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Cellular Accumulation is not Predictive of Intracellular Activity for Fluoroquinolones against *S. aureus* : Comparison of Gemifloxacin with Moxifloxacin and Ciprofloxacin

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Background : FQ accumulate in eucaryotic cells and are considered as drugs of choice for intracellular infections. GMF shows a larger distribution volume than other FQ, suggestive of tissular tropism. To examine the significance of this PK property, we have examined the accumulation and intracellular activity of GMF and 2 other FQ (CIP and MXF), and its subcellular distribution.

Methods : MIC measured in broth (CLSI method). Cellular accumul. and activity studied in human THP-1 monocytes and subcellular distribution in murine J774 macrophages. FQ assayed by validated microbiological or fluorimetric assay. Intracellular activity measured against phagocytized *S. aureus* ATCC25923 using a wide range of extracellular concentrations to obtain full dose-responses and PD parameters (see definition in Table and in AAC 2006;50:841-51). Cell fractionation made by differential centrifugation (JAC 2003;51:1167-73).

Results : Cellular accumulation and intracell. activity are shown in the Table. The distribution of GMF between nuclear, organelles and soluble fraction was 10, 20 and 70 %, as previously observed for CIP (JAC 2003; 51:1167–73) and other FQ (JAC 1990; 26(B):27-39).

fluoroquinolone	Accumulation [a]	Activity			MIC [e]
		E_{min} [b]	E_{max} [c]	C_s [d]	
GMF	16.2 ± 0.3	1.8 ± 0.4	-1.9 ± 0.3	0.10	0.008
MXF	10.4 ± 0.3	1.8 ± 0.2	- 2.2 ± 0.1	0.19	0.03
CIP	7.1 ± 0.8	2.1 ± 0.1	-1.6 ± 0.1	0.25	0.25

[a] using a conversion factor of 5 µl cell vol./mg prot; extracell. conc. 20 µg/ml
[b] increase in log CFU at 24h compared to time 0 for an infinitely low drug conc.
[c] decrease in log CFU at 24h compared to time 0 for an infinitely large drug conc.
[d] Static conc: extracell. conc. (mg/L) yielding no apparent change in the inoculum vs to time 0
[e] mg/L in broth (CLSI method)

Conclusion : Although more active in broth (lower MIC) and showing larger cellular accumulation, GMF is not more active than CIP of MXF against intracell. *S. aureus*. Since subcellular distributions are similar, this should be due to other factors such as local interferences or modulation of bacterial responsiveness. As for other antibiotics, this study reinforces the conclusion that drug accumulation and activity are not necessarily linked.

INTRODUCTION

- Fluoroquinolones are known to accumulate inside eucaryotic cells and are therefore considered as drugs of choice for the treatment of intracellular infections. Yet, the level of accumulation may considerably vary from one molecule to the other, depending probably on their capacity to diffuse through the membrane and/or to their recognition by active transporters.
- Gemifloxacin shows a higher volume of distribution than most fluoroquinolones currently used in the clinics, associated to a high level of cellular accumulation. Human pharmacokinetic data document indeed an accumulation factor in alveolar macrophages (ratio to serum level) of 90 for gemifloxacin vs 56 for moxifloxacin and 5-10 for ciprofloxacin [1,2].

AIM OF THE STUDY

To examine whether the accumulation of fluoroquinolones in macrophages is predictive of intracellular activity.

To this effect, we compared

- the accumulation of gemifloxacin, moxifloxacin, and ciprofloxacin in human THP-1 monocytes;
- their activity against intracellular *S. aureus,* a phagolysosomal bacterium;
- their subcellular localization within eucaryotic cells

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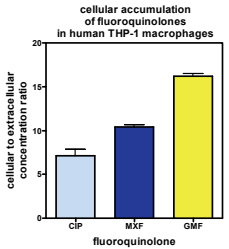
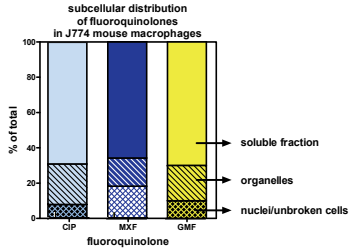
RESULTS

⇨cellular pharmacokinetics



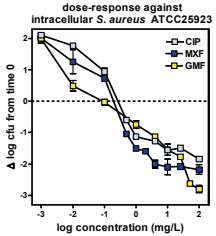


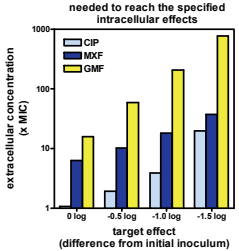
Left : cellular accumulation of ciprofloxacin (CIP), moxifloxacin (MXF), and gemifloxacin (GMF) in THP-1 macrophages incubated for 2 h with 20mg/L of each drug.

