Cellular accumulation and intracellular activity of finafloxacin (FIN), a novel fluoroquinolone (FQ) with enhanced activity at acid pH, against *S. aureus* (*S.a.*) and *L. pneumophila* (*L.p.*): comparison with ciprofloxacin (CIP) and moxifloxacin (MXF).

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Abstract

Background. FIN, in contrast to other FQ, shows markedly lower MIC's at acidic pH (Kresken et al, ICAAC 2008, poster F1-2037). We have measured its cellular accumulation and assessed its intracellular activity against organisms located in acidic (*S.a.*, pH 5-5.5 [phagolysosomes]) or moderately acidic (*L.p.*; pH 6 [phagosomes]) organelles in human THP-1 macrophages, in comparison with CIP and MXF.

Methods. Accumulation: uninfected cells exposed to neutral and acidic pH, and cells exposed to 10 mM NH_4Cl (to neutralize the phagolysosomal pH). Intracellular activity: in infected cells (*S.a.* ATCC 25923; *L.p.* ATCC 33153) exposed to antibiotic concentrations from 0.001 to 100 mg/L for 24 h (*S.a.*) or 48 h (*L.p.*), with changes in $\log_{10}$ CFU (compared to time 0) used to fit a Hill equation to determine static (C_s) and 1 $\log_{10}$ CFU drop (C_{90}) concentrations, and maximal relative efficacy [E_{max}] (Barcia-Macay et al, AAC 50:841-851).

Results. The main results are shown in the Table.

	FIN		CIP		MXF	
Cell. Accum.	**pH 7.4**	**pH 5.5**	**pH 7.4**	**pH 5.5**	**pH 7.4**	**pH 5.5**
Control (Cc/Ce) [a]	1.9 ± 0.1	8.1 ± 1.3	9.3 ± 0.1	5.1 ± 0.8	5.5 ± 1.7	1.6 ± 0.1
+ NH_4Cl (% of control)	39 ± 29	N.D.	229 ± 15	N.D.	256 ± 6	N.D.
Intr. Activity *	***S.a.***	***L.p.***	***S.a.***	***L.p.***	***S.a.***	***L.p.***
C_s (mg/L) [b]	1.8	0.08	0.26	0.35	0.2	N.D.
C_{90} (mg/L) [c]	7.5	0.38	1.00	3.59	0.54	0.93
E_{max} [d]	-1.6 ± 0.3	-2.7 ± 0.3	-1.7 ± 0.1	-2.0 ± 0.3	-2.2 ± 0.1	-2.9 ± 0.3

[a] Cellular to extracellular concentration ratio (Cc/Ce)
[b] concentration (mg/L) causing no apparent change from post-phagocytosis inoculum
[c] concentration (mg/L) causing a 1 $\log_{10}$ drop from post-phagocytosis inoculum
[d] change in $\log_{10}$ CFU per mg of cell protein from the original, post-phagocytosis inoculum for an infinitely large concentration of antibiotic (Hill equation, $R^2 > 0.95$ for all conditions)
* MIC (mg/L; broth): S. a. pH 7.4/pH 5.5 – FIN: 0.06/0.007; CIP: 0.125/1; MXF: 0.03/0.06

Conclusions. Although displaying lower MICs in acidic medium, FIN showed larger Cs and C_{90} than CIP and MXF against intracellular *S.a.* Conversely, FIN was more active against intracellular *L.p.* The divergent effect of NH_4Cl on accumulation suggests distinct pH-dependent transport behaviors and / or different subcellular localizations. The data show that, even within a pharmacological class, intracellular activity cannot be predicted based on intrinsic potency and/or accumulation only, but that other parameters (subcellular disposition, localization of the bacteria) may also be critical.

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Background and aim

Treatment of intracellular infections requires that antibiotics enter cells and express activity therein. Acidic conditions prevailing within the phagolysosomes (pH 5.0 – 5.5) or phagosomes (pH 6.0) of infected cells may, however, significantly impair the activity of many antibiotics such as gentamicin, azithromycin or clindamycin.[1-3]

Finafloxacin is a novel fluoroquinolone showing enhanced activity at acidic pH. [4] In this context, our aim was to measure assess the cellular accumulation of finafloxacin in macrophages and to measure its intracellular activity towards S.a (phagolysosomes) and L.p. (phagosomes).

Methods

Cells. Experiments were performed with human THP-1 cells, a myelomonocytic cell line displaying macrophage-like activity.[5]

Assay of cell-associated antibiotics. Fluoroquinolones were assayed by the disc-plate method using *S. aureus* ATCC 25923 as test organism or fluorimetry (for ciprofloxacin and moxifloxacin only).

Bacterial strain and susceptibility testing. *S. aureus* strain ATCC 25923 and *L. pneumophila* strain ATCC 33153 (Manassas, VA) were used throughout. MICs determinations were made, respectively, in Mueller Hinton broth (for *S.a.*, 24 h) or in α-ketoglutarate Buffered Yeast Extract broth adjusted at pH 6.9 (for *L. p.*, 48 h).

Cell infection and determination of the intracellular activities of antibiotics. Phagocytosis was initiated at a bacteria per macrophage ratio of 4 (for *S.a.*, 1 h) or 10 (for *L.p.*, 2 h), followed by elimination of non-phagocytosed bacteria by exposing the cells to 50 mg/L gentamicin (30-45 min). Cells were then transferred to fresh medium supplemented with increasing concentrations of antibiotics. Results, expressed as the change in the intracellular inoculum at 24 h (for *S. a.*) or 48 h (for *L.p.*) compared to time 0, were used to fit a Hill equation to allow determination of the values of key pharmacological descriptors of antibiotic activity (see ref. 2 for details).

