INFLUENCE OF TWO ABC TRANSPORTERS, ABCC4 AND ABCG2, ON CIPROFLOXACIN ACCUMULATION IN THE CONTEXT OF CYSTIC FIBROSIS

L. Garcia, B. Marquez, J. Buyck, M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke

Unité de Pharmacologie Cellulaire et Moléculaire, Louvain Drug Research Institute, Université Catholique de Louvain, Brussels, Belgium

Mailing address: F. Van Bambeke, Unité de Pharmacologie Cellulaire et Moléculaire, UCL 73.70 av. E. Mounier 73, 1200 Brussels – BELGIUM; francoise.vanbambeke@uclouvain.be

INTRODUCTION

Cystic fibrosis (CF) patients are particularly prone to develop bacterial infections with lung colonization by various opportunistic bacteria including *Pseudomonas aeruginosa*. These infections require the use of antibiotics, among which Ciprofloxacin (CIP).

Ciprofloxacin is subject to efflux mediated by ABC transporters in eukaryotic cells. It has been demonstrated that Ciprofloxacin is substrate for Abcc4 (Mrp4) in mouse macrophages [1] and for ABCG2 (BCRP), both in human and murine cells [2].

The aim of this study was to compare the expression of these two transporters and their influence on Ciprofloxacin accumulation in bronchial epithelial cells with no expression of CFTR (WT cells), expression of wild-type CFTR (CFTR cells) or expression of mutated CFTR (ΔF 508 cells).

MATERIALS & METHODS

- **Cells: We used human airway epithelial cell line CFBE41o$^-$ that do not express CFTR (WT) and its stable transfectants with wild-type CFTR (CFTR) or Delta F508 CFTR (ΔF508) [3].**
- **Real Time PCR: *CFTR*, *ABCG2* and *ABCC4* mRNA expression levels were quantified by real-time PCR using SYBR Green detection [4]. *GAPDH* was used as housekeeping gene (ref) to normalize gene expression as compared to WT cells (For *CFTR*, which is not detected in WT cells, CFTR cells were used for normalization) [5].**
- **Western Blots: CFTR, ABCG2 and ABCC4 proteins were detected in whole cell lysates, respectively with human CFTR C-terminus (clone 24-1), BXP-53 and M_4 I-10 monoclonal antibodies ; Anti-actin polyclonal antibody was used as a control.**
- **CIP Accumulation: Cells were incubated with 50 µM of CIP for 4 hours, at 37°C. Gemfibrozil (Gem, 500 µM) and Fumitremorgin C (FTC, 10 µM) were used to inhibit ABCC4 and ABCG2, respectively. Cellular concentrations of CIP were measured by fluorimetry and expressed by reference to the total protein content in each sample [1;6-7]. For ATP depletion experiments, cells were preincubated for 20 minutes with 5 mM NaN_3 and 60 mM 2-D-deoxyglucose before addition of CIP (50 µM of Ciprofloxacin in the continuing presence of NaN_3 and 2-D-deoxyglucose), for 2 hours [8].**
- **Confocal microscopy: CFTR protein was detected in cells fixed with 0.8 % formaldehyde in acetone and permeabilized with saponin [6] using a CFTR monoclonal antibody (clone 24-1) for CFTR (in green). Actin was stained with rhodamine-labeled phalloidin (in red), nucleus with TO-PRO-3 (in blue).**

RESULTS

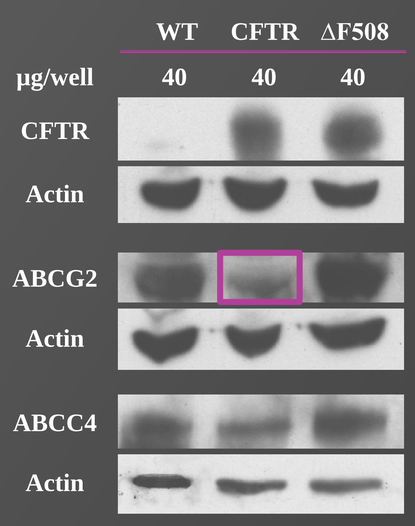
1. ABC transporters expression

1.1. Real time PCR

Cell Type	mRNA expression ratio		
	CFTR	ABCG2	ABCC4
WT	ND*	1.00	1.00
CFTR	1.00	0.35	0.81
ΔF508	1.64	1.14	0.79

