Build up and make use of a Molecular Pharmacology Lab

Françoise Van Bambeke, PharmD, PhD
Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
Université catholique de Louvain
Brussels, Belgium

http://www.facm.ucl.ac.be/

Presented at the University of Pharmacy, Hanoi, Vietnam

With the support of Wallonie-Bruxelles-International
Build up and make use of a Molecular Pharmacology Lab

**DRUG**
- analysis

**MOLECULAR TARGET**
- protein
  - receptor
  - transporter, canal,…
  - enzyme
- lipid
  - membrane target
- nucleic acid
  - transcription
  - integrity

**Biochemistry**
- molecular biology

**Biochemistry**
- biophysics

**Molecular Biology**
From genes to function .....
What are you interested in?

- **Proteins**
  - Detection of proteins
  - Change in expression level
  - Change in localization
  - Change in activation state; post-traductional modifications
  - Change in activity

- **ADN**
  - Amplification (for cloning, e.g.)
  - Detection of genes (« yes or no » reply)
  - Search for mutations

- **ARNm**
  - Expression levels
  - Post-transcriptional modifications
1. Build up ...
A. Plan what you need as material
A. Plan what you need as material

• **Proteins (activity)**
  
  • thermostatized bath, melting ice
  • spectrometer, fluorimeter, (microplate reader)
  • centrifuge

  • ultracentrifuge
  • cell culture room / animal house (depending on your model…)
  • scintillation counter, HPLC, mass spectrometry, …
A. Plan what you need as material

- **Proteins (detection)**

  - **western-blot**:
    - gel preparation, thank, gentle agitator,
    - system for detecting signal associated to secondary antibody
      (luminescence, absorbance, fluorescence, ...),
    - software for analysing band intensity

- **cell fractionation**: ultracentrifuge

- **2D-gels (proteomic analysis)**:
  - gel preparation, electrofocusing, thanks for running several gels in parallel
  - system for detecting signal (silver coloration, fluorophores)
  - protein identification (mass spectrometry)
  - appropriate software for gel analysis and protein identification
A. Plan what you need as material

• **DNA/RNA**

• electrophoresis thank
• PCR machine
• transluminator for band visualization + software for band analysis
• thermostatized bath (incubation over wide range of $t^\circ$ [4-90°C]

• real-time PCR
• fluorimeter for quantification of DNA/ARN [Qubit® for example]
• hood to prevent contaminations
B. Organize your lab in an efficient way
Contaminating is easy with DNA / RNA!

FIGURE 1. Outline of sample processing and analysis in a PCR laboratory.

How to prevent contamination?

1. Organize your lab!

FIGURE 2. Organization of a PCR laboratory with separate pre- and post-PCR rooms.

How to prevent contamination

2. Use appropriate material!

FIGURE 3. Use of barrier tips to prevent amplicon contamination in the PCR laboratory.
How to prevent contamination

3. Perform quality control!

→ **positive control**:  
  - matrix ~ gene of interest  
  - PCR mixture  
  - enzyme (polymerase)  

shows that PCR conditions are appropriate to detect the gene of interest

→ **negative control**:  
  - water used for sample dilution  
  - PCR mixture  
  - enzyme (polymerase)  

shows that your reagents are not contaminated
A practical example in our lab …

Absence of DNA in mRNA samples for real time PCR

Nucleic acids → purification → mRNA → reverse transcription → cDNA → Amplication → Evaluation of gene expression level

Samples (mRNA) + cDNA + PCR mix or H₂O + PCR mix

Positive control

Negative control

No interpretation possible …

contamination of water !

mRNA purified !

clean water !
How to prevent contamination

wear gloves … and change when needed!

Contamination source could be the skin!

→ DNA/RNAase could be present

→ proteins could be present
  → western blot detect specific proteins only
  → may be a problem in global analyses of proteome
    (detection of proteins absent from the sample itself)
A practical example in our lab ....

