Contrasting effect of acidic pH on the extracellular and intracellular activities of delafloxacin (DFX) vs moxifloxacin (MXF) towards *S. aureus*

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Abstract

Background. In contrast to currently marketed fluoroquinolones (FQ) that are zwitterionic, DFX (1-(6-Amino-3,5-difluoropyridin-2-yl)-8-chloro-6-fluoro-7-(3-hydroxyazetidin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid), highly active on Gram-positive bacteria, is an anionic compound (pKa's = 0.80 and 5.49). In this context, we have examined the effect of acidic pH on its accumulation in *S. aureus* and in human THP-1 cells, in parallel with its activity against extra- and intracell. *S. aureus* (localized in phagolysosomes, pH 5-5.5). MXF (pKa's = 6.04 and 10.61) was used as comparator.
Methods. MICs measured in pH-adjusted Mueller-Hinton broth by microdilution. FQ accumulation in *S. aureus* ATCC 25923 and in uninfected THP-1 cells determined by microbiological assay. Intracell. activity assessed after 24 h of incubation of cells in media at pH 7.4 or 5.5 (JAC 2005, 55:897-904).
Results. The Table shows that DFX is generally more potent than MXF, as it shows activity at lower conc. both extracell. and intracell. At reduced pH, this difference is enhanced as acidification (i) increases about 10-fold DFX accumulation in both bacteria and cells, and (ii) decreases DFX MIC (5 dil.), resulting in a marked increase in intracell. activity (0.8-2.4 $\log_{10}$). Opposite effects are seen for MXF.

Parameters studied	Moxifloxacin		Delafloxacin	
	pH 7.4	pH 5.5	pH 7.4	pH 5.5
MICs (mg/L)	0.03	0.125	0.0075	0.0002
Bact. conc.[a]	100.0 ± 4.1	57.8 ± 4.7	100.0 ± 9.7	1100.2 ± 180.7
Cell. accum.[b]	5.8 ± 0.5	1.5 ± 0.0	1.7 ± 0.4	19.8 ± 4.7
Intr. activity[c]				
At 0.1 mg/L	0.7 ± 0.2	0.5 ± 0.0	-0.5 ± 0.1	-1.3 ± 0.0
At 1 mg/L	-1.5 ± 0.1	0.1 ± 0.1	-0.6 ± 0.0	-3.0 ± 0.1

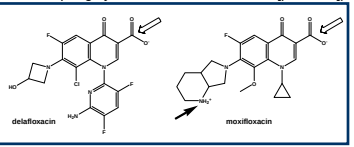
[a] Antibiotic uptake within *S. aureus* (30 min; % of control value [pH 7.4])
[b] Cellular to extracellular concentration ratio (30 min)
[c] A log cfu from time 0 (24 h)

Conclusions. DFX displays a markedly improved potency and activity at acidic pH, probably related to its anionic character and ensuing increased accumulation in both bacteria and eukaryotic cells. This may confer an advantage to DFX for the treatment of staphylococcal infections in territories where pH is acidic.

Background

Delafloxacin (DFX) is an investigational quinolone demontrating potent intrinsic activity, especially against Gram-positive bacteria (including MRSA isolates)[1-2]. On a chemical point of view, delafloxacin differs from others currently used quinolones by the absence of a protonable substituant, which confers a weak acid character to the molecule.
This property further increases its potency in acidic environments. This could be advantageous for *S. aureus* infections, as this bacterium has ability to survive and thrive in mildly acidic compartments[3-4] (such as the skin, the vagina, the urinary tract, or the phagolysosomes of infected cells [pH 5-5.5]).

Structural formulae.
The open arrows point to the acidic function of fluoroquinolones and the plain arrow, to the basic function of moxifloxacin that is protonated at physiological pH.



Aim of the study

to examine the effect of acidic pH
- **on the accumulation in bacteria and in eukaryotic cells,**
- **on the activity towards extracellular and intracellular *S. aureus***

of delafloxacin and moxifloxacin.

Methods

• **Bacteria, susceptibility testing, and extracellular activity**: *S. aureus* strain ATCC 25923 was used for all experiments. MICs were determined in pH-adjusted Mueller Hinton Broth by microdilution. Extracellular activity was determined as previously described (4).

• **Cell line:** Experiments were performed with THP-1 cells (ATCC TIB-202), a human myelomonocytic cell line displaying macrophage-like activity.

