Impact of ABCB1 and its polymorphism on fluoroquinolone transport in eukaryotic cells

Gwenaëlle Mahieu [1, 2], Françoise Van Bambeke [1], Laure Elens [1, 2]

[1] Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain - Brussels (Belgium)
[2] Integrated PharmacoMetrics, PharmacoGenomics and PharmacoKinetics

#03054

e-mail : gwenaelle.mahieu@uclouvain.be

33rd ECCMID Copenhagen, Denmark 15 – 18 April 2023

Background

- Due to their accumulation in eukaryotic cells, fluoroquinolones are antibiotics of choice against intracellular infections
- This accumulation can be counterbalanced by the activity of efflux transporters expressed in phagocytes cells such as the ATP-dependent transporter ABCB1 also know as P-glycoprotein (P-gp) or MDR1
- ABC transporters are subject to genetic polymorphisms (SNPs) that can explain differences in their activity, expression or substrate specificity between individuals
- **This study aims to evaluate the impact of ABCB1 (wild-type) and one of its polymorphism c.1199G>A (rs 2229109) affecting 4 to 6% of Caucasian population, on fluoroquinolone cellular accumulation (Fig. 1)**

Materials and methods

1) Transfection

- Two recombinant cell lines, Human Embryonic Kidney (HEK293) cells overexpressing ABCB1 carrying either the wild-type [1199G] or its variant [1199A] allele were generated by stable transfection using lipofectamine 3000 as transfection reagent and compared to HEK293 control cells [pvide]

2) Model validation

- ABCB1 protein expression was checked by FACS and western-blot (Fig. 2)
- *ABCB1* gene expression was assessed by quantitative RT-PCR (Fig. 3)
- Functionality of the transporter was assessed by measuring the cell accumulation of Rhodamine 123, a known substrate (Fig. 4)

3) Fluoroquinolones accumulation in transfected cells

- Cells were incubated with moxifloxacin or ciprofloxacin over a wide range of concentrations during 2 hrs, after which fluoroquinolone cellular concentration was determined using a fluorometric assay(1) (Fig. 5)

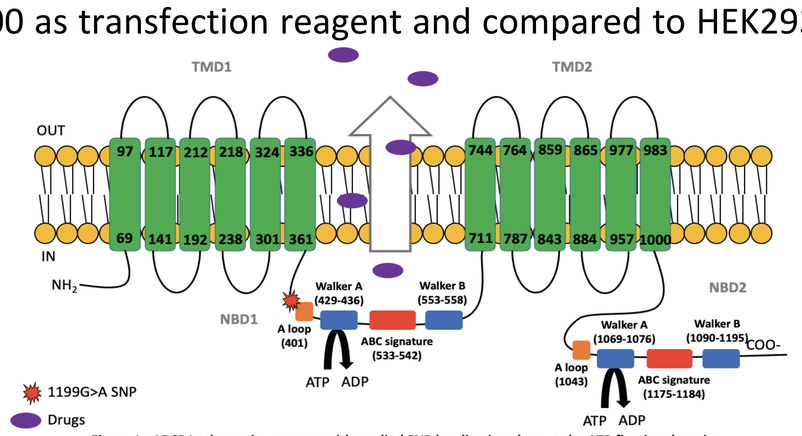

Figure 1 : ABCB1 schematic structure with studied SNP localization close to the ATP fixation domain
Adapted from : GU et al., DOI : 10.3390/md17050259

Results

1 & 2) Transfection & Model validation

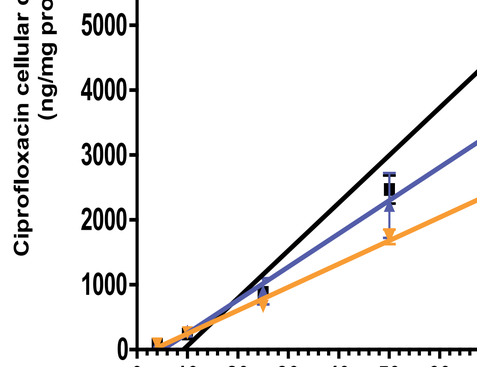

Figure 2 : Qualitative analysis by western-blot of ABCB1 expression (180kDA) in transfected wild-type (1199G) and variant cells (1199A) in comparison to control cells (pvide).

