the PK/PD of Ciprofloxacin in the neutropenic thigh infection model following IV bolus and infusion administrations at human equivalent doses.

Methods

- **Microorganisms:** *Escherischia coli* (ATCC25922) (Ec), *Pseudomonas aeruginosa* (ATCC27853)(Pa), *Acinetobacter baumannii* (ATCC19606) (Ab), *Klebsiella pneumoniae* (ATCC13883) (Kp) were from ATCC.
- **Minimum Inhibitory Concentration (MIC)** was performed in microtiter plate format using standard microdilution methods.
- **Chemicals:** Ciprofloxacin (CIP) and Cyclophosphamide were from Sigma and TCI chemicals, respectively. All other reagents were of analytical grade.
- **Animal Studies**: Study protocols were approved by the Institutional Animal Ethics Committee. Male Wistar rats (6-8 weeks) were used in PK and PD studies.
- **Neutropenic Rat thigh infection (RTI) model** (2): Neutropenia was induced by cyclophosphamide (1). Ec/Pa/Ab/Kp was injected into thighs (~2 x 10^6 CFU/animal). Treatment was initiated at 2 h post infection (PI);animals were terminated at 26 h PI (24 h post dose), thighs collected, weighed, homogenised and plated for bacterial enumeration (Log_{10}CFU/g thigh).
- **IV PK/PD:**
  - ❑PD: Two h PI, rats infected with Ec or Pa, were given CIP at 10, 30 and 100 mg/kg as IV bolus, 30 and 60 min IV infusions (constant rate). Infusions were administered to anesthetized rats (Ketamine (60 mg/kg IP) + Xylazine (10 mg/kg IP)). Bacterial densities in thighs were estimated at 24 h post dose.
  - ❑PK: PK was performed in PD animals. Serial blood samples were drawn at different time points and plasma harvested. CIP was quantified in plasma by LC/MS/MS. Non-compartmental PK parameters were estimated using WinNonlin (Certara)
  - ❑PD of CIP at rat equivalent human doses in rats (3): The doses of CIP used in the clinic were converted to rat doses using the formula :

Rat dose (mg/kg) = Human dose (mg/kg)/ (rat weight in kg/human weight in kg)$^{0.33}$

CIP Human dose (4,5)	Rat equivalent total dose/regimen
200 mg *bid*, IV=Total dose 400 mg (7 mg/kg)	Total dose 50 mg/kg (*uid*), 1 h IV inf
	Total dose 50 mg/kg (25 mg/kg, *bid*) 1 h IV inf
400 mg *bid*, IV =Total dose 800 mg (14 mg/kg)	Total dose 90 mg/kg (*uid*), 1 h IV inf
	Total dose 90 mg/kg (45 mg/kg *bid*), 1 h IV inf

Rats infected with Ec, Pa, Ab and Kp were treated with indicated doses/regimens. Bacterial densities in thighs were estimated at 24 h post dose.

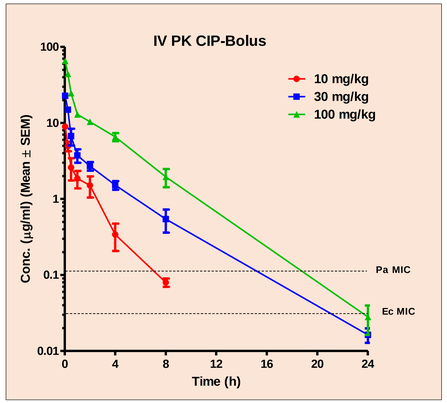
Data analysis: 1 way ANOVA (95 % confidence levels)

RESULTS

MIC

Organism	MIC range (µg/ml)
***E. coli* (ATCC25922)**	**0.0156 - 0.03125**
***P. aeruginosa* (ATCC27853)**	**0.0625 - 0.125**
***A. baumannii* (ATCC19606)**	**0.25 - 0.5**
***K. pneumoniae* (ATCC13883)**	**0.125 - 0.25**

