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Activity of fluoroquinolones (finafloxacin, levofloxacin, ciprofloxacin) *vs*. that of imipenem against extracellular and intracellular *Burkholderia thailandensis*

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Introduction & Purpose

Burkholderia pseudomallei (BP) is the agent of melioidosis (a lethal infection in humans). *B. thailandensis* (BT) is considered as a surrogate for BP, as both species are facultative intracellular bacteria that can escape from phagosomes and thrive in the cytosol of the host cells [1-2].

The aim of this work was to compare the activity of fluoroquinolones to that of a typical β-lactam (imipenem) against the extracellular and intracellular forms of infection by *B. thailandensis,* both antibiotic classes having access to eukaryotic cell cytosol [3]. Among fluoroquinolones, we selected finafloxacin (showing enhanced activity at acidic pH [4]; currently in development for the treatment of serious bacterial infections in the hospital and critical care setting), and compared it with levofloxacin and ciprofloxacin.

Materials & Methods

Bacterial strain: BT ATCC 700388 (reference strain).

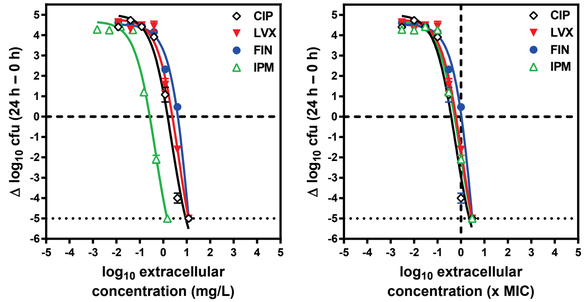
Extracellular infection: (i) incubation of bacteria (initial inoculum : 10^6 cfu/mL) during 24h with antibiotics (0.003-100 x MIC) in MHB-CA; (ii) cfu counting after appropriate dilution and overnight incubation on agar plates containing 0.4% charcoal (to mitigate carry-over effect).

Intracellular infection in human THP-1 cells: (i) phagocytosis of human serum-opsonized bacteria (1 h; 10 bacteria/cell); (ii) elimination of non-phagocytosed bacteria by incubation with gentamicin (1h; 100 x MIC); (iii) 24h incubation of infected cells with antibiotics (0.003-100 x MIC).

Pharmacodynamic parameters: Maximal efficacy (E_{max}) and relative potency (C_s [apparent static concentration]) calculated from the Hill function fitted to the data (GraphPad Prism ® [5]).

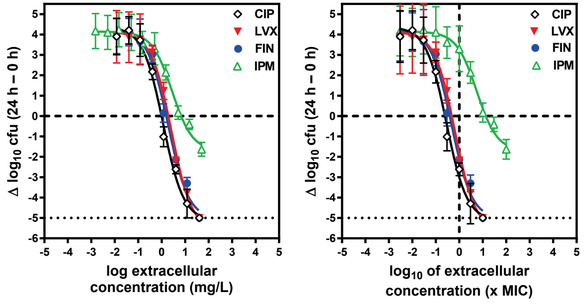
Results

Extracellular concentration-response curves



Antibiotic	MIC (mg/L)	Extracellular C_s* (x MIC)	Extracellular E_{max}** (Δ $\log_{10}$ cfu)
Finafloxacin	4	0.8±0.3	< -5
Levofloxacin	4	0.5±0.2	< -5
Ciprofloxacin	4	0.4±8.7	< -5
Imipenem	0.5	0.5±0.2	< -5

Intracellular concentration-response curves



Antibiotic	MIC (mg/L)	Intracellular C_s* (x MIC)	Intracellular E_{max}** (Δ $\log_{10}$ cfu)
Finafloxacin	4	0.4±0.1	< -5
Levofloxacin	4	0.5±0.2	< -5
Ciprofloxacin	4	0.3 ±0.1	< -5
Imipenem	0.5	13.4 ±15.3	-1.7 ±0.2

- **Extracellularly,** all antibiotics showed a C_s close to their MIC and E_{max} values below the detection level (5 $\log_{10}$ cfu decrease [apparent complete eradication]).
- **Intracellularly,** fluoroquinolones were as potent and effective as extracellularly while imipenem was less potent (C_s at 13x MIC) and considerably less effective (less negative E_{max}).

* **C_s** (relative potency): extracellular concentration resulting in no apparent bacterial growth as compared to the initial inoculum.

** **Emax** (maximal relative efficacy): cfu change (in $\log_{10}$ units) at 24 h from the initial inoculum as extrapolated for an infinitely large antibiotic concentration, based on the Hill equation (slope factor set to 1)

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Conclusions

- Fluoroquinolones are remarkably effective against intracellular *B. thailandensis,* consistent with their activity already demonstrated for intracellular *Listeria monocytogenes,* which also thrives in the cytosol [6].
- In contrast, imipenem, although part of the β-lactam class of antibiotics often recommended for the treatment of *Burkholderia* infections, was unable to eradicate intracellular bacteria.
- **This work rationalizes the recently demonstrated efficacy of finafloxacin in an animal model of *B. pseudomallei* infection [7].**

References

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