Antibiotic efflux pumps in eucaryotic cells: consequences for activity against intracellular bacteria
Magic bullets need to reach their target

Paul Ehrlich (1854–1915)

“...the goal is...to find chemical substances that have special affinities for pathogenic organisms and that, like magic bullets, go straight to their targets...”
Magic bullets need to reach their target

- glycopeptides
- β-lactams
- quinolones
- macrolides
- aminoglycosides
Target accessibility is critical for intracellular activity.
Active efflux reduces antibiotic cellular concentration
Why efflux transporters?
Why efflux transporters?

Physico-chemical properties are inadequate for reaching an intracellular target!

Why efflux transporters?

Amphipathic drug

Most drugs are amphipathic by design, to be able to cross membrane barriers!

Why efflux transporters?

But a diffusible compound may have potentially harmful effects!

Why efflux transporters?

Extrusion by efflux pumps

Why efflux transporters?

Extrusion by efflux pumps

general mean of protection against cell invasion by diffusible molecules

Typical ‘toxic’ diffusible substances as substrates for efflux pumps

- Antibiotics
- Antifungals
- Anticancer agents
Mechanisms of active efflux

Most antibiotics are amphiphilic!

- Cationic amphiphiles: macrolide, fluoroquinolone, rifampicin, lincosamide, sulfamide
- Anionic amphiphiles: tetracycline, fluoroquinolone, β-lactam, fusidic acid

<table>
<thead>
<tr>
<th>Antibiotic class</th>
<th>bacteria Gram (+)</th>
<th>bacteria Gram(-)</th>
<th>fungi</th>
<th>superior eucaryotes</th>
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</thead>
<tbody>
<tr>
<td>β-lactams, fusidic acid, macrolides, streptogramins, tetracyclines, aminoglycosides, chloramphenicol, rifamycins, sulfamides, trimethoprim, fluoroquinolones</td>
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Consequences of antibiotic efflux from eucaryotic cells

• alteration of pharmacokinetics
  • single cell: accumulation, localization
  • whole organism: absorption, distribution, elimination

• alteration of pharmacodynamics
  • cellular level: activity against intracellular bacteria
  • body level: drug concentration in the infected compartment

### Antibiotics as substrates of efflux pumps

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<td>chloramphenicol</td>
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<td>rifamycins</td>
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<td>sulfamides</td>
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<td>trimethoprim</td>
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<td>fluoroquinolones</td>
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Antibiotics as substrates of efflux pumps

azithromycin

ciprofloxacin

moxifloxacin
Macrolides and quinolones as cell-associated antibiotics

Clinical relevance of intracellular and extracellular concentrations of macrolides.

Carbon C.

The serum levels of the three macrolides--roxithromycin, clarithromycin and azithromycin--vary considerably. The prediction of the antibacterial effect against extracellular pathogens is based on circulating concentrations of free drug, peak and trough levels, the rate of killing, and the presence of a post-antibiotic effect. Intracellular activity depends on the distribution of the antibiotic and the localization of the bacteria, and is variable. Roxithromycin uptake is greater than that of erythromycin. The intracellular half-life may be long for some compounds (azithromycin > roxithromycin). The intracellular distribution is bimodal, both in the lysosomes and the cytoplasm, but the mechanisms of uptake have not yet been established. At low pH, accumulation is low and macrolides are less active in an acidic medium. Intracellular concentrations cannot readily be predicted on the basis of extracellular levels. Different models have shown that the greater the intracellular concentration, the better the clinical effect. In addition, the transport of macrolides by cells into the infected focus may play an important role in the therapeutic outcome. These factors influence the clinical indications for macrolides, their dosing regimens and breakpoints. In future, macrolides will be developed that are more selective for intracellular infections, while others, which will achieve significant serum levels, will be useful for a broader range of diseases. However, new compounds should be evaluated in different models of infection before clinical studies are instituted. The analysis of failures remains the most important approach in defining concentration/effect relationships.

Quinolones in the treatment of lower respiratory tract infections caused by intracellular pathogens.

Chidiac C, Mouton Y.
Department of Infectious Diseases, University of Lille II, Central Hospital, Tourcoing, France.

