Antibiotic accumulation and efflux in eukaryotic cells:

a journey at the frontier of pharmacokinetics and pharmacodynamics
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

for appropriate time and in sufficient concentration …
Birth of antibiotic “PK-PD”

Dosage regimen

Concentration versus time in serum
- absorption distribution elimination

Concentration versus time in tissues and other body fluids

Concentration versus time at site of infection

Pharmacologic or toxicologic effect

Antimicrobial effect versus time

PHARMACOKINETICS

PHARMACODYNAMICS

Craig (1998) CID 26:1-10
ISAP classical view of PK/PD

Table: PK/PD Parameters Correlating with Efficacy in Murine Thigh and Lung Infections

<table>
<thead>
<tr>
<th><em>Penicillins</em></th>
<th><em>Aminoglycosides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Above MIC</td>
<td>AUC (Peak)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Fluoroquinolones</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>Metronidazole</td>
</tr>
<tr>
<td>Monobactams</td>
<td>Daptomycin</td>
</tr>
<tr>
<td>Tribactams</td>
<td>Ketolides</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td>Streptogramins</td>
<td>Macrolides</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td></td>
</tr>
</tbody>
</table>

Image: PPT Slide - Netscape


Slide 11 of 31
but classical PD predicts concentration-effects for all drugs ....
but classical PD predicts concentration-effects for all drugs.

S. aureus; 24 h

Can we conciliate both theories?

**Figure:**

- **Δ log CFU (24 h - 0 h)**
- **log concentration (X MIC)**
- Points represent data for **ampicillin** (blue squares) and **gentamicin** (red circles).
- The figure illustrates the conc.-dependent and time-dependent PD profiles in the clinics.

**Notes:**

- **S. aureus; 24 h**
- Barcia-Macay *et al*, submitted; Lemaire *et al* (2005) JAC in press
Target accessibility becomes critical for intracellular activity
Main routes of drug entry in cells

- Passive diffusion
- Channel transporter
- Endocytosis transporter
- Active diffusion
Intracellular “PK-PD”

- **Dosage regimen**
- Concentration versus time in **cells**
- Concentration versus time in **non-infected cells**
  - Pharmacologic or toxicologic effect
  - Antimicrobial effect versus time
- Concentration versus time at **intracellular site** of infection
- Penetration distribution efflux

**Pharmacokinetics**

**Pharmacodynamics**
Intracellular “PK-PD”

- **Dosage regimen**
- **glycopeptides**
- **Concentration versus time in cells**
- **Concentration versus time in non-infected cells**
- **Concentration versus time at intracellular site of infection**

**Pharmacokinetcis**
- **Pharmacologic or toxicologic effect**
- **Antimicrobial effect versus time**

**Pharmacodynamics**
- **penetration**
- **distribution**
- **efflux**
Intracellular “PK-PD”

- Dosage regimen
- Concentration versus time in non-infected cells
- Glycopeptides penetration distribution efflux
- Concentration versus time at intracellular site of infection
- Pharmacologic or toxicologic effect
- Antimicrobial effect versus time

**PHARMACOKINETICS**

**PHARMACODYNAMICS**
Intracellular “PK-PD”

Gamuts of glycopeptides:
- Dosage regimen
- Concentration versus time in cells
- Concentration versus time in non-infected cells
- Concentration versus time at intracellular site of infection

Gamuts of pharmacokinetics:
- Penetration
- Distribution
- Efflux

Gamuts of pharmacodynamics:
- Pharmacologic or toxicologic effect
- Antimicrobial effect versus time

Gamuts of pharmacokinetics and pharmacodynamics:
- PHARMACOKINETICS
- PHARMACODYNAMICS
Intracellular “PK-PD”

Dosage regimen

Concentration versus time in cells

Concentration versus time in non-infected cells

Concentration versus time at intracellular site of infection

Pharmacologic or toxicologic effect

Antimicrobial effect versus time

macrolides
quinolones

penetration distribution efflux

PHARMACOKINETICS

PHARMACODYNAMICS
Intracellular “PK-PD”

- Dosage regimen
- Concentration versus time in non-infected cells
- Concentration versus time in cells
- Concentration versus time at intracellular site of infection
- Pharmacologic or toxicologic effect
- Antimicrobial effect versus time

