# Changes in the expression of MDR transporters in J774 mouse macrophages selected by chronic exposure to ciprofloxacin (CIP) or moxifloxacin (MXF), two fluoroquinolone antibiotics

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## INTRODUCTION

Active efflux of cytotoxic substances is a well-known mechanism of multidrug resistance in bacteria (antibiotic efflux) [1] and eukaryotic cells (anticancer drug efflux) [2]. Recently, it has been shown in our laboratory that ciprofloxacin (CIP), a fluoroquinolone antibiotic, is substrate for a MDR efflux pump in mouse macrophages [3,4], while moxifloxacin (MXF), another fluoroquinolone, is not [4]. The transporter was identified as being Mrp4 [5]. CIP-resistant cells obtained by chronic exposure to this antibiotic show a decreased CIP accumulation, and increased CIP efflux, asssciated with an overexpression of Mrp4 [5].

The aim of this study was to compare the phenotype and the genotype of MXF-resistant cells with that of WT and ciprofloxacin-resistant cells.

## MATERIALS AND METHODS

**Cells** : we used wild type mouse macrophages J774 (WT), and cells made resistant to CIP and MXF. These obtained by chronical exposure to increasing concentrations of one of these two antibiotics (0.1 to 0.2mM of CIP or MXF) [6].

**Accumulations** : cells were incubated with 50µM of CIP or MXF, or with 10µM of rhodamine 123 (Rho; preferential P-gp substrate) for 2h, and with 0.1µM of Bodipy-Prazosin (BP; preferential Bcrp substrate) for 1h. Specific inhibitors used were gemfibrozil (Gem, 500µM) for Mrp, verapamil (V, 100µM) for P-gp, and fumitremorgin C (FTC, 10µM) for Bcrp. Cellular concentrations of drugs and reference substrates were measured by fluorimetry and expressed by reference to the total protein content in each sample.

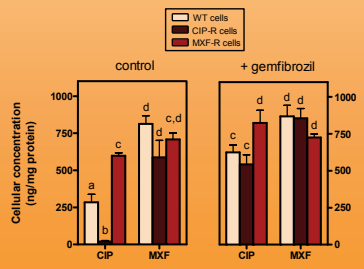
**CIP efflux** : cells were incubated for 2h at 37°C with 50µM of CIP alone or in combination with 500µM gemfibrozil (only for WT cells), and reincubated to CIP-free medium for up to 30 min. Results are expressed as the percentage of the CIP cell content measured before transfer to CIP-free medium.

**Real Time PCR** : ABC transporters mRNA expression levels were quantified by TaqMan Low Density Array on a 7900HT Fast Real Time PCR System (Applied Biosystems). Values are the mean of duplicates from two biological samples, and two housekeeping genes (*Gapdh* and *Gusb*) were used to normalize gene expression as compare to WT cells.

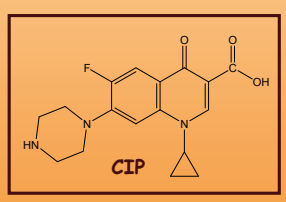
**Western Blot** : Mrp4 was detected in whole cell lysates with $M_4I$-10 monoclonal antibody (anti-actin antibody was used as control), while P-gp and Bcrp were detected in enriched membrane preparations with C219 and BXP-53 antibodies respectively (anti-prohibitin antibody was used as control).

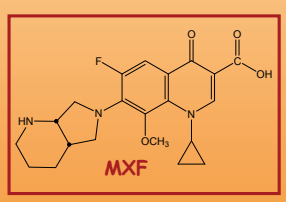
## RESULTS

### CIP and MXF accumulation

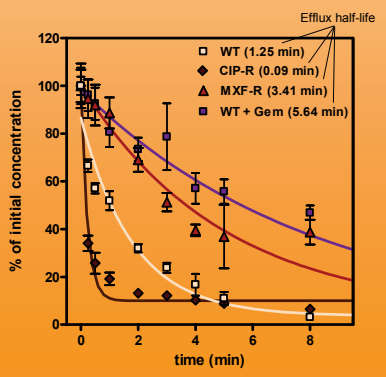



Statistical analysis : one-way ANOVA, Tukey post-hoc test.
Different letters indicates significantly different values with p<0.05.

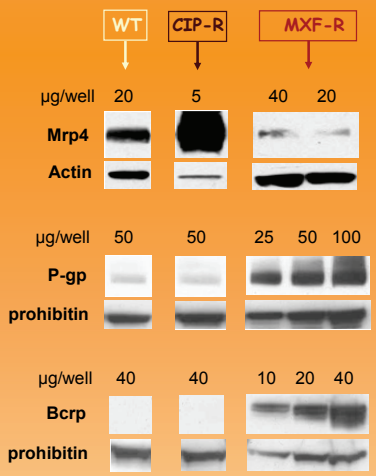



### CIP efflux in CIP-R and MXF-R cells




### Mrp4, P-gp and Bcrp proteins expression

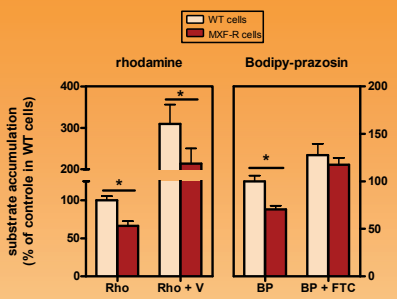



### ABC transporters mRNA expression

| | gene | CIP-R cells | MXF-R cells |
|---|---|---|---|
| | Abca1 | -1.53 | -3.51 |
| | Abca8b | 1.39 | 76.2 |
| P-gp → | Abcb1a | -1.63 | 80.38 |
| | Abcb2 | 1.1 | 4.3 |
| | Abcb3 | -1.35 | 3.17 |
| | Abcb9 | 6.08 | 9.83 |
| Mrp4 → | Abcc4 | 14.59 | -1.82 |
| | Abcg1 | -1.93 | -4.92 |
| Bcrp → | Abcg2 | 1.14 | 103.94 |

mRNA variation expression of 9 of the 47 ABC transporters examined as compared to WT cells.
Red background : MDR transporters mRNA with over- or under-expression
White background : ABC transporters not involved in MDR but showing over- or under-expression
No background : no difference in mRNA level between WT and CIP-R or MXF-R cells.

### P-gp and Bcrp activity




Statistical analysis : one-way ANOVA, Tukey post-hoc test.
* indicates significantly different values between 2 bars with p<0.05.

## CONCLUSIONS

Chronic exposure of WT macrophages to a Mrp4 substrate like CIP and a non substrate like MXF induces opposite changes in the expression of Mrp4 (the CIP transporter), and has different effects on the expression of other transporters :

- In CIP-R cells, we observe a markedly decreased accumulation and a faster efflux of CIP, which are mainly due to Mrp4 over-expression.

- In MXF-R cells, we show here :

× a higher accumulation and a slower efflux of CIP than observed in WT cells; this phenotype can be ascribed to an under-expression of the Mrp4 protein (sequencing of the Mrp4 ORF shows no mutation which could have altered CIP transport).

× much more modifications in ABC transporters mRNA levels among which P-gp and Bcrp are over-expressed and of main importance in multidrug transport. The functionality of P-gp and Bcrp in MXF-R cells have been demonstrated by the lower accumulation of specific substrates (Rhodamine 123 for P-gp and Bodipy-Prazosin for Bcrp) in comparison to that in WT cells.

As a conclusion to this work, we can observe that two closely related fluoroquinolone antibiotics (namely CIP and MXF) can differentially affect ABC transporters expression, suggesting complex regulatory mechanisms which will do the object of future investigations.

## REFERENCES

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