General lab

L2 (pathogens) micro-biology cell culture balances

storage sterilie material autoclaves centrifuges -80°C -20°C 4°C

14C-3H biophysics

mol bio 3 mol bio 1

mol bio 2

General lab

spectrometer fluorimeter plate readers HPLC
A practical example in our lab ....
A practical example in our lab …. 

Pre-PCR steps in a lab 1
A practical example in our lab ….

Lab 2 organized with specific benches
- Proteins
- DNA
- RNA

mol bio 3
mol bio 1
mol bio 2
A practical example in our lab ....

PCR and post-PCR in lab 3
A practical example in our lab ....

Nice place to work!
Don’t forget computers …

Many machines are now piloted with computers …
A series of databases on the web ...

<table>
<thead>
<tr>
<th>Database</th>
<th>URL</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleotide Sequence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GenBank</td>
<td><a href="http://www.ncbi.nlm.nih.gov">www.ncbi.nlm.nih.gov</a></td>
<td>All publicly available nucleotide and protein sequences</td>
</tr>
<tr>
<td>EMBL Nucleotide Sequence Database</td>
<td><a href="http://www.ebi.ac.uk/embl.html">www.ebi.ac.uk/embl.html</a></td>
<td>All publicly available nucleotide and protein sequences</td>
</tr>
<tr>
<td>DNA Data Bank of Japan (DDBJ)</td>
<td><a href="http://www.ddbj.nig.ac.jp">www.ddbj.nig.ac.jp</a></td>
<td>All publicly available nucleotide and protein sequences</td>
</tr>
<tr>
<td><strong>DNA Sequences: Genes, Motifs and Regulatory Sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIGR Gene Indices</td>
<td><a href="http://www.tigr.org/tdb/tgi.shtml">www.tigr.org/tdb/tgi.shtml</a></td>
<td>Organism-specific databases of EST and gene sequences</td>
</tr>
<tr>
<td>ExInt</td>
<td><a href="http://sege.ntu.edu.sg/wester/iekb/">http://sege.ntu.edu.sg/wester/iekb/</a></td>
<td>Exon-intron structure of eukaryotic genes</td>
</tr>
<tr>
<td>TRANSFAC</td>
<td><a href="http://www.gene-regulation.com">http://www.gene-regulation.com</a></td>
<td>Transcription factors and binding sites</td>
</tr>
<tr>
<td>RDP</td>
<td>rdp.cme.msu.edu</td>
<td>Ribosomal database project: rRNA sequences data</td>
</tr>
<tr>
<td><strong>Gene Expression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIR</td>
<td>pir.georgetown.edu</td>
<td>A collection of protein sequence databases</td>
</tr>
<tr>
<td>SWISS-PROT</td>
<td><a href="http://www.expasy.ch/sprot">www.expasy.ch/sprot</a></td>
<td>Curated protein sequence databases</td>
</tr>
<tr>
<td>PROSITE</td>
<td><a href="http://www.expasy.ch/prosite">www.expasy.ch/prosite</a></td>
<td>Biologically-significant protein patterns and profiles</td>
</tr>
<tr>
<td>Pfam</td>
<td><a href="http://www.sanger.ac.uk/Software/Pfam/">www.sanger.ac.uk/Software/Pfam/</a></td>
<td>Sequence alignments and profile hidden Markov models</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCSD</td>
<td>bssv01.lancs.ac.uk/gig/pages/gag/carbbank.htm</td>
<td>Complex carbohydrate structure databases (CarbBank)</td>
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<tr>
<td><strong>Protein Structure</strong></td>
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<td>PDB</td>
<td><a href="http://www.rcsb.org/pdb/">www.rcsb.org/pdb/</a></td>
<td>All available 3D structures of proteins and nucleic acids</td>
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<td><strong>Genomics</strong></td>
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<td>GO</td>
<td><a href="http://www.geneontology.org">www.geneontology.org</a></td>
<td>Gene ontology consortium database</td>
</tr>
<tr>
<td>KEGG</td>
<td><a href="http://www.genome.ad.jp/kegg">www.genome.ad.jp/kegg</a></td>
<td>Databases of genes, proteins, and metabolic pathways</td>
</tr>
<tr>
<td>EcoCyc</td>
<td>ecocyc.org</td>
<td><em>E. coli</em> K-12 genes, metabolic pathways, transporters, regulation</td>
</tr>
<tr>
<td>Ensembl</td>
<td><a href="http://www.ensembl.org">www.ensembl.org</a></td>
<td>Annotated information on eukaryotic genomes</td>
</tr>
</tbody>
</table>
C. Have a responsible person with skills in molecular biology to conduct projects
C. Have a responsible person with skills in molecular biology