- macrolides
- quinolones
- penetration distribution
- efflux

PHARMACOKINETICS

PHARMACODYNAMICS
Accumulation of magic bullets in eukaryotic cells

glycopeptides
Vancomycin, the parent compound
The glycopeptide oritavancin, a voluminous, amphiphilic molecule
From vancomycin to oritavancin

epi-vancosamine
From vancomycin to oritavancin

4-epi-vancosamine ➔ self-association capacity

LY264626
From vancomycin to oritavancin

lipophilic side chain
→ activity
including against resistant enterococci

Oritavancin, a cationic amphiphile

- Lipophilic side chain
- Additional protonable amine
Oritavancin, a cationic amphiphile

New chemical entity

- New pharmacodynamic properties?
- New pharmacokinetic profile?
- But also ... new potential side effects?
## Spectrum of activity

<table>
<thead>
<tr>
<th>bacteria</th>
<th>resistance</th>
<th>vancomycin</th>
<th>oritavancin</th>
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<tbody>
<tr>
<td>enterococci</td>
<td>susc.</td>
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<td>0.06-0.25</td>
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<td></td>
<td>VanA</td>
<td>&gt;128</td>
<td>1-4</td>
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<tr>
<td></td>
<td>VanB</td>
<td>8-128</td>
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<td>S. aureus</td>
<td>Methi-S</td>
<td>1-2</td>
<td>1</td>
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<tr>
<td></td>
<td>Methi-R</td>
<td>1-4</td>
<td>1-2</td>
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<tr>
<td></td>
<td>GISA</td>
<td>8</td>
<td>1-8</td>
</tr>
<tr>
<td></td>
<td>GRSA</td>
<td>&gt; 128</td>
<td>0.5</td>
</tr>
</tbody>
</table>

- highly active on susc. enterococci
- active on VAN-resistant strains

Pharmacodynamic profile

VANCOMYCIN: modestly bactericidal
- slow
- no or little conc. effect

ORITAVANCIN: highly bactericidal:
- rapid
- conc. dependent

S. aureus

Barcia-Macay et al. submitted
## Pharmacokinetic properties

<table>
<thead>
<tr>
<th>parameter</th>
<th>Vancomycin (15 mg/kg)</th>
<th>Oritavancin (3 mg/kg)</th>
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<tr>
<td>peak (mg/L)</td>
<td>20-50</td>
<td>31</td>
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<tr>
<td>trough (mg/L)</td>
<td>5-12 (24 h)</td>
<td>1.7 (24 h)</td>
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<tr>
<td>protein binding</td>
<td>10-55 %</td>
<td>90 %</td>
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<tr>
<td>terminal t½ (h)</td>
<td>4-8</td>
<td>360</td>
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</tbody>
</table>

- daily administration
- retention in the organism?

Aim of the study

- activity against (multi-resistant) Gram-positive (S. aureus)
- rapid bactericidal activity
- retention in the organism

any place for intracellular infections?

**cellular pharmacokinetics:**
accumulation and subcellular distribution in eukaryotic cells

**cellular pharmacodynamics:**
activity against intracellular S. aureus

**cellular toxicity:**
morphological and biochemical alterations
Aim of the study

- activity against (multi-resistant) Gram-positive (S. aureus)
- rapid bactericidal activity
- retention in the organism

any place for intracellular infections?

- cellular pharmacokinetics: accumulation and subcellular distribution in eukaryotic cells

- cellular pharmacodynamics: activity against intracellular bacteria

- cellular toxicity: morphological and biochemical alterations
Oritavancin accumulation and release are slow.