Intracellular pathogens are inhibited to varying degrees, depending upon the strain of the organism and the quinolone tested. Quinolones achieve levels in the lower respiratory tract that equal or exceed serum concentrations, and they also achieve good intracellular concentrations. Experimental models of intracellular infection have demonstrated the efficacy of ciprofloxacin, daptomycin, levofloxacin, ofloxacin and pefloxacin. Animal models of experimental legionellosis have confirmed in vivo their efficacy in this field. Thus, quinolones appear to be a safe and efficacious alternative treatment in lower respiratory tract infection (LRTI) due to intracellular pathogens. Considering the in vitro and experimental studies, quinolones should play an important role in the treatment of LRTI caused by intracellular pathogens, and prospective controlled studies are strongly recommended.
Characterization of antibiotic efflux pumps in macrophages
Efflux pumps expressed in J774 macrophages
ABC multidrug transporters

cationic amphiphiles

MDR-1 (P-glycoprotein)

anionic amphiphiles

MRP1-10
How to inhibit ABC transporters?

- MDR-1 (P-glycoprotein)
- MRP1-10

Cationic amphiphiles
- Deoxyglucose
- NaN₃

Anionic amphiphiles
- ATP
- ADP
How to inhibit ABC transporters?

cationic amphiphiles

MDR-1 (P-glycoprotein)

verapamil

GF120918

ATP

ADP
How to inhibit ABC transporters?

- Probenecid
- Gemfibrozil
- MK571
- MRP1-10

Chemical structures and ATP/ADP interactions.
Differential recognition by MDR pumps

Influence of ATP-depletion and pump inhibitors on accumulation at equilibrium

Azithromycin & P-glycoprotein
extracell. conc. 5 mg/L;
AZM 3 h; CIP 2 h

Ciprofloxacin & MRP

Efflux of macrolides: azithromycin
Kinetics of accumulation

Azithromycin accumulates to high levels in eucaryotic cells

extracell. conc. 5 mg/L

Macrolide subcellular distribution

Macrolides accumulate in the lysosomal compartment

Carlier et al, JAC (1987) 20 Suppl B:47-56
Mechanism of accumulation for macrolides

Macrolide accumulation proceeds by diffusion / segregation

Kinetics of accumulation and efflux for azithromycin

Azithromycin concentration is high but still suboptimal ...

extracell. conc. 5 mg/L

Kinetics of accumulation and efflux for azithromycin

Inhibition of P-gp by verapamil increases accumulation

extracell. conc. 5 mg/L; verapamil 20 µM

Kinetics of accumulation and efflux for azithromycin

Accumulation markedly increased; efflux marginally affected

extracell. conc. 5 mg/L; verapamil 20 µM

Azithromycin, ‘kick-back’ model

Efflux of quinones (ciprofloxacin, moxifloxacin)

ciprofloxacin

moxifloxacin
Kinetics of accumulation

Quinolones accumulate to moderate levels in eucaryotic cells

Carlier et al JAC (1990) 26 Suppl B:27-39
Quinolone subcellular distribution

Quinolones are found in the soluble fraction

LDH (cytosol)  NABgase (lysosomes)  proteins

Carlier et al JAC (1990) 26 Suppl B:27-39
Kinetics of accumulation and efflux for ciprofloxacin

both accumulation and efflux markedly affected

extracell. conc. 17 mg/L; probenecid 5 mM

Ciprofloxacin, classical model

Kinetics of accumulation and efflux for moxifloxacin

neither accumulation nor efflux affected

extracellular conc. 17 mg/L; probenecid 5 mM

Michot et al. AAC (2005) 49:2429-37
Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin

Michot et al. AAC (2005) 49:2429-37
Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin
- moxifloxacin not affected

Michot et al. AAC (2005) 49:2429-37
Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin

Michot et al. AAC (2005) 49:2429-37
Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin and moxifloxacin

Moxifloxacin also able to interact with the transporter!

