Characterization of the active efflux of fluoroquinolones in eukaryotic cells

Coralie VALLET

Promotor: Françoise Van Bambeke
INTRODUCTION

RESISTANCE: What? When? Why?

• Defence mechanism against cellular invasion by toxic substances.

• Chronic use of chemotherapy leads to resistance
  ➢ anticancer agents ——> eukaryotic cells
  ➢ antibiotics ———> prokaryotic cells (bacteria)

• Cell adaptation in order to avoid death
INTRODUCTION

RESISTANCE: How?

- enzymes $\Rightarrow$ inactive drugs
- target mutation $\Rightarrow$ ineffective drugs
INTRODUCTION

RESISTANCE: How?

• enzymes $\Rightarrow$ inactive drugs

• target mutation $\Rightarrow$ ineffective drugs

• **active efflux** of the toxic substance $\Rightarrow$ drugs cannot reach their target
INTRODUCTION

RESISTANCE: How?

• enzymes $\Rightarrow$ inactivate drugs

• target mutation $\Rightarrow$ ineffective drugs

• active efflux of the toxic substance $\Rightarrow$ drugs cannot reach their target

  ABC (ATP Binding Cassette) transporters
INTRODUCTION

RESISTANCE: How?

• enzymes ⇒ inactivate drugs

• target mutation ⇒ ineffective drugs

• active efflux of the toxic substance ⇒ drugs cannot reach their target
  ABC (ATP Binding Cassette) transporters
  MRP = Multidrug resistance associated proteins
INTRODUCTION

Why study the active efflux of **antibiotics** in **eukaryotic** cells?

- **Antibiotics**
  - Prokaryotic cells (bacteria)

- **Anticancer agents**
  - Eukaryotic cells (animal)
INTRODUCTION

Why study the active efflux of **antibiotics** in **eukaryotic** cells?

![Diagram showing the active efflux of antibiotics and anticancer agents between prokaryotic (bacteria) and eukaryotic (animal) cells.](image-url)
INTRODUCTION

Why study the active efflux of **antibiotics** in **eukaryotic** cells?

- **Antibiotics**
  - Prokaryotic cells (bacteria)
  - Eukaryotic cells (animal)

- **Anticancer agents**

**Intracellular bacteria**
INTRODUCTION

Antibiotics = fluoroquinolones

Inhibition of topoisomerase activity ⇒ bacteria’s death
CONTEXT OF THE STUDY

Characterization of fluoroquinolone efflux in macrophages

CIPROFLOXACIN

MOXIFLOXACIN
CONTEXT OF THE STUDY

Characterization of fluoroquinolone efflux in macrophages

(Michot et al., 2004 ; Michot et al., 2005)
CONTEXT OF THE STUDY

Characterization of fluoroquinolone efflux in macrophages

(Michot et al., 2004; Michot et al., 2005)

MRP substrate

Non MRP substrate
CONTEXT OF THE STUDY

Can we make eukaryotic cells resistant to fluoroquinolones?

Phenotype?
CONTEXT OF THE STUDY

Can we make eukaryotic cells resistant to fluoroquinolones?

Phenotype?

**Phenotype?**

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CONTEXT OF THE STUDY

Can we make eukaryotic cells resistant to fluoroquinolones?

Mrp4 overexpression

CIP accumulation 🆙

Phenotype?
OBJECTIVES OF THE STUDY

1. Characterization of ciprofloxacin- and moxifloxacin-resistant cells

- Mechanism of Mrp4 overexpression?
- ABC transporters profile/phenotype?
- CIP and MXF effect on eukaryotic topoisomerases?

