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Cellular pharmacokinetics of quinolones revisited : Role of the MDR efflux pumps expressed in macrophages

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Dosing
the magic bullets

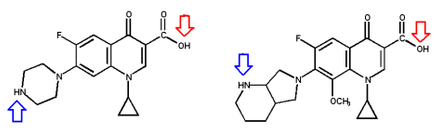
ABSTRACT

Background : Efflux pumps can modulate the cellular pharmacokinetics of drugs in general, and of antibiotics in particular, which may have deleterious effects on their activity against intracellular infections. In this respect, we recently showed that ciprofloxacin is substrate for an efflux pump with MRP (Multidrug Resistance Protein)-like properties in J774 mouse macrophages (AAC 48: 2673-82). In the present work, we have compared the cellular pharmacokinetics of CIP to that of MXF and examined its modulation by active efflux pumps.
Methods : Quinolones were assayed by fluorimetric assay, and their cell-associated concentration was calculated by reference to the cell protein content.
Results : Both quinolones entered J774 macrophages at a similar rate but reached different accumulation levels ranging from 3 (CIP) to 15-fold (MXF). Inhibitors of MRP (probenecid, MK 571) markedly increased CIP accumulation (~ 450 %), but did not influence that of MXF. Accordingly, probenecid notably slowed down the efflux of CIP only. Heating of the cells to 56°C prior incubation brought the accumulation of both drugs to a similarly high value (15 to 20-fold). In cells incubated at 4°C, MXF accumulation remained high while that of CIP was drastically reduced.
Conclusions: quinolone level of accumulation markedly differs in J774 macrophages, which may be a consequence of (a) their capacity to interact and/or cross the membrane, as suggested by accumulation data at 4°C or (b) their differential recognition by MRP efflux pumps, as suggested by the variable effect of inhibitors and the influence of cell heating (as a mean to denature proteins). These data may enlighten structure-pharmacokinetics relationships for quinolones and hence contribute to the definition of appropriate dosing.

INTRODUCTION

MDR (multidrug resistance) efflux pumps are well known to reduce the cellular accumulation of anticancer agents in eucaryotic cells, and of antibiotics in bacteria, compromizing the pharmacological activity of these drugs [1]. Due to their very large substrate specificity, MDR pumps expressed in macrophages can also recognize antibiotics, modulating their accumulation in these cells and impairing their activity against intracellular bacteria [2].
Quinolone are zwitterionic amphiphilic molecules, and could therefore serve as substrates for these pumps, which recognize either cationic amphiphiles (P-glycoprotein) or anionic amphiphiles (MRP; Multidrug Resistance Proteins) [1].

CHEMICAL STRUCTURE OF THE QUINOLONES STUDIED



ciprofloxacin moxifloxacin

Moxifloxacin is more lipophilic than ciprofloxacin. The red arrows points to the deprotonable function (anionic character), and the blue arrow, to the protonable function (cationic character)

METHODS

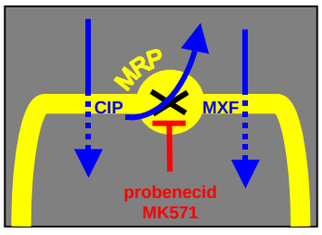
- J774 macrophages were incubated with quinolones at an extracellular concentration of 5 mg/L (or 17 mg/L for efflux experiments). At the end of the incubation, cell sheets were rinced in cold PBS, scrapped and collected in 0.1 M glycine-HCl pH 3 buffer.
- Cell content in quinolone was determined using fluorimetry [3] and concentrations were calculated by reference to the protein cell content. Apparent accumulation was calculated using a conversion factor of 3.08 µl/mg protein [3].

AIM OF THE STUDY

- to compare the accumulation and efflux of CIP and MXF in macrophages
- to evaluate the impact of efflux pumps on their cellular pharmacokinetics.

CONCLUSIONS

- Accumulation of quinolones reaches variable levels, with the more lipophilic molecule (MXF) reaching higher accumulation levels.
- Differences in this accumulation level may result from
  - a variable capacity to interact with the cell surface, as suggested by the reduction in apparent cell-associated amount of CIP but not of MXF at 4°C .
  - a variable recognition by efflux pumps, as suggested by
    1) the differential effect of inhibitors of MRP on accumulation and rate of efflux,
    2) the high and almost similar level of accumulation reached for both quinolones in heated cells (heating used as a mean to denature cell proteins, among which efflux pumps).
- The data suggests that macrophages possess in their membrane an efflux pump with MRP-like properties, which can recognize (probably from the membrane environment), quinolones with appropriate structural determinants, reducing thereby their apparent cellular accumulation.

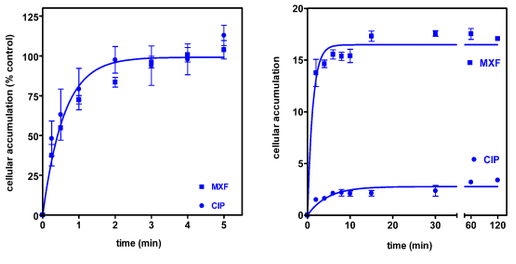


- Further studies are needed to better delineate the structure-activity relationship governing the recognition of quinolones by efflux pumps, but these data need to be taken into account for selecting appropriate quinolone dosage especially when dealing with intracellular infections.

RESULTS

1. going in …

quinolones enter cells at a similar fractional rate, but reach different accumulation ratios

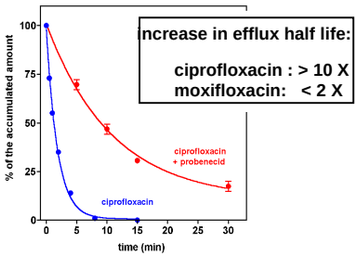


kinetics of accumulation of quinolones in cells exposed to an extracellular concentration of 5 mg/L for up to 5min. Results are expressed in % of the maximal level of accumulaltion reached for each drug (see righht panel)

kinetics of accumulation of quinolones in cells exposed to an extracellular concentration of 5 mg/L for up to 2 h.

2. going out …

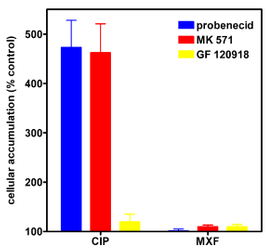
probenecid differently affects the efflux of quinolones



kinetics of efflux of ciprofloxacin from cells incubated during 2 h with CIP 17 mg/L alone and then placed in a fresh medium without antibiotic, or from cells incuabted during accumulation and efflux in the presence of probenecid 5 mM.

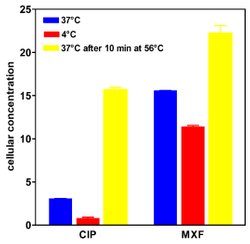
3. accumulation at equilibrium

Inhibitors of MRP but not of P-gp increase CIP accumulation and do not affect that of MXF



influence of inhibitors of MRP (probenecid 5 mM; MK571 100 µM) or of P-glycoprotein (GF120918 2 µM) on the accumulation of quinolones after 2 h of incubation (extracellular concentration: 5 mg/L)

Heated cells accumulate both quinolones to a high level.
At 4°C, CIP accumulation is much more reduced than that of MXF



accumulation of quinolones after 2 h of incubation (extracellular concentration: 5 mg/L) at 37°C, 4°C or 37°C after 10 minutes exposure at 56°C

REFERENCES

1. Van Bambeke et al (2000) Biochem. Pharmacol. 60: 457-70
2. Seral et al (2003) JAC 51:1167-73.
3. Michot et al (2004) AAC 48: 2673-82.