Why?

• design of a molecular biology experiment is not easy if you are not an expert
  • loss of time
  • loss of money

• data interpretation requires appropriate background

• quality of the data critically depends on
  • the way the experiment was performed
  • the quality of the controls

• many fields are highly competitive; be efficient!
• science is progressing exponentially in that field [databases !!!]

• molecular biology is highly expensive!
  • competitive grant applications need to be submitted ….
C. Have a responsible person with skills in molecular biology

How?

• Think twice to the best approach to answer your specific question

• Study in depth literature and take examples from high quality papers to design your experiments

• Make use of existing tools → kits → strains, cell lines, plasmids, …

• Combine molecular biology with biochemistry/pharmacology to link genetic or proteomic changes with phenotype
2. Make use …

« Magic bullets » meeting; Nuremberg, Germany
Some applications in our team

**Pharmacokinetics**: antibiotics and multidrug transporters eucaryotic cells: Identification of transporters and modulation of their expression [cellular biology, drug analysis, real-time PCR, western blot, proteomics, genomics]

**Antibiotic resistance**: Identification and characterization of resistance by efflux [microbiology, real time PCR, gene disruption]

**Pharmacodynamics**: Modulation of antibiotic activity against intracellular bacteria [cellular biology, microbiology, cell fractionation, proteomics]

Other examples of integrated pharmacology
Pharmacological, proteomic and genomic characterization of fluoroquinolone transporters in eucaryotic cells

• Pharmacologie cellulaire et moléculaire, UCL, Bruxelles
  C. Vallet, N.E. Caceres and B. Marquez
  supervision: M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke
• Laboratorium voor Eiwitbiochemie en Eiwitengineering, Ugent, Ghent
  M. Aerts; supervision: B. Devreese
• Centre de Génétique, cliniques St Luc, UCL, Bruxelles
  Geneviève Ameye, Hélène Antoine-Poirel
Chemical structure of fluoroquinolones

- **Ciprofloxacin**
  - Molecular structure with highlighted nitrogen, fluorine, and functional groups.

- **Moxifloxacin**
  - Molecular structure with highlighted nitrogen, fluorine, and functional groups.

Functional groups highlighted for comparison:
- Carboxylate (-COO⁻) groups.
- Other functional groups indicated with arrows.
Ciprofloxacin is substrate for an MRP-like transporter in J774 macrophages

Transport

… ATP-dependent

• of anion transporters
• of MRP

Identification of ciprofloxacin transporter: « resistant cells » as a tool

Chronic exposure to increasing concentrations of ciprofloxacin

Michot et al., AAC (2006) 50:1689-1695
Ciprofloxacin-resistant cells: phenotypic analysis

† efflux

† gemfibrozil IC$_{50}$

Marquez et al., AAC (2009) 53: 2410-2416
Ciprofloxacin-resistant cells: genomic analysis

ARNm expression levels by Real-Time PCR

↑ expression of Mrp2 and Mrp4, BUT Mrp4 from far most abundant

Marquez et al., AAC (2009) 53: 2410-2416
Ciprofloxacin-resistant cells: proteomic analysis

Detection of the corresponding proteins by

Western-Blot of membrane fractions

Mrp2

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

Confocal microscopy

Marquez et al., AAC (2009) 53: 2410-2416
Ciprofloxacin-resistant cells: which is the ciprofloxacin transporter?