2. Mrp4: fluoroquinolone recognition and transport
OBJECTIVES OF THE STUDY

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2. Mrp4: fluoroquinolone recognition and transport

Phenotype?
RESULTS I: resistance and mechanisms

Characterization of Abcc4 Gene Amplification in Stepwise-Selected Mouse J774 Macrophages Resistant to the Topoisomerase II Inhibitor Ciprofloxacin

Béatrice Marquez\(^1\), Geneviève Ameye\(^2\), Coralie M. Vallet\(^1\), Paul M. Tulkens\(^1\), Hélène A. Poirel\(^2\), Françoise Van Bambeke\(^1\*\)

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RESULTS I: resistance and mechanisms

CIP accumulation and Mrp4 expression
RESULTS I: resistance and mechanisms

CIP accumulation and Mrp4 expression

- CIP accumulation
- Mrp4 (gene and protein)
RESULTS I: resistance and mechanisms

CIP accumulation and Mrp4 expression

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CIP accumulation and Mrp4 expression

- CIP accumulation
- Mrp4 (gene and protein)

Over-representation of the Mrp4 protein in CIP-R cells ᝲ due to a duplication of the mrp4 gene
SUMMARY

1. Characterization of ciprofloxacin- and moxifloxacin-resistant cells
   - Mechanism of Mrp4 overexpression?
   - CIP and MXF effect on eukaryotic topoisomerase?
   - ABC transporters profile/phenotype?

2. Mrp4: fluoroquinolone recognition and transport

Phenotype?
Modulation of the expression of ABC transporters in murine (J774) macrophages exposed to large concentrations of the fluoroquinolone antibiotic moxifloxacin

Coralie M. Vallet¹,², Béatrice Marquez³,⁴, Naïma Nhiri³, Ahalieyah Anantharajah³, Marie-Paule Mingeot-Leclercq³, Paul M. Tulkens⁵, Jean-Yves Lallemand⁶, Eric Jacquet⁷,⁸, Françoise Van Bambeke⁹,¹⁰

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² Centre de recherche de Gif, Institut de Chimie des Substances naturelles, avenue de la Terrasse, 91198 Gif-sur-Yvette, France
³ IMAGIF qPCR Platform, CNRS UPR2301, avenue de la Terrasse, 91198 Gif-sur-Yvette, France
RESULTS I: resistance and mechanisms

MXF and CIP accumulation

MXF accumulation constant

➔ CIP accumulation in MXF-R cells
RESULTS I: resistance and mechanisms

MXF and CIP accumulation

- CIP accumulation in MXF-R cells

MXF accumulation constant

CIP efflux slower in MXF-R cells
RESULTS I: resistance and mechanisms

MXF and CIP accumulation

MXF accumulation constant

→ CIP accumulation in MXF-R cells

CIP efflux

CIP efflux slower in MXF-R cells

Mrp4 protein in MXF-R cells

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1. Characterization of ciprofloxacin- and moxifloxacin-resistant cells

- Mechanism of Mrp4 overexpression?
- ABC transporters profile/phenotype?

- CIP and MXF effect on eukaryotic topoisomerases?

2. Mrp4: fluoroquinolone recognition and transport
RESULTS I: resistance and mechanisms

Topoisomerase activity in wild-type, ciprofloxacin- and moxifloxacin-resistant cells
RESULTS I: resistance and mechanisms

Topoisomerase activity in WT, CIP-R and MXF-R cells incubated with CIP, MXF, CPT, ETO or combination of anticancer agent and fluoroquinolones
RESULTS I: resistance and mechanisms

Topoisomerase activity in WT, CIP-R and MXF-R cells incubated with CIP, MXF, CPT, ETO or combination of anticancer agent and fluoroquinolones
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Topoisomerase activity in WT, CIP-R and MXF-R cells incubated with CIP, MXF, CPT, ETO or combination of anticancer agent and fluoroquinolones

<table>
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<th>WT</th>
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<th>MXF-R</th>
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<tr>
<td>CT</td>
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<td>+ CIP</td>
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<td>+ CPT</td>
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<td>+ ETO</td>
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Type I topo

Type II topo

No topoisoermerase inhibition

No topoisoermerase inhibition ⇒ resistance mechanism??

active topoisoermerase

inhibited topoisoermerase
### RESULTS I: resistance and mechanisms

Topoisomerase activity in WT, CIP-R and MXF-R cells incubated with CIP, MXF, CPT, ETO or combination of anticancer agent and fluoroquinolones

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FQ protection against induced toxicity of CPT or ETO

- Except in CIP-R cells with CIP

No topoisomerase inhibition

- Resistance mechanism??