References

(1) Baudoux *et al*, J. Antimicrob Chemother. (2007) 59: 246-253.
(2) Barcia-Macay *et al*, Antimicrob Agents Chemother (2006), 50: 841-851.
(3) Lemaire *et al*, Antimicrob Agents Chemother (2009), jun 29 (in press).
(4) Kresken *et al*, ICAAC 2009, poster F1-2037
(5) Tsuchiya *et al*, *Intern. J Cancer* (1980) 26: 171-176.

Results

Cellular accumulation of fluoroquinolones

A. Influence of the pH medium

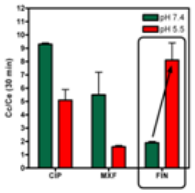

Fig. 1. Influence of the pH medium (7.4 vs. 5.5) on the cellular accumulation of finafloxacin and comparators in THP-1 cells (37°C, 30 min). Results were expressed as cellular to extracellular concentration ratio (Cc/Ce).

➔ **Decreasing the pH of the culture medium from 7.4 to 5.5 markedly enhanced the cellular accumulation of finafloxacin, with an opposite effect for CIP and MXF.**

B. Influence of ammonium chloride

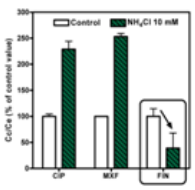

Fig. 2. Influence of ammonium chloride **(known to neutralize the phagolysosomal pH)** on the cellular accumulation of finafloxacin and comparators (37°C, 2 h). Results were expressed as a change from control. percentage of control value.

➔ **Exposure of cells to ammonium chloride reduced the cellular accumulation of finafloxacin, and had the opposite effect on CIP and MXF**

Susceptibility testings

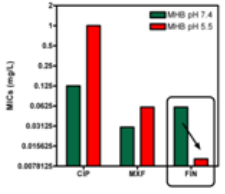

Fig. 3. Influence of pH on the intrinsic activity of finafloxacin and comparators against *S. aureus* ATCC 25923.

➔ **Decreasing the pH from 7.4 to 5.5 significantly increased the activity of finafloxacin towards *S. aureus* while the activity of CIP is markedly and that of MXF slighly reduced.**

Intracellular activity of antibiotics

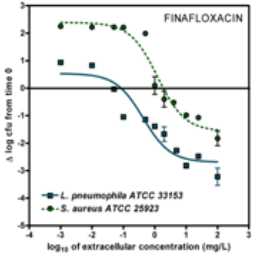

Fig. 4. Dose-response curves of finafloxacin against *S. aureus strain* ATCC 25923 (phagolysosomal infection) or *L. pneumophila* strain ATCC 33153 (phagosomal infection) phagocytized by human THP-1 macrophages after 24 h (for *S.a.*) or 48 h (for *L.p.*) incubation of the cells in the presence of increasing concentrations of antibiotic. The ordinate shows the change in CFU (in $\log_{10}$ units) per mg of cell protein.

Finafloxacin shows larger static conc. and C_{90} than CIP or MXF against intracellular *S. aureus*, but considerably lower values against intracellular *L. pneumophila*. In all cases, these concentrations are lower than the expected C_{max} of FIN in patients

Table 1. Pharmacological descriptors of antibiotic activity of the dose-response curves of finafloxacin (FIN), ciprofloxacin (CIP), and moxifloxacin (MFX)and against *S.a.* or *L.p.*

Parameters	*S.a.*			*L.p.*		
	FIN	**CIP**	**MXF**	**FIN**	**CIP**	**MXF**
Cs (mg/L [multiples of C_{max}]) [a,d]	1.8 [0.16]	0.26 [0.06]	0.20 [0.05]	0.08 [0.007]	0.35 [0.07]	N.D.
C_{90} (mg/L [multiples of C_{max}]) [b,d]	7.5 [0.67]	1.00 [0.23]	0.54 [0.13]	0.38 [0.003]	3.59 [0.81]	0.93 [0.23]
E_{max} [c]	-1.6 ± 0.3	-1.7 ± 0.1	-2.2 ± 0.1	-2.7 ± 0.3	-2.0 ± 0.3	-2.9 ± 0.3

[a] concentration (mg/L) causing no apparent change from post-phagocytosis inoculum (static concentration)
[b] concentration (mg/L) causing a 1 $\log_{10}$ drop from post-phagocytosis inoculum
[c] change in $\log_{10}$ CFU per mg of cell protein from the original, post-phagocytosis inoculum for an infinitely large concentration of antibiotic (Hill equation, $R^2 > 0.95$ for all conditions)
[d] based on C_{max} values of 11.1 mg/L for FIN (phase I study), and 4.4 and 4 mg/L for CIP, and MXF (see ref. 2).

Conclusions

- **The effect of pH on FIN uptake shown here suggests that its accumulation could be increased *in vivo* in acidic environments (infected or inflamed tissue, cutaneous abscesses, …).**
- **Despite its higher activity in acidic medium (broth), finafloxacin was less potent (larger Cs and C_{90} values) than ciprofloxacin or moxifloxacin towards *S. aureus* (phagolysosomial infection; pH ~ 5-5.5) but more potent towards *L. pneumophila* (phagosomal infection; pH ~ 6).**
- **This shows that intracellular activity cannot be predicted from intrinsic potency (MIC) and/or cellular pharmacokinetics only, but that other parameters not analyzed here are critical.**
- **Further work will need to examine the intracellular localization and pharmacodynamics of FIN at different external pHs.**

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