**Accumulation**

- Cellular concentration (μg/mg prot) vs. time (h)

**Efflux**

- Extracellular conc. 25 mg/L; 24 h

Van Bambeke et al. (2004) AAC 48:2853-60
Comparison with other antibiotics

Oritavancin reaches exceptional cellular accumulation levels

J774 macrophages; extracell. conc. 25 mg/L; 24 h

Van Bambeke et al. (2004) AAC 48:2853-60
Subcellular localization

- N-acetyl-β-glucosaminidase
- lysosomes
- mitochondria
- membrane
- cytochrome c-oxidase
- inosine diphosphatase

Van Bambke et al. (2004) AAC 48:2853-60
Oritavancin is a lysosomotropic antibiotic

N-acetyl-β-glucosaminidase

Van Bambeke et al. (2004) AAC 48:2853-60
Mechanism of cellular accumulation

chloroquine
azithromycin

HRP
latex beads
Mechanism of cellular accumulation

Oritavancin kinetics of accumulation are very similar to those of tracers of (adsorptive) endocytosis

Van Bambeke et al. (2004) AAC 48:2853-60
Aim of the study

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- rapid bactericidal activity
- retention in the organism

any place for intracellular infections?

cellular pharmacokinetics:
- accumulation and distribution in eukaryotic cells

cellular pharmacodynamics:
- activity against intracellular bacteria

cellular toxicity:
- morphological and biochemical alterations
Intracellular activity on *S. aureus* control or itavancin. Oritavancin can destroy intracellular bacteria in THP-1 macrophages; extracellular conc. 25 mg/L; 24 h. Barcia-Macay *et al.* submitted.
Oritavancin shows time- and concentration-dependent intracellular bactericidal effects.

Dose-effect for extracell. vs intracell. activity

Intracellular activity < extracellular activity

THP-1 macrophages; 24 h

Barcia-Macay et al. submitted
intracellular activity < extracellular activity, but bactericidal effects reached at clinically-relevant concentrations
oritavancin is one of the most active drugs against intracellular *S. aureus*.
Aim of the study

- activity against multi-resistant Gram-positive (S. aureus)
- rapid bactericidal activity
- retention in the organism

any place for intracellular infections?

cellular pharmacokinetics:
accumulation and subcellular distribution in eukaryotic cells

cellular pharmacodynamics:
activity against intracellular bacteria

cellular toxicity:
morphological and biochemical alterations
Morphological studies

Van Bambeke et al. (2005) AAC – in press

Rat embryo fibroblasts; 25 mg/L; 3 days
J774 macrophages, 25 mg/L; 1 day

polar lipids
macrophages
fibroblasts
Biochemical studies: time-effects

Accumulation of phospholipids and cholesterol develops in parallel with oritavancin cellular concentration.

Rat embryo fibroblasts; 25 mg/L

Van Bambeke et al. (2005) AAC – in press
Biochemical studies: dose-effects

Rat embryo fibroblasts, 3 days
J774 macrophages, 1 day

Van Bambeke et al. (2005) AAC – in press
Model of the interaction of oritavancin with eukaryotic cells
Can we dissociate activity from toxicity?

cellular alterations co-exist with destroyed bacteria ....

THP-1 macrophages; 25 mg/L; 24 h
Can we dissociate activity from toxicity?

Comparison with two other lysosomotropic cationic antibiotics

**Gentamicin**
- Polycationic, hydrophilic
- Endocytosis
- Phospholipidosis

**Azithromycin**
- Dicationic
- Diffusion/segregation
- Accumulation of phospholipids and cholesterol
Lysosomotropic antibiotics and activity on *S. aureus*

GEN and ORI are both conc.-dependent intracellularly

*J774 macrophages*
Lysosomotropic antibiotics and activity on *S. aureus*

GEN and ORI are both conc.-dependent intracellularly in J774 macrophages.
Lysosomotropic antibiotics and activity on \textit{S. aureus}

But GEN activity limited at clinically-relevant conditions
Lysosomotropic antibiotics and phospholipidosis

Phospholipidosis developing on a conc.-dependent manner

Rat embryo fibroblasts
Lysosomotropic antibiotics and phospholipidosis

Toxic potential variable at clinically-relevant conditions

Rat embryo fibroblasts
Lysosomotropic antibiotics and phospholipidosis

Toxic potential variable at clinically-relevant conditions

Rat embryo fibroblasts
Lysosomotropic antibiotics and phospholipidosis

Toxic potential variable at clinically-relevant conditions

Rat embryo fibroblasts
Can we dissociate activity from toxicity?

