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Activity of finafloxacin, ciprofloxacin, and imipenem against intracellular *Burkholderia thailandensis*, *Yersinia pseudotuberculosis*, and *Francisella philomiragia* in media at neutral or acidic pH

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Introduction & Purpose

The biothreat pathogens *Burkholderia pseudomallei* (agent of melioidosis), *Yersinia pestis* (agent of plague), and *Francisella tularensis* (agent of tularemia), are all facultative intracellular organisms that reside in the cytosol [1], phagolysosomes [2] and phagosomes and cytosol [3], respectively. *B. thailandensis*, *Y. pseudotuberculosis,* and *F. philomiragia* can be used as their surrogates in the laboratory.

The aim of this work was to examine the activity of fluoroquinolones in comparison with that of a typical β-lactam (imipenem) against the intracellular forms of infection of these bacteria, knowing that fluoroquinolones show a high intracellular activity against many intracellular bacteria, irrespective of their subcellular localisation [4].

Among them, finafloxacin (currently in development for the treatment of serious bacterial infections in acute and critical care hospital settings) was selected because of its enhanced activity at acidic pH [5], which may confer to it an advantage in infected sites where acidic microenvironments are common.

We compared finafloxacin with ciprofloxacin and imipenem, in broth and in infected THP-1 monocytes using culture media maintained at neutral or acidic pH.

Materials & Methods

Extracellular infection: (i) incubation of bacteria (initial inoculum: 10^6 cfu/mL) during 24h with antibiotics (0.003-100 x MIC) in CA-MHB; (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 (1h; 5-10 bacteria/cell); (ii) elimination of non-phagocytised bacteria by incubation with gentamicin (1h; 15-100 x MIC); (iii) 24h incubation of infected cells with antibiotics (0.003-100 x MIC) to obtain full concentration-effects realtionships. Accumulation of finafloxacin in THP-1 cells was measured using [14C]labeled drug [6].

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 ®) [7].

Results

Pharmacodynamic parameters of finafloxacin *vs.* ciprofloxacin and imipenem

Antibiotic	pH of the medium	*B. thailandensis* ATCC 700388			*Y. pseudotuberculosis* ATCC 29833			*F. philomiragia* ATCC 25015		
		Broth	Intracellular		Broth	Intracellular		Broth	Intracellular	
		MIC (mg/L)	E_{max}[a] (Δlog cfu)	C_s[b] (mg/L)	MIC (mg/L)	E_{max}[a] (Δlog cfu)	C_s[b] (mg/L)	MIC (mg/L)	E_{max}[a] (Δlog cfu)	C_s[b] (mg/L)
Finafloxacin	7.4	4	-5*	1.6	0.25	-5*	0.15	0.06	-4*	na[c]
	5.5	1	-5*	0.7	0.12	-5*	0.03	0.008	-4*	na[c]
Ciprofloxacin	7.4	4	-5*	1	0.06	-3.3	0.08	0.015	-4*	na[c]
	5.5	32	-5*	3.7	1	-3.6	0.14	0.06	-4*	na[c]
Imipenem	7.4	0.5	-1.7	0.7	0.25	-0.8	1.9	0.25	-1.8	na[c]
	5.5	8	-1.5	2.2	8	-0.9	2.1	32	-0.1	na[c]

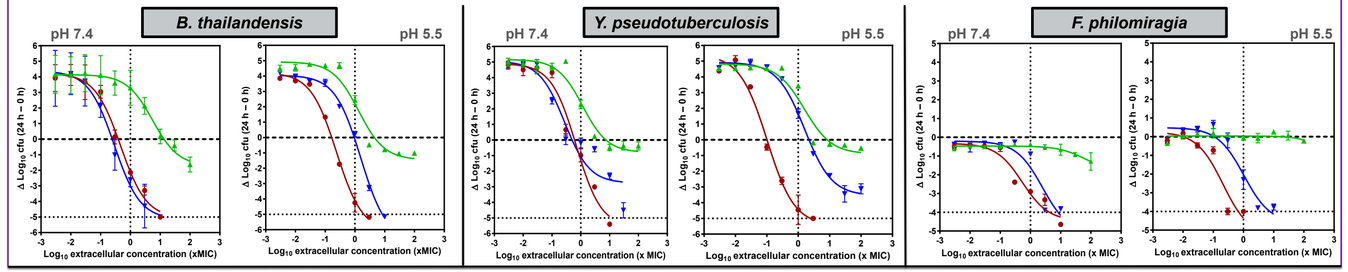
a E_{max} (maximal relative efficacy): cfu change (in $\log_{10}$ units) at 24 h from the initial inoculum as extrapolated for an infinitely large antibiotic concentration. When cfu counts fell below the lowest detection level within the range of extracellular drug concentrations investigated, E_{max} was not calculated but arbitrarily set at -5 (or -4 for intracellular *F. philomiragia*) and marked with an *.

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

c Not applicable because the intracellular inoculum at 24h is lower than the initial inoculum.

- **Extracellularly (broth),** MICs of finafloxacin were 1-3 doubling dilutions lower while those of ciprofloxacin and imipenem were 2-4 and 4-7 doubling dilutions higher, respectively, at acidic pH compared to neutral pH.
- **Intracellularly**,
  - Finafloxacin was more potent (lower C_s) when cells are incubated in a medium at acidic pH than at neutral pH and was highly effective, causing a complete eradication of all three bacterial species (E_{max} reaching the limit of detection) at both pHs.
    Finafloxacin accumulated 15-20 times in THP-1 cells when using medium adjusted to pH 5.5 but only 5 times at pH 7.4 (not illustrated).
  - Ciprofloxacin was less potent (higher C_s) at acidic pH and remained capable of eradicating *B. thailandensis* and *F. philomiragia* but not *Y. pseudotuberculosis.*
  - Imipenem was also less potent at acidic pH and considerably less effective against all intracellular bacteria (E_{max} < 1.8 log cfu decrease).

Concentration-effects of finafloxacin (FIN) *vs.* ciprofloxacin (CIP) and imipenem (IPM) towards intracellular bacteria in media at pH 7.4 or 5.5



Main messages

- In broth, and in contrast to ciprofloxacin and imipenem, finafloxacin shows higher intrinsic activity (lower MICs) at acidic pH than at neutral pH against the three bacterial species studied here.
- Intracellularly, finafloxacin is remarkably effective against the three species, both in media at neutral or acidic pH. Its higher accumulation at acidic pH rationalizes its increased intracellular potency when cells are incubated in acidic medium.
- This work confirms the interest of fluoroquinolones in general, and of finafloxacin in particular, for the treatment of infections by intracellular bacteria, whatever their subcellular localization, as previously shown for other intracellular species [8], and whatever the pH of the environment. It rationalizes the recently demonstrated efficacy of finafloxacin in an animal model of infection with *B. pseudomallei* [9].

References

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Acknowledgments

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