Activity of finafloxacin (FNX), a fluoroquinolone with enhanced activity at acid pH, against intracellular *Legionella pneumophila*: comparison with azithromycin,clarithromycin, telithromycin, ciprofloxacin and moxifloxacin

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Abstract

Background:
LP invades macrophages and multiplies in moderately acidic vacuoles (Sturgill-Koszycki & Swanson, J Exp Med 2000, 192:1261-72). Macrolides and quinolones are commonly recommended treatment for LP infections. In this context, our aim was to examine the activity FNX (a novel fluoroquinolone exhibiting increased activity under acidic conditions [Higgins et al., AAC 2010, 54:1613-5]) against intracellular LP.

Methods:
L. pneumophila ATCC 33153 and THP-1 cells were used. MICs were measured in α-ketoglutarate buffered yeast extract (pH 6.9). Infection of THP-1 cells was performed as described earlier (Lemaire et al. AAC 2009, 53:3734-3743). Briefly, cells were allowed to phagocytize bacteria at a 10:1 bacteria per cell ratio (2 h). Non-phagocytized bacteria were eliminated by incubation in Phosphate Buffer Saline (PBS) supplemented with 50 mg/L gentamicin (1 h, MIC: 0.25 mg/L) and 4 successive washings with PBS. Infected cells were then transferred in fresh culture medium containing FNX and comparators covering a wide range of concentrations to obtain full concentrationdependent effects. After 48 h, cells were harvested, washed with PBS, lysed and used for enumeration of CFUs and assay of cell protein. Data were used to determine the apparent static concentration (Cs) of each antibiotic and its activity (change in log cfu) at a concentration corresponding to the reported human Cmax.

Results:
MICs, Cs and change in log cfu at Cmax are shown in the Table. Except for AZM, all antibiotics achieved a static effect at concentration similar to (CIP, MXF) or slightly above their MIC (CLR, TEL and FNX). At Cmax, AZM was unable to control intracellular bacterial growth, CLR and TEL were modestly and equally effective, whereas quinolones showed a clear ranking of efficacy (CIP < MXF < FNX), with FNX yielding an close to bactericidal effect (defined by a 3 log cfu decrease).

Antibiotics	MIC (mg/L)	Cmax (mg/L)	Intracell. Activity (48 h)	
			Cs (mg/L)	Δ Log cfu at Cmax
azithromycin (AZM)	0.01-0.03	0.5	~ 3.03 (300 x MIC)	+ 0.75 ± 0.05
clarithromycin (CLR)	0.008	1	~ 0.06 (7 x MIC)	- 0.43 ± 0.04
telithromycin (TLR)	0.008	1	~ 0.05 (6 x MIC)	-0.44 ± 0.01
ciprofloxacin (CIP)	0.01	4	< 0.01 (1 x MIC)	-0.70 ± 0.01
moxifloxacin (MXF)	0.01	4	< 0.005 (0.5 x MIC)	-1.81 ± 0.02
finafloxacin (FNX)	0.01	4	~ 0.05 (5 x MIC)	-2.27 ± 0.05

[a] Concentration resulting in no apparent bacterial growth (no change of CFU)

Conclusions:
Compared to macrolides, quinolones appear more effective against intracellular LP. Amongst them, FNX shows the greatest activity at clinically-relevant concentrations, perhaps in relation to its improved activity at acidic pH.

Background and aim

Legionella pneumophila, the causative agent of Legionnaire's disease, shows ability to survive intracellularly within phagosomes and weakly acidic vacuoles.[1] Treatment of these infections remains however challenging, as the activity of many antimicrobials (such as macrolides) may be partially defeated by the acidic environment.

Finafloxacin is a novel fluoroquinolone antibiotic showing enhanced antibacterial activities under acidic conditions, as previously demonstrated for *S. aureus*[2-3] and its intracellular forms.[3] In this context, we have investigated the activity of finafloxacin towards the intracellular forms of *L. pneumophila*. Macrolides and conventional quinolones were used as comparators.

Methods

Cells. Experiments were performed with human THP-1 cells, a myelomonocytic cell line displaying macrophage-like activity.[4]

Bacterial strain and susceptibility testing. *L. pneumophila* strain ATCC 33153 was used throughout. MICs determinations were made in α-ketoglutarate buffered yeast extract (pH 6.9) after 48 h incubation.