*both processes are dependent on cellular concentration...*

... and develop in parallel
amphiphilic glycopeptides, a new type of « magic bullets»

**Conclusion**

**Pharmacodynamics:**
- Bactericidal, conc-dep. activity

**Pharmacokinetics:**
- Lysosomotropic accumulation

**Cellular Pharmacodynamics:**
- Conc. and time-dependent bactericidal activity towards extra AND intra S. aureus

**Cellular Toxicity:**
- Conc. and time-dependent cellular toxicity

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**Morphological Studies**
- Rat embryo fibroblasts; 25 mg/L; 3 days
- J774 macrophages, 25 mg/L; 1 day

Van Bambeke et al. (2005) AAC – in press
Questions for future work

- reasons for reduction in activity intracellularity
- binding site
- mechanism of accumulation
- in vivo
- oritavancin
- S. aureus
- polar lipids
- undefined material
Take home message

cellular accumulation, the best and the worse of properties…

drug development

activity

toxicity
Intracellular “PK-PD”

Dosage regimen → Concentration versus time in cells

macrolides quinolones → Concentration versus time in cells

penetration distribution efflux

Concentration versus time in non-infected cells → Pharmacologic or toxicologic effect

Concentration versus time at intracellular site of infection → Antimicrobial effect versus time

PHARMACOKINETICS

PHARMACODYNAMICS
Intracellular “PK-PD”

Dosage regimen

Concentration versus time in cells

macrolides quinolones

Concentration versus time in non-infected cells

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Pharmacologic or toxicologic effect

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PHARMACOKINETICS

PHARMACODYNAMICS
Efflux of magic bullets from eukaryotic cells

macrolides quinolones
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

Mechanisms of active efflux

### Antibiotics as substrates of efflux pumps

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

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, Meunon 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, difloxacin, fleroxacin, 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.
Aim of the study

amphiphilic antibiotics
  • accumulating in eucaryotic cells
  • considered as useful for treating intracellular infections
  • known substrates of efflux pumps in bacteria

efflux from macrophages ?

macrolides
  • phenotypic characterization of the active efflux
  • consequences for intracellular activity

quinolones
Aim of the study

amphiphilic antibiotics
  • accumulating in eucaryotic cells
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efflux from macrophages?

macrolides

quinolones

• *phenotypic characterization of the active efflux*

• consequences for intracellular activity
Efflux pumps expressed in J774 macrophages
ABC multidrug transporters

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

Cationic amphiphiles

Anionic amphiphiles

ATP → ADP
How to inhibit ABC transporters?

cationic amphiphiles

MDR-1 (P-glycoprotein)

anionic amphiphiles

MRP1-10

deoxyglucose

NaN₃

ATP → ADP
How to inhibit ABC transporters?

cationic amphiphiles

MDR-1 (P-glycoprotein)

ATP

ADP

verapamil

GF120918
How to inhibit ABC transporters?

- Probenecid
- Gemfibrozil
- MK571
- MRP1-10

Anionic amphiphiles

ATP

ADP
Differential recognition by MDR pumps

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

**azithromycin**

- control
- ATP-depl
- VER
- GF
- PROB

**ciprofloxacin**

- control
- ATP-depl
- VER
- GF
- PROB
- MK

extracell. conc. 5 mg/L; AZM 3 h; CIP 2 h

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  • phenotypic characterization of the active efflux
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quinolones
Models of intracellular infection

\[ L. \text{ monocytogenes} \quad S. \text{ aureus} \]

- Cytosol
- Phagolysosomes
Influence of pump inhibitors on intracellular activity of azithromycin and L. monocytogenes

verapamil 20 µM; 24 h

Influence of pump inhibitors on intracellular activity of azithromycin and *S. aureus*

verapamil 20 µM; 24 h

Influence of pump inhibitors on intracellular activity

ciprofloxacin and \textit{L. monocytogenes}

gemfibrozil 250 µM; 24 h

Influence of pump inhibitors on intracellular activity

ciprofloxacin and S. aureus

gemfibrozil 250 µM; 24 h

Influence of pump inhibitors on antibiotic distribution

Verapamil enhances azithromycin concentration in cytosol and vacuoles

Influence of pump inhibitors on antibiotic distribution

gemfibrozil enhances ciprofloxacin cytosolic content

L. monocytogenes

S. aureus

Are these effects clinically-relevant?

