Antibiotic efflux pumps in eucaryotic cells: consequences for activity against intracellular bacteria

Françoise Van Bambeke

on behalf of J.M. Michot, C. Seral, M. Heremans, M.P. Mingeot-Leclercq, P.M. Tulkens

Unité de Pharmacologie cellulaire et moléculaire

Université catholique de Louvain
Brussels, Belgium

www.md.ucl.ac.be/facm
Bienvenue à l'UCL-Bruxelles

pharmacy
antibiotics as magic bullets

already concerned in efflux?
Why drug efflux transporters?

Physico-chemical properties are inadequate for reaching an intracellular target!
Why drug efflux transporters?

Amphipathic drug

Most drugs are amphipathic by design, to be able to cross membrane barriers!
Why drug efflux transporters?

But a diffusible compound may have potentially harmful effects!
Why drug efflux transporters?

Extrusion by efflux pumps
Why drug efflux transporters?

Extrusion by efflux pumps

general mean of protection against cell invasion by diffusible molecules
Typical ‘toxic’ diffusible substances well known as substrates for efflux pumps

- Antibiotics
- Antifungals
- Anticancer agents
Most antibiotics are amphiphilic!

cationic amphiphiles
- macrolide
- tetracycline
- rifampicin
- lincosamide

anionic amphiphiles
- fluoroquinolone
- β-lactam
- fusidic acid

### Antibiotic classes recognized by efflux pumps in different types of organisms

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

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

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

Main multidrug resistance efflux pumps in eucaryotic cells

ATP-binding Cassette (ABC) Superfamily

MDR-1 (P-glycoprotein)
MRP1-9

cationic amphiphiles
anionic amphiphiles
Inhibitors and substrates share the same physicochemical properties.

- MDR-1 (P-glycoprotein)
- MRP1-9
- Cationic amphiphiles: verapamil
- Anionic amphiphiles: probenecid
Macrophages express at least MRP-1 and P-gp
1. Influence of efflux pumps on antibiotic cellular pharmacokinetics

macrolides
Azithromycin is a dicationic amphiphilic molecule
Azithromycin accumulates to high levels in eucaryotic cells

macrolides accumulate mainly in the lysosomal compartment

Carlier et al, JAC (1987) 20 Suppl B:47-56
macrolide accumulation proceeds by diffusion / segregation

Azithromycin concentration is high but still suboptimal …

Inhibition of P-gp by verapamil increases accumulation

Inhibition of P-gp by verapamil increases accumulation and slightly slows down efflux

Similar effects are obtained with more specific inhibitors of P-gp

How can efflux pumps increase azithromycin accumulation without markedly affecting its efflux?

 mechanism of action of efflux pumps
Efflux pumps as pores?  

Probably not ...

Experimental evidences

• substrates and inhibitors are amphiphilic

• rates and kinetics of efflux are not directly related to the cytosolic drug content

Bolhuis et al, EMBO J (1996) 15:4239-4245
Efflux pumps as vacuum cleaners?

Possibly ...
Efflux pumps as flippases? 

Possibly ...

X-Ray structure of the lipid A transporter from *E. coli*, an homolog of P-glycoprotein

substrates potentially extruded from the membrane!
substrates potentially extruded from the membrane without having seen the cytosol!
2. Influence of efflux pumps on antibiotic cellular pharmacokinetics

quinolones
Ciprofloxacin is a zwitterionic amphiphilic molecule.
Quinolones accumulate to moderate levels in eucaryotic cells

Carlier et al JAC (1990) 26 Suppl B:27-39
Quinolones are found in the soluble fraction of cell homogenates

LDH (cytosol)  NABgase (lysosomes)  proteins

Carlier et al JAC (1990) 26 Suppl B:27-39
Ciprofloxacin facilitates its own uptake

Ciprofloxacin accumulation and efflux in ATP-depleted cells

Ciprofloxacin accumulation and efflux in probenecid-treated cells

Differential effects of pump inhibitors on ciprofloxacin and azithromycin accumulation

Data from Michot et al, AAC, 2004
Have all quinolones been made equal?

ciprofloxacin

moxifloxacin

levofloxacine

garenoxacin
Quinolones markedly differ by their accumulation level.
contrasting effect of quinolone concentration on their accumulation
contrasting effect of ATP-depletion on quinolone accumulation
comparative effect of pump inhibition on quinolone accumulation

2 h incubation, 5 mg/L, inhibitors:

Is this the max level of quinolone accumulation?
contrasting effect of temperature on quinolone accumulation – cooling

2 h incubation, 5 mg/L, temperature:

- **37°C**
  - cipro: 18%
  - levo: 55%
  - gareno: 45%
  - moxi: 78%

- **4°C**
  - cipro: 18%
  - levo: 55%
  - gareno: 45%
  - moxi: 78%

slowed diffusion through membranes
contrast effect of temperature on quinolone accumulation – heating

2 h incubation at 37°C, 17 mg/L, preexposure to 56°C:

Is this the max level of quinolone accumulation?

active processes impaired in death cells
quinolone accumulation is passive but efflux is active

actual sorting site may be at the cell surface
accumulation is inversely related to recognition by efflux transporters

Is the amount of drug reaching the cellular medium high enough to kill intracellular bacteria?
3. Influence of efflux pumps on antibiotic cellular pharmacodynamics
Does efflux from macrophages confer ‘resistance’ against intracellular infections?

Does efflux affect the intracellular activity of these antibiotics?

- Listeria monocytogenes
- Staphylococcus aureus
Verapamil increases azithromycin activity against *L. monocytogenes*
Verapamil increases azithromycin activity against *S. aureus*

Verapamil increases azithromycin conc. both in the soluble and granular fractions

Gemfibrozil increases ciprofloxacin activity against *L. monocytogenes*

*Seral et al, JAC (2003) 51:1167-73*
Gemfibrozil does not increase ciprofloxacin activity against *S. aureus*

*S. aureus*

CIP

Gemfibrozil increases ciprofloxacin conc. in the soluble fraction only

Inhibition of efflux pumps may increase antibiotic activity in the compartments where they accumulate.
Strategies for the future of antibiotherapy of intracellular infections

• use of poor substrates of efflux pumps (moxi vs cipro)

• caution for « cross – resistance » with other substrates (over – expression of efflux pumps)

• development of specific inhibitors of efflux pumps
moxi/gareno are more active than cipro/levo against *L. monocytogenes* et *S. aureus*
Strategies for the future of antibiotherapy of intracellular infections

• use of poor substrates of efflux pumps (moxi vs cipro)

• caution for « cross – resistance » with other substrates (over – expression of efflux pumps)

• development of specific inhibitors of efflux pumps
Over-expression of efflux pumps as mechanism of resistance
Ciprofloxacin selects over-expression of efflux pumps as mechanism of resistance

2 h incubation at 37°C

macrophages exposed to increasing conc. of cipro (up to 80 mg/L)
Strategies for the future of antibiotherapy of intracellular infections

- use of poor substrates of efflux pumps (moxi vs cipro)
- caution for « cross – resistance » with other substrates (over – expression of efflux pumps)
- development of specific inhibitors of efflux pumps
Inhibitors of efflux transporters ... should help you to keep your stuff in ...
Inhibitors of efflux transporters ...

But be careful not to turn off a useful pump ...
come and see us at <www.md.ucl.ac.be/facm>