Michot et al. AAC (2005) 49:2429-37
Moxifloxacin, ‘futile-cycle’ model

Eytan et al. (1996) JBC 271:12897-902
Influence of efflux pumps on antibiotic activity against intracellular infections
Efflux from eucaryotic cells and intracellular activity

Models of intracellular infection

$L.\ monocytogenes$  
S. aureus

cytosol  
phagolysosomes
Influence of pump inhibitors on intracellular activity

azithromycin and *L. monocytogenes*

*L. monocytogenes*

**AZM**

verapamil 20 µM; 24 h

Δ log CFU (5 h – 0 h)

Influence of pump inhibitors on intracellular activity

azithromycin and S. aureus

S. aureus

verapamil 20 µM; 24 h

Influence of pump inhibitors on intracellular activity
ciprofloxacin and *L. monocytogenes*

*CIP*

*L. monocytogenes*


gemfibrozil 250 µM; 24 h

\[ \Delta \log \text{CFU (5 h - 0 h)} \]

\[ \text{ciprofloxacin concentration (x MIC)} \]
Influence of pump inhibitors on antibiotic distribution

verapamil enhances azithromycin concentration in cytosol and vacuoles

L. monocytogenes

S. aureus

Influence of pump inhibitors on antibiotic distribution

gemfibrozil enhances ciprofloxacin cytosolic content

*L. monocytogenes*  
*S. aureus*

Conclusion

Constitutive efflux of antibiotics in macrophages

Pharmacokinetics:
- Suboptimal cellular accumulation

Pharmacodynamics:
- Suboptimal intracellular activity

Pharmacology:
- Differences in affinity within a AB class

Pharmacokinetics:
- Wide spectrum transporters

Resistance?  
Drug interactions?
Perspectives for the future of chemotherapy

Grand Prix de Tours
2005
Perspectives for the future of chemotherapy

- use of poor substrates of efflux pumps (moxi vs cipro)
- development of specific inhibitors of efflux pumps
- caution for « cross – resistance » with other substrates (over – expression of efflux pumps)
Quinolones differ by the susceptibility to efflux

Michot et al. AAC (2005) 49:2429-37
Quinolones differ by their activity against intracellular Listeria
Perspectives for the future of chemotherapy

• use of poor substrates of efflux pumps (moxi vs cipro)
• development of specific inhibitors of efflux pumps
• caution for « cross – resistance » with other substrates (over – expression of efflux pumps)
Specific inhibitors

GF120918: a specific MDR inhibitor currently in clinical evaluation in cancer chemotherapy
Specific inhibitors

GF120918 increases efficacy of doxorubicin in mice with resistant tumors

Specific inhibitors

GF120918 is more potent than verapamil to increase azithromycin cellular accumulation

Seral et al, AAC (2003) 47:1047-1051
Perspectives for the future of chemotherapy

- use of poor substrates of efflux pumps (moxi vs cipro)
- development of specific inhibitors of efflux pumps
- caution for « cross – resistance » with other substrates (over – expression of efflux pumps)
Over-expression of efflux pumps as mechanism of resistance

- anticancer agent
- antibiotic

- antibiotic
- anticancer agent
Over-expression of efflux pumps as mechanism of resistance

How to get resistant cells?

- step-wise increase in CIP concentration
- several passages at each step

multifactorial multidrug resistance

Over-expression of efflux pumps as mechanism of resistance

Ciprofloxacin accumulation is reduced in resistant cells

Heremans et al. ICAAC 2004
Over-expression of efflux pumps as mechanism of resistance

influence of probenecid on quinolone accumulation in wild-type cells

Heremans et al. ICAAC 2004
Over-expression of efflux pumps as mechanism of resistance

Influence of probenecid on quinolone accumulation in wild-type and CIP-resistant cells

Heremans et al. ICAAC 2004
Over-expression of efflux pumps as mechanism of resistance

CIP is ineffective in CIP-resistant cells infected by *L. monocytogenes*

Heremans et al. ECCMID 2005
Over-expression of efflux pumps as mechanism of resistance

Probenecid restores CIP activity in CIP-resistant cells infected by \textit{L. monocytogenes}

Heremans et al. ECCMID 2005
Over-expression of efflux pumps as mechanism of resistance the CIP-resistant phenotype is not easily reversible

Heremans et al. ICAAC 2004
Over-expression of efflux pumps as mechanism of resistance

the CIP-resistant phenotype is not easily reversible

Heremans et al. ICAAC 2004
Take home message

constitutive efflux is part of the game

→ Take it into account

- in the choice of your « magic bullets » …
- for their optimal targeting
Thanks to...

F Van Bambeke  JM Michot  C Seral  M Heremans  MP Mingeot-Leclercq  PM Tulkens

come and see us at <www.md.ucl.ac.be/facm>