Right: subcellular distribution of fluoroquinolones in the nuclei / unbroken cell fraction, the MLP [mitochondria, lysosomes, peroxisomes] fraction containing the bulk of the organelles, or the soluble fraction of a cell homogenate of J774 macrophages.

- gemifloxacin accumuates to higher extent than moxifloxacin and ciprofloxacin
- the three fluoroquinolones have a similar subcellular distribution, with most of the drug being found in the soluble fraction of the cells

⇨cellular pharmacodynamics





drug	MIC (pH 7.4)	MIC (pH 5.5)
CIP	0.25	0.5
MXF	0.03	0.125
GMF	0.008	0.125

Left : intracellular activity of fluoroquinolones against intracellular *S. aureus* ATCC 25923 after 24 h incubation in the presence of increasing drug concentrations. Data are expressed as the change in cfu in comparison with the initial inoculum.

Right: comparison of the concentration of each drug that is needed in the culture medium to reach a similar intracellular effect. Concentrations are expressed in X MIC and are intrapolated from the calculated sigmoidal regression of the dose-response (using a Hill factor of 1 for CIP and MXF and a variable slope for GMF).

- the activity of all drugs develops on a concentration-dependent manner.
- higher extracellular concentrations of GMF than of MXF or CIP are needed to reach a given intracellular effect
- gemifloxacin reaches an intracellular bactericidal effect at the highest concentration tested, in contrast with MXF and CIP.
- MIC of GMF is more affected than that of MXF or of CIP by acidic pH

MATERIALS & METHODS

- **cells:** we used both human THP-1 macrophages (accumulation; intracellular activity) and J774 mouse macrophages (cell fractionation studies).
- **Fluoroquinolone accumulation:** cells were incubated with the drug at an extracellular concentration of 20 mg/L for 2 h. The extracellular drug was eliminated by centrifugation [3]. Fluoroquinolones were assayed by fluorimetry as previously described [4] (with λexc set at 275 nm, 298 nm, and 270 nm and λem set at 450 nm, 504 nm, and 402 nm for CIP, MXF, and GMF, respectively) and drug content were expressed by reference to protein content. Cellular accumulation was calculated using a conversion factor of 5 µl/mg cell protein.
- **Cell fractionation:** Homogenates of J774 macrophages were fractionated by low speed centrifugation in a nuclei/unbroken cells fraction and a cytoplasmic extract which was further separated in a MLP [mitochondria, lysosomes, peroxisomes] fraction containing the organelles and a soluble fraction. Drug concentration was measured in each fraction together with the activity of enzymes used as markers of the main cell compartments [5].
- **Intracellular activity:** Phagocytosis was initiated at a bacteria/ macrophage ratio of 10, followed by elimination of non-phagocytosed bacteria by exposing the cells to 50 mg/L gentamicin. Activity was determined by measuring CFU per mg of cell lysates obtained from cells exposed to over time to fixed concentrations of antibiotics or for fixed time (24 h) to increasing concentrations of antibiotic. Results were expressed as the change in the inoculum at 24 h compared to time 0 [4].
- **Susceptibility testing:** MIC were determined by microdilution in MH broth adjusted to pH 7.4 or 5.5 [4].

CONCLUSIONS

- The fluoroquinolones under study markedly differ in their accumulation level but share a similar subcellular distribution.
- Low MIC and high accumulation is not predictive of intracellular potency, as the drugs with higher accumulation and lower MIC require higher extracellular concentrations to reach a comparable intracellular effect. This cannot be explained by the defeating effect of acidic pH on activity (*S. aureus* being located in acidic vacuoles inside the cells) but rather suggests a poor cellular bioavailability for drugs that accumulate to large extent.

REFERENCES

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