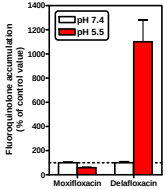
• **Determination of antibiotic accumulation in *S. aureus* and THP-1 cells:** Antibiotic accumulation was measured after 30 min incubation. The content in antibiotic was determined by microbiological assay (disc-plate). The cellular concentration of fluoroquinolones was expressed by reference to the total cell protein, as determined using the Folin-Ciocalteu / biuret method. Cellular accumulation in THP-1 cells was calculated using a converson factor of 5 µL per mg of cell protein.

• **Cell infection and assessment of intracellular activity:** Cell infection was performed exactly as described previously (4) with a starting inoculum of ~ 1 to 2x10^6 CFU/mg prot. Antibiotic activities was examined after 24 h in the presence of fixed concentration of antibiotics in media at pH 7.4 or 5.5, and results were expressed as the change in the inoculum at 24 h compared to time 0.

Results

A) Influence of acidic pH on the accumulation of moxifloxacin and delafloxacin

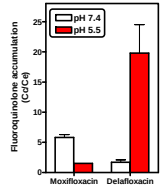
A.1. Accumulation of antibiotics inside bacteria (*S. aureus*)



Accumulation in *S. aureus*

Growing bacteria were incubated for 30 min in pH-adjusted broth (pH 7.4 vs 5.5) with moxifloxacin (50 mg/L) or delafloxacin (100 mg/L).
Values are means ± SD. Results are expressed as the cellular to extracellular concentration ratio (% of control value [pH 7.4]).

A.2. Accumulation of antibiotics inside THP-1 cells



Accumulation in THP-1 cells

THP-1 macrophages were incubated for 30 min in cell culture medium (pH 7.4 vs 5.5) with moxifloxacin (20 mg/L) or delafloxacin (20 mg/L).
Values are means ± SD. Results are expressed as the cellular to extracellular concentration ratio (Cc/Ce).

Acid pH markedly increases the accumulation of delafloxacin in both bacteria (*S. aureus*) or THP-1 cells. Opposite effects are observed for moxifloxacin.

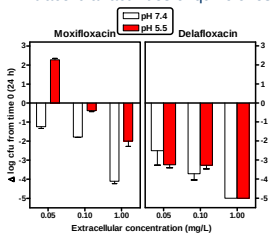
B) Influence of acidic pH on the activity of moxifloxacin and delafloxacin

B.1. Intrinsic activities of quinolones

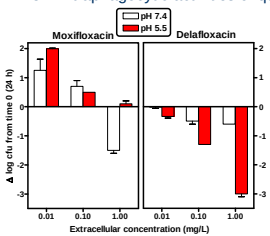
MICs (mg/L)	pH 7.4	pH 5.5
Moxifloxacin	0.03	0.125
Delafloxacin	0.0075	0.0002

Acidic pH increases the activity of delafloxacin towards both extracellular and intracellular *S.aureus*, but impairs the activity of moxifloxacin.

B.2. Extracellular activities of quinolones



B.3. Intraphagocytic activities of quinolones



Extracellular (broth) and intracellular (THP-1 cells) activity of quinolones:

Change in bacterial counts from the initial inoculum in broth (B2) or in infected cells (B3) after 24 h incubation in medium at pH 7.4 vs pH 5.5 containing fixed extracellular concentration of quinolones. All values are means ± SD of 3 independent determinations. Positive value denote a gain, and negative values, a loss in activity. Limit of detection: -5 log CFU

Conclusions

Delafloxacin is highly intrinsically active against *S. aureus*. In contrast to moxifloxacin, it shows enhanced potency and efficacy at acidic pH against both extracellular and intracellular bacteria, in relation with its higher accumulation in both procaryotic and eucaryotic cells. This may confer an advantage to delafloxacin for the treatment of staphylococcal infections in territories where pH is acidic, including those involving intracellular reservoirs.

References

1) Ohshita et al, abstr. F-84, *38th ICAAC* (1998)
2) Burak et al, P-1080, *19th ECCMID* (2009)
3) Weinrick et al, *J Bacteriol* (2004), 186:8407-23
4) Barcia-Macay et al, *Antimicrob Agents Chemother.* (2006), 50:841-51

This poster will be made available for download after the meeting:
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