IV (Bolus) Pharmacokinetics of CIP in Infected Rats

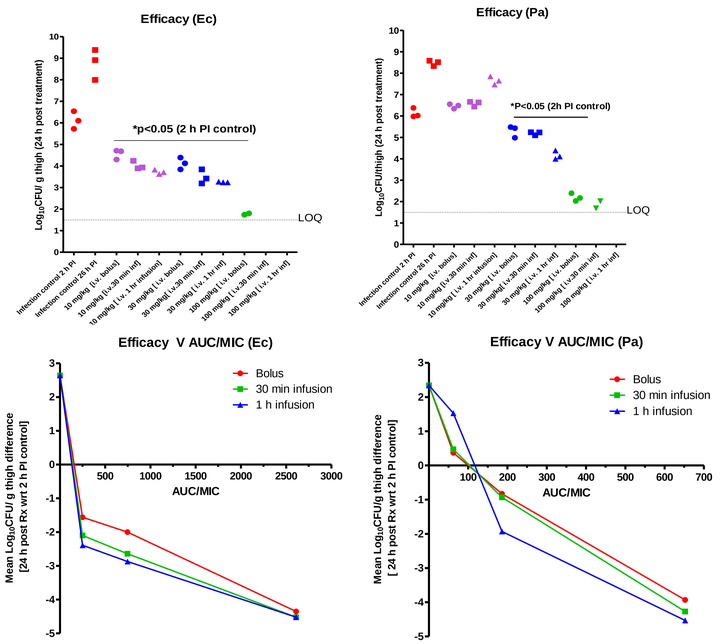


NCA PK parameters and PK/PD indices of CIP in rats infected with Ec : IV Bolus

Dose	10 mg/kg	30 mg/kg	100 mg/kg
C_{max} (µg/ml)	8.95 ± 0.74	22.8± 2.0	65.43 ± 4.5
AUC_{inf} (µg.h/ml)	7.78 ± 2.14	23.3 ± 5.1	81.52 ± 2.41
$t_{1/2}$ (h)	1.54 ± 0.18	3.0 ± 0.29	2.48 ± 0.5
V_{ss} (l/kg)	2.3 ± 0.74	3.95 ± 0.5	3.64 ± 0.33
CL(l/h/kg)	1.35 ± 0.33	1.33 ± 0.3	1.23 ± 0.04
AUC_{24}/MIC_{Ec}	249	746	2609
AUC_{24}/MIC_{Pa}	62	186	652
C_{max}/MIC_{Ec}	286	730	2094
C_{max}/MIC_{Pa}	72	182	523

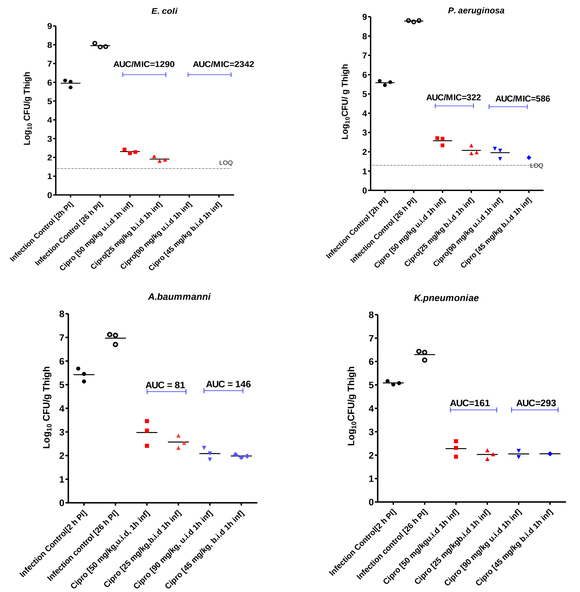
- **CIP showed linear PK with high V_{ss} and moderate clearance**
- **Pharmacologically active PK/PD targets achieved against Ec and Pa**

IV Pharmacodynamics of CIP in RTI model



- ❑ **CIP showed significant dose dependent efficacy when given as bolus and infusions**
- ❑ **Efficacy of CIP was comparable when administered as bolus, 30 min and 60 min infusions, consistent with PK/PD index**
- ❑ **AUC/MIC ~200 associated with 1 Log_{10}CFU/g thigh reduction**

Efficacy at Rat Equivalent Human Doses



Species	Dose/regimen/IV Infusion	AUC_{24h} (µg.h/ml)	AUC_{24}/MIC	C_{max} (µg/ml)	C_{max}/MIC	Efficacy
Humans*	**Total dose 400 mg (7 mg/kg -200 mg *bid*, IV)**	**49 (9.0-229)**	**802 (6.2-5541)**	**4.8 (1.9-15.4)**	**100 (0.9-769)**	**88% Clinical cure/ 81 % microbiological cure**
Rat (human equivalent dose)	**Total dose 50 mg/kg (*uid*)**	**40**	**81-1290**	**34**	**69-1102**	**100% survival ;> 3 Log_{10}CFU/g thigh reduction at 24 h post treatment**
	Total dose 50 mg/kg (25 mg/kg *bid*)	**40**	**81-1290**	**19**	**38-604**	**100% survival ;> 3 Log_{10}CFU/g thigh reduction at 24 h post treatment**
	Total dose 90 mg/kg (*uid*)	**73**	**146-2342**	**59**	**119-1900**	**100% survival ;> 3 Log_{10}CFU/g thigh reduction at 24 h post treatment**
	Total dose 90 mg/kg (45 mg/kg *bid*)	**73**	**146-2342**	**31**	**62-992**	**100% survival ;> 3 Log_{10}CFU/g thigh reduction at 24 h post treatment**

* Data are from ref 4

Conclusions

- ❑ **The 24 h exposures of CIP in infected rats at human equivalent doses were similar to human exposures (4) when administered as IV infusions.**
- ❑ **Range of 24 h PK/PD indices and efficacies achieved in rats were comparable to humans at equivalent doses (4).**
- ❑ **PK/PD of CIP was similar to mice (1) and humans (4) - significant efficacy observed when AUC/MIC > 125.**

The rat thigh infection model can be useful for a) Evaluation of PK/PD of anti-infectives intended for IV infusions, b) Simultaneous recording of PK and PD in the same animal, c) Developing PK/PD models for anti-infectives, d) Prediction of PK/PD in humans, and e) Additional species for proof of concept studies.

References

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