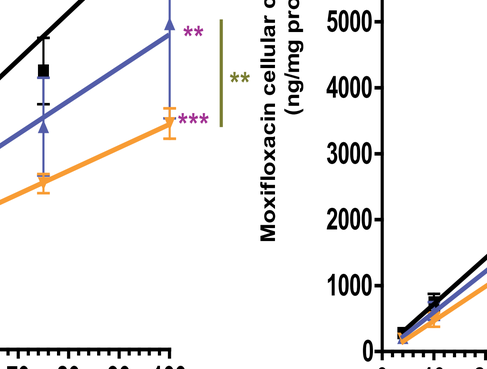

Figure 3: Quantification of ABCB1 transporteur expression in HEK293 control cells (pvide) and transfected with ABCB1 WT (1199G) or SNP (1199A). All data are the mean ± SEM (N=1 & n+3). Results are normalized according to 18s and compared to control cells .

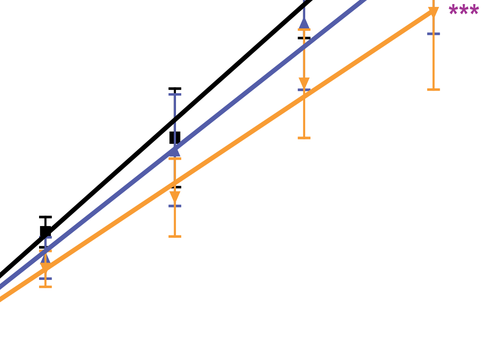

Figure 4: Accumulation of Rhodamine 123 in HEK293 control cells (pvide) and transfected with ABCB1 WT (1199G) or SNP (1199A).
All data are the mean ± SEM (N=3 & n=3).
Statistical analysis : one way ANOVA with Tukey's post-hoc test, comparison between cell lines (*p<0.001).

Observation 1 : ABCB1 overexpression level was not significantly different between cells transfected by the plasmid carrying the wild-type [1199G] or variant [1199A] ABCB1

Observation 2 : Fluorescence intensity in either wild-type or variant cells was lower than intensity obtained in control cells. This result supports the fact that a transporter expressed in transfected cells decreases rhodamine accumulation in comparison to control cells

3) Fluoroquinolones accumulation in transfected cells

Figure 5 : Fluoroquinolone cellular accumulation (after 2 hours of incubation over a wide range of concentrations) in cells overexpressing WT ABCB1 (1199G) and SNP ABCB1 (1199A) in comparison to control cells
All data are the mean ± SEM (N=3 & n=3, when nonvisible, error bars are smaller than the symbol size). **pvide :** cells transfected with empty plasmid (not coding for *ABCB1*), 1199G : cells transfected with plasmid encoding for *ABCB1* wild-type allele, 1199A : cells transfected with plasmid encoding for *ABCB1* variant allele. Statistical analysis : one way ANOVA with Tukey's post-hoc test, comparing slopes vs pvide and slopes between 1199G and 1199A (*p<0.05 ; **P<0.01 ; ***p<0.001).

Observation 3 : Cellular concentrations of both fluoroquinolones increased linearly over the range of extracellular concentrations investigated and were significantly reduced in cells overexpressing wild-type ABCB1 (1199G) and even more in those overexpressing the variant (1199A) as compared to control cells.

Observation 4 : The effect of the SNP was more important for ciprofloxacin than for moxifloxacin (slope of linear regressions in percentage of value in control cells: 70% [1199G] and 49% [1199A] for ciprofloxacin; 89% [1199G] and 74% [1199A] for moxifloxacin).

References

1. **Michot et al.** doi: 10.1128/AAC.49.6.2429-2437.2005
2. **Sun et al.** doi: 10.1002/cpdd.848
3. **Naidoo et al.** doi:10.2217/pgs- 2017-0144

Conclusion

Ciprofloxacin, and to a lower extent, moxifloxacin, are **substrates of ABCB1**, and **even more of its 1199G>A variant. Affecting 4 to 6% of Caucasian population,** the clinical significance of this observation needs to be established. Noteworthy in this context, **other SNPs in ABCB1 have already been shown to reduce exposure to fluoroquinolones in humans** [2, 3] (c.3435C>T (rs1045642) affecting 50% of the Caucasian population and c.2677T>G/A (rs2032582) affecting between 0,13 % (A) and 55 % (G) of the Caucasian population).