Silencing of MRP expression in resistant cells by siRNA

Marquez et al., AAC (2009) 53: 2410-2416
Stable Isotope Labeling Aminoacid in Culture

\[ ^{13}\text{C}_6\text{-Lys} \quad ^{13}\text{C}_6\text{-Arg} \]
\[ ^{12}\text{C}_6\text{-Lys} \quad ^{12}\text{C}_6\text{-Arg} \]

WT CIP-macrophages

sample mixing 1:1

protein digestion

identification in mass spectrometry and determination of the relative abundance
SILAC: proteins with modified expression

**Light/Heavy ratios in pooled F1**

- Frequency distribution with bin ranges from -4.0 to 4.0 on the x-axis and frequency on the y-axis.

**Light/Heavy ratios in pooled F2**

- Similar to F1, with bin ranges from -4.0 to 4.0 on the x-axis and frequency on the y-axis.

**Biological function of proteins with differential abundance**

- Pie chart showing the percentage distribution of proteins across different biological functions:
  - Transport: 23%
  - Cell adhesion and localization: 11%
  - Immune response: 11%
  - Signal transduction: 3%
  - Cytoskeleton organization: 3%
  - Lipid metabolism: 9%
  - Protein metabolism: 5%
  - Carbohydrate metabolism: 5%
  - Nucleic acid metabolism: 3%
  - Other: 3%
  - Unknown: 9%

Mrp4 and Dnajc3 are the most upregulated proteins!
Gene amplification of part of chromosome 14 in CIP-R cells

Mrp4 and Dnaj3 co-amplified in CIP-resistant cells
Active efflux as a mechanism of resistance to fluoroquinolones in *S. pneumoniae*

- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**
  L. Avrain, F. El Garch, A. Lismond, S. Delvigne
  supervision: P.M. Tulkens, F. Van Bambeke
- **Unité des agents antibactériens, Institut Pasteur, Paris, France**
  P. Courvalin
- **School of Immunity & Infection, University of Birmingham, UK**
  M. Garvey
  supervision: L. Piddock

www.fcm.ucl.ac.be
# MIC of fluoroquinolones

- or + Reserpine as efflux pump inhibitor

<table>
<thead>
<tr>
<th>FQ strains</th>
<th>NOR</th>
<th>CIP</th>
<th>LVX</th>
<th>MXF</th>
<th>GMF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-R</td>
<td>+R</td>
<td>-R</td>
<td>+R</td>
<td>-R</td>
</tr>
<tr>
<td>49619</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>SP334</td>
<td>32</td>
<td>4</td>
<td>2</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>SP335</td>
<td>64</td>
<td>32</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>SP295</td>
<td>16</td>
<td>2</td>
<td>1</td>
<td>0.125</td>
<td>0.063</td>
</tr>
<tr>
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<td>0.25</td>
</tr>
</tbody>
</table>

- NOR and CIP show elevated MICs in the 4 resistant strains
- LVX MIC is close to the EUCAST Bkpt (±1 dil) in all strains
- MXF and GMF consistently show low MICs

*El Garch et al., JAC (2010) 65: 2076–2082*
### MIC of fluoroquinolones

- or + Reserpine as efflux pump inhibitor

<table>
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<tr>
<th>FQ strains</th>
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<td>SP335</td>
<td>64</td>
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<td>32</td>
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<td>0.5</td>
</tr>
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<td>16</td>
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</tr>
</tbody>
</table>

- Reserpine reverses resistance but only partially in 2 strains
- MFX not affected; LVX and GMF poorly affected
- Efflux contributes to resistance in the 4 strains
- Other mechanisms also present in 2 strains

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*El Garch et al., JAC (2010) 65: 2076–2082*
Target mutations?