*** ND: Not Detected**
**** mRNA expression ratio > 2: overexpression; mRNA expression ratio < 0.5: lower expression**

1.2. Western Blots

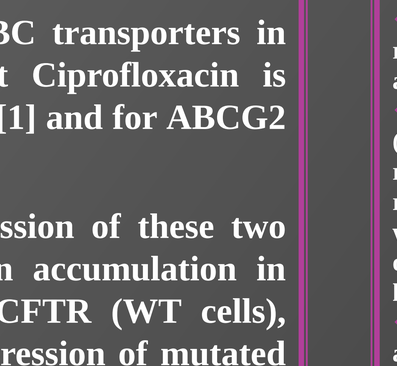


⇨ **Confirmation of cells phenotype**

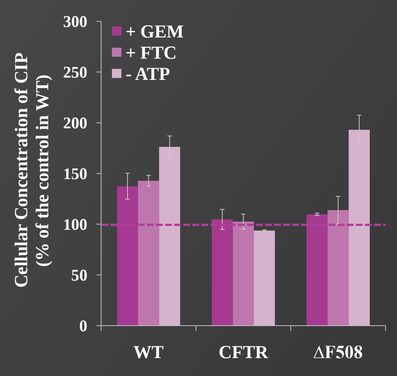
⇨ **Lower expression of ABCG2 in CFTR cells**

⇨ **No change in ABCC4 expression**

2. CIP accumulation



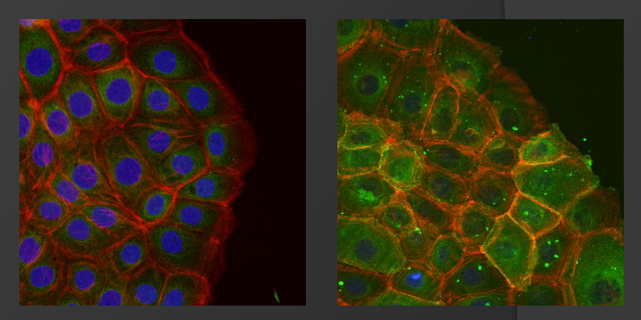
⇨ **CIP accumulation is higher in CFTR cells than in WT and ΔF508 cells;**



⇨ **GEM and FTC modestly increase CIP accumulation in WT cells;**

⇨ **ATP depletion markedly increases CIP accumulation in WT and ΔF508 cells.**

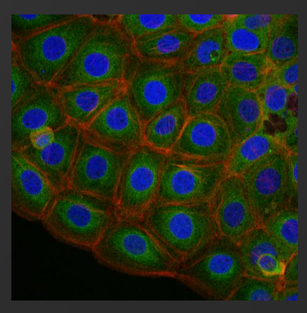
3. Localization of CFTR



No specific signal

Localization at the pericellular membrane

ΔF508 cells



Perinulear localization

*** CFTR, in green**
Actin, in red
Nucleus, in blue

CONCLUSIONS

ABCG2 shows a lower expression at both the mRNA and protein levels in cells expressing CFTR. This could account for the higher CIP accumulation and for the absence of effect of ATP depletion on CIP accumulation in these cells.

The modest effect of FTC as compared to ATP depletion on CIP accumulation in WT and ΔF508 cells may be due to the low concentration of inhibitor used (10 µM FTC vs 50 µM CIP).

REFERENCES

[1] Marquez *et al.* (2009) Identification of the efflux transporter of the fluoroquinolone antibiotic ciprofloxacin in murine macrophages: studies with ciprofloxacin-resistant cells. Antimicrob. Agents Chemother. 53: 2410-2416.
[2] Merino et al. (2006) Breast cancer resistance protein (BCRP/ABCG2) transports fluoroquinolone antibiotics and affects their oral availability, pharmacokinetics, and milk secretion. DMD. 34 : 690-695.
[3] Bedok *et al.* (2005) Failure of cAMP agonists to activate rescued ΔF508 CFTR in CFBE41o– airway epithelial monolayers. J. Physiol. 2005 569: 601-615.
[4] Szakacs *et al.* (2004) **Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells.** Cancer Cell. 6: 129-137.
[5] Huang *et al.* (2006) **Vincristine transcriptional regulation of efflux drug transporters in carcinoma cell lines.** Biochem Pharmacol 71: 1695-1704.
[6] Michot *et al.* (2005) Influence of efflux transporters on the accumulation and efflux of four quinolones (Ciprofloxacin, Levofloxacin, Garenoxacin, and Moxifloxacin) in J774 macrophages. Antimicrob. Agents Chemother. 49: 2429-2437.
[7] Michot *et al.* (2006) Cellular accumulation and activity of quinolones in Ciprofloxacin-resistant J774 macrophages. Antimicrob. Agents Chemother. 50: 1689-1695.
[8] Michot *et al.* (2004) Active Efflux of Ciprofloxacin from J774 Macrophages through an MRP-Like Transporter. Antimicrob. Agents Chemother. 48: 2673-2682.

ACKNOWLEDGEMENT

We thank J.P. Clancy (Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham, USA), R.W. Robey (Department of Anatomy and Cell Biology, Saarland University, Homburg, Germany) for the kind gift of human bronchial epithelial cells and Fumitremorgin C, respectively.
This research was supported by the Belgian *Fonds National de la Recherche Scientifique Médicale* and the *Région Wallonne* (Biowin programm [BioFina project] and First postdoc programm [Modupump project]). LG is recipient of a doctoral fellowship from the Belgian *Fonds pour la Recherche dans l'Inductrie et l'Agriculture*.