Influence of Inhibitors of Efflux Pumps on the Accumulation of Quinolones in a model of Calu-3 Human Airway Epithelial Cells

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ABSTRACT

Background:
Quinolones are used for the control of respiratory tract infections caused by extracellular or intracellular pathogens. Based on previous observations that they accumulate in phagocytic cells but are potential substrates for efflux pumps, we have now examined how their accumulation is affected by inhibitors of efflux pumps in Calu-3 cells as a model of the airways epithelium.

Methods:
Accumulation of quinolones was measured at equilibrium in Calu-3 cells incubated with an extracellular concentration of 20 mg/l. Active efflux was inhibited using inhibitors of the P-glycoprotein (verapamil; GF120916) and of MRP (probenecid; MK571).

Results:
Accumulation of GAR (garenoxacin, BMS284756), CIP (ciprofloxacin), and LVX (levofloxacin) in Calu-3 cells was approx. 4.5, 3.5, and 3 after 2 h. GAR accumulation was significantly increased by all inhibitors, CIP by the 2 MRP inhibitors and verapamil, and LVX by verapamil and MK571 only. The effect of inhibitors were more marked on CIP than on LVX and GAR.

Inhibitor[a]	Accumulation (% control)		
	GAR	CIP	LVX
Verapamil 100 μM	133 ± 5*	153 ± 8*	130 ± 4*
GF 120918 2 μM	122 ± 8*	111 ± 21	117 ± 10
Probenecid 5mM	136 ± 6*	178 ± 18*	105 ± 17
MK571 100 μM	151 ± 5*	163 ± 12*	132 ± 8*

[a]concentration showing maximal effect on CIP accumulation;
*p < 0.05 (Student's t test)

Conclusions:
Accumulation of quinolones in Calu-3 cells is suboptimal due to active efflux. Efflux inhibitors could therefore be tested for improve activity of quinolones against intracellular organisms. Transporters differ, however, in their ability to recognise specific quinolones, which may have important implications for the rational development of new molecules.

INTRODUCTION

- Quinolones are large spectrum antibiotics, largely used in clinics for the treatment of respiratory tract infections caused by extracellular but also by intracellular pathogens, taking advantage of their ability to accumulate inside eucaryotic cells.
- Cellular accumulation and intracellular activity of quinolones may however be defeated by the action of efflux pumps present at the surface of epithelial cells.[1] Calu-3 cells, as a model of epithelial cells of the bronchial mucosa, have been shown to express MDR1 (P-glycoprotein) at their apical membrane and MRP1 (Multidrug resistance-related protein-1) at their basolateral membrane (see figure for a schematic view).[2]
- Being zwitterionic molecules, quinolones are potential substrates of both transporters, since MDR mainly recognizes cationic amphiphiles, and MRP, anionic amphiphiles.[3]

AIM OF THE STUDY

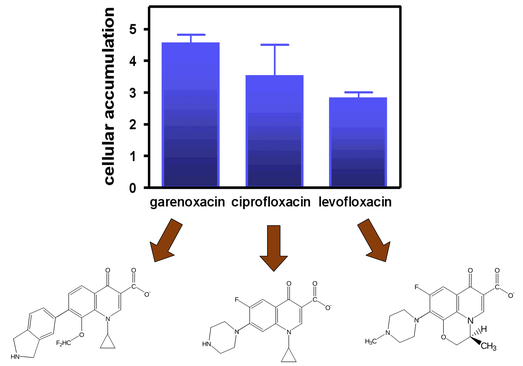
- To compare the accumulation of quinolones in Calu-3 cells
- To compare the influence of efflux pumps on their accumulation, using
  - Inhibitors of MRP[4,5]:
    - Probenecid (non specific inhibitor of organic anion transporters)
    - MK571 (specific inhibitor of MRP)
  - Inhibitors of MDR[6,7]:
    - Verapamil (non-specific inhibitor of MDR)
    - GF120918 (specific inhibitor of MDR)

METHODS

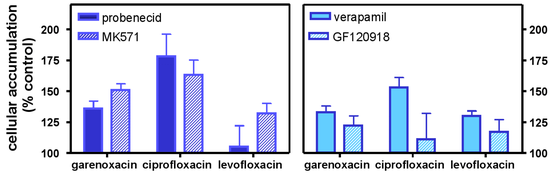
- Calu-3 cells were cultured in MEM medium supplemented with 10 % foetal calf serum in a 5 % CO_2 atmosphere. All experiments were performed with non-polarized cells, used at confluency.
- Cells were incubated with the quinolone in the absence or in the presence of one of the inhibitors, washed in ice-cold PBS, harvested by scraping and lyzed by sonication.
- Cells lysates were used for determination of cell content in total protein[8] and in quinolone. Levofloxacin and ciprofloxacin concentrations were measured by fluorimetry[9] (λex; λem = 298nm; 500nm and 275nm; 450nm respectively) and garenoxacin concentration, by scintillation counting (cells having been incubated with the radiolabeled product).
- Cellular accumulation was calculated using a conversion factor of 5μl/mg cell protein.[10]

RESULTS

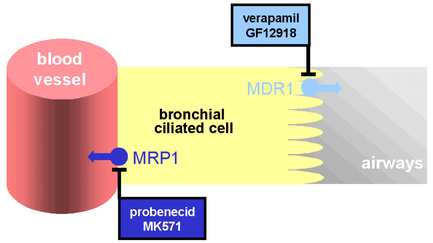
Cellular accumulation of three quinolones in Calu-3 cells
(2 h of incubation, extracellular concentration: 20 mg/l)



Influence of inhibitors of multidrug resistance pumps on the accumulation of quinolones in Calu-3 cells
(2 h of incubation, 20 mg/l quinolone; 5 mM probenecid; 100 μM MK571; 100 μM verapamil; 2 μM GF120918)



Pharmacological inhibition of multidrug resistance pumps : application to polarized Calu-3 cells



CONCLUSION

- Quinolone accumulate to various extents in Calu-3 cells.
- Quinolone accumulation in Calu-3 cells is suboptimal due to active efflux.
- Quinolones are probably preferential substrates for MRP, since specific inhibition of MRP is more prone to increase quinolone accumulation than specific inhibition of MDR.
- Transporters differ in their ability to recognize closely related molecules like quinolone derivatives.
- These data may have important implications for the rational design of new derivatives with optimal pharmacokinetic profile.

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