No topoisomerase inhibition

- Resistance mechanism??
SUMMARY

1. Characterization of ciprofloxacin- and moxifloxacin-resistant cells

- Amplification of the Mrp4 gene
- Mrp4 protein
- Type II topoisomerase alteration

Protection of CIP and MXF against CPT and ETO toxic effects

2. Mrp4: fluoroquinolone recognition and transport
**SUMMARY**

1. Characterization of ciprofloxacin- and moxifloxacin-resistant cells
   - Mechanism of Mrp4 overexpression?
   - ABC transporters profile/phenotype?
   - CIP and MXF effect on eukaryotic topoisomerases?

2. Mrp4: fluoroquinolone recognition and transport
Cellular accumulation of fluoroquinolones is not predictive of their intracellular activity: studies with gemifloxacin, moxifloxacin and ciprofloxacin in a pharmacokinetic/pharmacodynamic model of uninfected and infected macrophages

Coralie M. Vallet, Béatrice Marquez¹, Eva Ngabirano, Sandrine Lemaire, Marie-Paule Mingeot-Leclercq, Paul M. Tulkens*, Françoise Van Bambeke

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RESULTS II: fluoroquinolone structure and PK profile

GMF, MXF and CIP accumulation
RESULTS II: fluoroquinolone structure and PK profile

GMF, MXF and CIP accumulation

→ High gemifloxacin accumulation level
→ As MXF, GMF accumulation is not affected by an Mrp transporter in WT cells
RESULTS II: fluoroquinolone structure and PK profile

GMF, MXF and CIP accumulation

⇒ High gemifloxacin accumulation level
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Has GMF a higher intracellular activity?
RESULTS II: fluoroquinolone structure and PK profile

GMF, MXF and CIP accumulation

- High gemifloxacin accumulation level
- As MXF, GMF accumulation is not affected by an Mrp transporter in WT cells

Has GMF a higher intracellular activity?

Intracellular activity of GMF, MXF and CIP against S. aureus and L. monocytogenes

Intracellular killing over time
RESULTS II: fluoroquinolone structure and PK profile

GMF, MXF and CIP accumulation

Has GMF a higher intracellular activity?

Intracellular activity of GMF, MXF and CIP against S. aureus and L. monocytogenes

NO !!
SUMMARY

1. Characterization of ciprofloxacin- and moxifloxacin-resistant cells
   - Mechanism of Mrp4 overexpression?
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2. Mrp4: fluoroquinolone recognition and transport
   Molecular determinants for recognition by an efflux pump?
RESULTS II: fluoroquinolone structure and PK profile

Substrate recognition by efflux pumps in prokaryotes (NorA in \textit{S. aureus}, PatA/PatB in \textit{S. pneumoniae}, Mex/Opr in \textit{P. aeruginosa}) and in eukaryotes (Mrp4 in murine J774 macrophages): a combined biological and structural study with 25 fluoroquinolones

(The structural study was performed in collaboration with Martine Prévost and Julien Dupont, \textit{Structure et Fonction des Membranes biologiques}, Université Libre de Bruxelles)
RESULTS II: fluoroquinolone structure and PK profile

Bacteria efflux pumps

- PatA/PatB from *Streptococcus pneumoniae*
- NorA from *Staphylococcus aureus*
- Mex/Opr system from *Pseudomonas aeruginosa*
RESULTS II: fluoroquinolone structure and PK profile

Bacteria vs mouse macrophages:
Δ accumulation levels maximal efflux (CIP-R) and minimal efflux (WT + Gem)

- eukaryotes / Gram+ ⇒ good correlation
- eukaryotes / Gram- ⇒ no correlation
**RESULTS II: fluoroquinolone structure and PK profile**

**Bacteria vs mouse macrophages:**

\[ \Delta \text{accumulation levels maximal efflux (CIP-R) and minimal efflux (WT + Gem)} \]

- eukaryotes / Gram+ ⇒ good correlation
- eukaryotes / Gram- ⇒ no correlation

Is there one physicochemical parameter which govern the sensitivity to efflux by Mrp4, NorA and PatA/PatB?