constitutive efflux makes AZM and CIP activity suboptimal in a clinically-meaningful range of concentrations.
Aim of the study

amphiphilic antibiotics
- accumulating in eucaryotic cells
- considered as useful for treating intracellular infections
- known substrates of efflux pumps in bacteria

macrolides ↔ quinolones
- cellular pharmacokinetics
- model of interaction with the transporters

efflux from macrophages?
Aim of the study

amphiphilic antibiotics
  • accumulating in eucaryotic cells
  • considered as useful for treating intracellular infections
  • known substrates of efflux pumps in bacteria

efflux from macrophages ?

macrolides

quanolones

• cellular pharmacokinetics
• model of interaction with the transporters
Kinetics of accumulation and efflux for azithromycin

accumulation markedly increased; efflux marginally affected

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

Kinetics of accumulation and efflux for ciprofloxacin

both accumulation and efflux markedly affected

extracellular conc. 17 mg/L; probenecid 5 mM

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) – in press
Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin

Michot et al. AAC (2005) – in press
Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin
- moxifloxacin not affected

Michot et al. AAC (2005) – in press
Quinolones as inhibitors of 
ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin

Michot et al. AAC (2005) – in press
Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin
  moxifloxacin

Moxifloxacin also able to interact with the transporter!

Michot et al. AAC (2005) – in press
## Comparison of kinetic parameters

<table>
<thead>
<tr>
<th>drug</th>
<th>influx</th>
<th>efflux</th>
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<tbody>
<tr>
<td></td>
<td>flux (pmol/mg prot/min)</td>
<td>half-life (min)</td>
<td>flux (pmol/mg prot/min)</td>
<td>half-life (min)</td>
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<tr>
<td></td>
<td>control</td>
<td>inhibitor</td>
<td>control</td>
<td>inhibitor</td>
</tr>
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<td>44</td>
<td>71</td>
<td>1</td>
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<tr>
<td></td>
<td>49</td>
<td>53</td>
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<td>flux (pmol/mg prot/min)</td>
<td>half-life (min)</td>
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<td>control</td>
<td>inhibitor</td>
</tr>
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<td>44</td>
<td>71</td>
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<tr>
<td>CIP</td>
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<tr>
<td>MXF</td>
<td>68</td>
<td>0.2</td>
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## Comparison of kinetic parameters

<table>
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</tr>
</tbody>
</table>
Azithromycin, ‘kick-back’ model

Ciprofloxacin, classical model

Moxifloxacin, ‘futile-cycle’ model

Eytan et al. (1996) JBC 271:12897-902
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?
Questions for future research

- mechanism of transport
- cooperation with bacterial efflux pumps
- identification of transporter
- metabolic alterations
- drug interactions
- questions
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 ...
Evaluating magic bullets

- **pharmacokinetics**
  - H. Chanteux, M. Heremans, J.M. Michot

- **pharmacodynamics**
  - M. Barcia, N. Bles, S. Carryn, S. Lemaire, A. Olivier, C. Seral, S. Van de Velde

- **toxicodynamics**
  - J.P. Montenez, H. Servais, D. Tyteca

- **biophysics & molecular biology**
  - N. Caceres, N. Fa
Thanks to …

New magic bullets

• **chemistry**
  E. Colacino, C. Dax, L. Efron, T. Happaerts, M. Renard

• **pharmacology**
  I. Tytgat, D. Van Ackeren

• **modeling**
  M. Prévost, M. Rooman, S. Vandevuer
Thanks to …

Resistance to magic bullets

• efflux
  L. Avrain, N. Mesaros

• glycopeptides
  P. Courvalin and his team
Clinical use of magic bullets

- clinical pharmacy
  E. Ampe, V. Basma, A. Spinewine
Thanks to …

Playing with magic bullets

• **technical staff**
  N. Aguilera, M.C. Cambier, O. Meert, F. Renoird, M. Vergauwen

• **secretary**
  M. Breugelmans
Thanks to …

Ehrlich’s colleagues
Thanks to …

Inspiring research on magic bullets
Evaluating research on magic bullets

Thanks to …

Paying for research on magic bullets

FNRS

UCL
Managing research on magic bullets

M.P. Mingeot-Leclercq
P.M. Tulkens
Thank you for your attention.