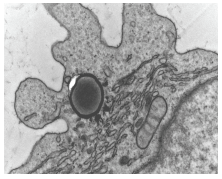
Cell infection and determination of the intracellular activities of antibiotics. Cell infection was initiated at a bacteria per macrophage ratio of 10, followed by elimination of non-phagocytosed bacteria by exposing the cells to 50 mg/L gentamicin (30-45 min) and 4 successive washings with Phosphate Buffered Saline (PBS). Cells were then exposed to increasing concentrations of antibiotics. After 48h, cells were harvested, washed free from antibiotics and used for determination of cfu and cell protein.

Concentration-response modeling. Data (change in the intracellular inoculum at 48 h compared to time 0) were used iterative "best fitting" of a single or a double Hill equation (sigmoids) using GraphPad software (version 4.2) with a-priori setting of initial parameters (E_{max}, E_{min}, EC_{50}, and Hill factor) based on visual inspection of the data.

Electron microscopy. Cells were infected as described above, except that the initial inoculum was increased to 20 bacteria per macrophage. Sample handling was then performed as described previously.[5]

Results

Intracellular localization



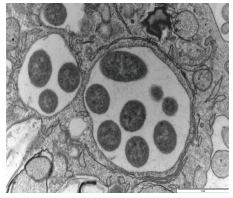


Fig.1 (top): Morphological appearance of *Legionella pneumophila* (strain ATCC 33153) internalized by human THP-1 macrophages (48 h incubation).

Intracellular activity of antibiotics

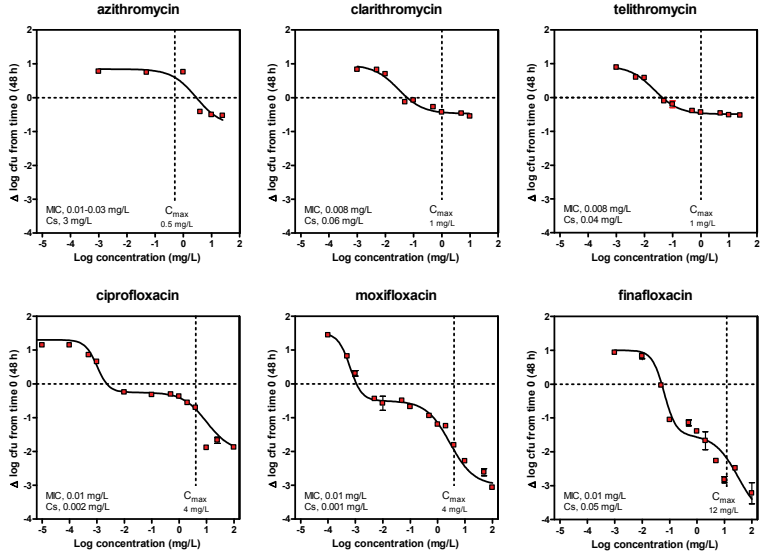


Fig.2 (right): Dose-response curves of 6 antibiotics towards the intracellular forms of *L. pneumophila* (human THP-1 macrophages). Antibiotic activities were determined after 48 h incubation in the presence of increasing concentrations of antibiotics, and the results (means ± standard deviations of three independent determinations) are expressed as the change in cfu compared to the initial, post-phagocytosis inoculum (time 0 h). The horizontal dotted line corresponds to a static effect (no apparent change from post-phagocytosis inoculum). For macrolides, dose-response could be modeled by a single Hill equation (sigmoid). For fluoroquinolones, manual fit was best for a double Hill equation. The vertical dotted line corresponds to the most commonly observed C_{max} of each drug in patients or volunteers (finafloxacin) treated with conventional doses.

Conclusions

- **Azithromycin fails to control the intracellular growth of *L. pneumophila* at concentrations equivalent to human C_{max}. Clarithromycin and telithromycin only poorly eradicate these forms at C_{max}.**
- **The activity of fluoroquinolones develops in a bimodal fashion, suggesting a double mode of action.**
- **Moxifloxacin and finafloxacin yield a 2 to 2.2 log_{10} cfu reduction at C_{max}, with further increase in activity (≥ 3 log_{10} cfu reduction) at larger concentrations.**
- **These data call for animal and clinical studies aiming at substantiating the superiority of fluroquinolones over macrolides if sufficiently large serum concentrations can be safely obtained.**

References

1. Sturgill-Koszycki and Swanson, *J Exp Med* (2000), 192:1261-72
2. Dalhoff *et al*, *Antimicrob Agents Chemother*. (2011), 55:1814-8
3. Lemaire *et al*, *International Journal of Antimicrobial Agents*, In press
4. Tsuchiya *et al*, *Intern. J Cancer* (1980), 26: 171-176
5. Barcia-Macay *et al*, *Antimicrob Agents Chemother*. (2006),50:841-51.

This poster will be made available for download after the meeting: http://www.facm.ucl.ac.be/posters.htm

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