⇒ NO
RESULTS II: fluoroquinolone structure and PK profile

Interactions between fluoroquinolones and Mrp4
RESULTS II: fluoroquinolone structure and PK profile

Interactions between fluoroquinolones and Mrp4

CIP: 3 interactions with Mrp4
RESULTS II: fluoroquinolone structure and PK profile

Interactions between fluoroquinolones and Mrp4

CIP: 3 interactions with Mrp4

MXF: 6 interactions with Mrp4
RESULTS II: fluoroquinolone structure and PK profile

Interactions between fluoroquinolones and Mrp4

CIP: 3 interactions with Mrp4

MXF: 6 interactions with Mrp4

Efflux ⇒ less interactions
No efflux ⇒ more interactions
TAKE HOME MESSAGE

✓ 2 molecules from the same class of antibiotics are able to induce **opposite phenotypes**
TAKE HOME MESSAGE

✓ 2 molecules from the same class of antibiotics are able to induce **opposite phenotypes**

CIP (Mrp4 substrate) ⇒ ☢ Mrp4

MXF (not Mrp4 substrate) ⇒ ☢ Mrp4
TAKE HOME MESSAGE

✓ 2 molecules from the same class of antibiotics are able to induce **opposite phenotypes**

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mayı Altered type II topoisomerase
**TAKE HOME MESSAGE**

- 2 molecules from the same class of antibiotics are able to induce **opposite phenotypes**
  - CIP (Mrp4 substrate) $\Rightarrow$ Mrp4
  - MXF (not Mrp4 substrate) $\Rightarrow$ Mrp4
  - Altered type II topoisomerase

- Fluoroquinolones can **protect** cells from anticancer agents toxicity
TAKE HOME MESSAGE

- 2 molecules from the same class of antibiotics are able to induce **opposite phenotypes**
  
  CIP (Mrp4 substrate) $\Rightarrow$ Mrp4
  
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- Fluoroquinolones can “**protect**” cells from anticancer agents toxicity
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CIP (Mrp4 substrate) ⇒ 🔄 Mrp4

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 Altered type II topoisomerase

✓ Fluoroquinolones can “**protect**” cells from anticancer agents toxicity ⇒ HOW ????

*Diagram showing the interaction between CIP and CPT with different molecular markers.*
TAKE HOME MESSAGE

- 2 molecules from the same class of antibiotics are able to induce **opposite phenotypes**

CIP (Mrp4 substrate) ⇒ 🤸 Mrp4

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様々 Altered type II topoisomerase

- Fluoroquinolones can “protect” cells from anticancer agents toxicity ⇒ HOW ????

- Accumulation of fluoroquinolones is **not predictive** of their **intracellular activity** ⇒ bioavailability ????
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TAKE HOME MESSAGE

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CIP (Mrp4 substrate) ⇒  Mrp4

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◇ Altered type II topoisomerase

✓ Fluoroquinolones can “**protect**” cells from anticancer agents toxicity ⇒ HOW ????

✓ **Accumulation** of fluoroquinolones is **not predictive** of their **intracellular activity** ⇒ bioavailability ???

✓ **All fluoroquinolones** are “**substrates**” of efflux pumps

⇒ Efflux is linked to the **number of interactions** the molecule does with the binding site of the efflux pump
PERSPECTIVES

- All fluoroquinolones are recognized by the Mrp4 efflux pump, but following their sensitivity to efflux, the resistance mechanism in cells exposed to FQs can differ.

What else now??

✓ MXF-R cells: what is the mechanism leading to Mrp4 reduction in expression? type II topoisomerase = resistance mechanism?

✓ How do fluoroquinolones protect cells against anticancer agents toxic effects?

✓ Intracellular bioavailability of fluoroquinolones?

✓ Transport of fluoroquinolones by Mrp4: Mrp4 crystal structure?
THANKS TO …

- Françoise Van Bambeke
- Prof. P. Tulkens, M-P Mingeot-Leclercq
- Members of the jury
- Collaborators: Martine Prévost and Julien Dupont (ULB), Dr. Wetzstein (Bayer)
- F.S.R. and F.R.I.A.
- My family
THANKS TO ...

All old and new FACMists... My colleagues and friends