<table>
<thead>
<tr>
<th>FQ strains</th>
<th>NOR -R</th>
<th>NOR +R</th>
<th>CIP -R</th>
<th>CIP +R</th>
<th>LVX -R</th>
<th>LVX +R</th>
<th>MXF -R</th>
<th>MXF +R</th>
<th>GMF -R</th>
<th>GMF +R</th>
</tr>
</thead>
<tbody>
<tr>
<td>49619</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
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<td>0.25</td>
<td>0.25</td>
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<td>2</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>0.125</td>
<td>0.125</td>
<td>0.063</td>
<td>0.032</td>
</tr>
<tr>
<td>SP13</td>
<td>64</td>
<td>16</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- reserpine reverses resistance but only partially in 2 strains

- efflux contributes to resistance in the 4 strains
- target mutations evidenced in 2 strains

Basal expression level of efflux systems

- all strains overexpress \textit{patA}/\textit{patB} to variable level
- SP335 and SP13 show a low level of \textit{pmrA} overexpression

\textit{El Garch et al., JAC (2010) 65: 2076–2082}
Induced expression level

4 h with $\frac{1}{2}$ MIC

- Induction of *patA*/*patB*
  - in all strains but to highly variable levels
  - by all FQ, whether substrates or not

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*El Garch et al., JAC (2010) 65: 2076–2082*
Induced expression level … response to stress

- concomitant overexpression of genes involved in response to stress

Efflux by PatA/PatB causes resistance!

Disruption of patA or patB restores susceptibility to fluoroquinolones in a resistant strain while disruption of pmrA does not

→ efflux of fluoroquinolones mediated by patA/patB in S. pneumoniae

---

**Table 1.** Susceptibility of *S. pneumoniae* to fluoroquinolones and substrates of efflux pumps in the absence (−R) or presence (+R) of reserpine (20 mg/L)

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant characteristics</th>
<th>Mutations in QRDR</th>
<th>norfloxacin</th>
<th>ciprofloxacin</th>
<th>levofloxacin</th>
<th>moxifloxacin</th>
<th>gemifloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC49619</td>
<td>Wild-type</td>
<td>None</td>
<td>4</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>ATCC49619patA</td>
<td>ATCC49619 patA::magellan2, SPT&lt;sup&gt;R&lt;/sup&gt;</td>
<td>None</td>
<td>4</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>ATCC49619patB</td>
<td>ATCC49619 patB::magellan2, SPT&lt;sup&gt;R&lt;/sup&gt;</td>
<td>None</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>ATCC49619pmrA</td>
<td>ATCC49619 pmrA::magellan2, SPT&lt;sup&gt;R&lt;/sup&gt;</td>
<td>None</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>SP334</td>
<td>ATCC49619 after 13-days exposure to ciprofloxacin, CIP&lt;sup&gt;R&lt;/sup&gt;</td>
<td>None</td>
<td>32</td>
<td>4</td>
<td>4</td>
<td>0.5</td>
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<tr>
<td>SP334patA</td>
<td>SP334 patA::magellan2, SPT&lt;sup&gt;R&lt;/sup&gt;</td>
<td>None</td>
<td>4</td>
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<tr>
<td>SP334patB</td>
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<td>None</td>
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<td>SP334pmrA</td>
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<td>None</td>
<td>32</td>
<td>4</td>
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</tr>
</tbody>
</table>

*El Garch et al., JAC (2010) 65: 2076–2082*
Why do beta-lactams regain activity against intracellular *Listeria monocytogenes*?

- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**  
  S. Van de Velde, S. Carryn  
  supervision: P.M. Tulkens, F. Van Bambeke
- **Laboratorium voor Eiwitbiochemie en Eiwitengineering, Ugent, Ghent**  
  M. Aerts; supervision: B. Devreese
- **Unité de recherche en biologie cellulaire, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium**  
  Edouard Delaive, Marc Dieu  
  supervision: M. Raes
Ampicillin is more active against intracellular than extracellular Listeria

Changes in intracellular metabolism?
Purifying intracellular Listeria by cell fractionation

low speed centrifugation

nuclei / unbroken cells

extract

high speed centrifugation on sucrose gradient

Van de Velde et al., Proteomics (2009) 9, 5484–5496
Separating and quantifying proteins

Van de Velde et al., Proteomics (2009) 9, 5484–5496
Interpreting changes in protein expression …. 

Van de Velde et al., Proteomics (2009) 9, 5484–5496
Examples of integrated programs of molecular pharmacology

**Oritavancin:**
a new antibiotic with novel mode of action and unusual cellular pharmacokinetic profile

**Beta-lactams:**
How do beta-lactams regain activity against MRSA in the intracellular milieu

**Aminoglycosides:**
from molecular mechanisms of toxicity to clinical implications
Pharmacology of oritavancin

- Pharmacologie cellulaire et moléculaire, UCL, Bruxelles
  C. Seral, H.A. Nguyen, S. Lemaire, P. Baudoux, O. Domenech
  supervision: M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke
- Faculté d'ingénierie biologique, agronomique et environnementale,
  Unité de chimie des interfaces, Louvain-La-Neuve
  Y. Dufrêne

www.facm.ucl.ac.be
Our main research interests...

Antibiotics: from molecules to man

Oritavancin story
Our main research interests...

- Antibiotics: from molecules to man
- Antibiotic toxicity
- Novel bacterial targets
- Cellular pharmacokinetics
- Cellular pharmacodynamics
- Resistance
- Clinical applications

Antibiotics: from molecules to man

Oritavancin story
Oritavancin, a novel lipoglycopeptide ...
A new mode of action ….

Bacteriostatic effect

Time- and concentration-dependent killing

Vancomycin

Oritavancin

Baudoux, Nguyen et al.
ICAAC 2009, Poster C1 1354
A new mode of action ....

release of entrapped calcein

membrane depolarization

Membrane permeabilization and depolarization

Baudoux, Nguyen et al.
ICAAC 2009, Poster C1 1354
A new mode of action ....
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Antibiotics: from molecules to man

Oritavancin story
Cellular pharmacokinetics of antibiotics

Subcellular distribution

uptake

efflux

Cellular pharmacokinetics of oritavancin

Lysosomal tropism

Huge cellular accumulation

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Oritavancin story
Cellular toxicity of oritavancin

Accumulation of lipids

Our main research interests...

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Antibiotics: from molecules to man

Oritavancin story
Cellular pharmacodynamics of antibiotics

SCV isolated from a cystic fibrosis patient

Comparison of antibiotics at their human Cmax

Most effective against NP and SCV

Dose effect relationship

Dual mode of action?

Conclusion of the oritavancin story and links with the clinics ….

• highly bactericidal and active against resistant strains thanks to a novel mode of action
  → Useful for infection by super bugs

• high accumulation in cells
  → Prolonged half life for once a day or once a week administration

• active against intracellular staphylococci
  → Interest for persistent infections

• lysosomal storage disorder
  → Significance in the clinics ?
  → FDA asks for more safety data before registration ….
How do beta-lactams regain activity against MRSA intracellularly?

- Pharmacologie cellulaire et moléculaire, UCL, Bruxelles
  S. Lemaire
  supervision: P.M. Tulkens, F. Van Bambeke
- Department of Chemistry and Biochemistry, Université de Notre-Dame
  Notre Dame, IN
  C. Fuda, S. Mobashery
- Centre d’ingénierie des protéines, Université de Liège
  B. Jooris
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Antibiotics: from molecules to man

cellular pharmacokinetics

antibiotic toxicity

novel bacterial targets

Beta-lactam activity against intracellular MRSA

cellular pharmacodynamics

resistance

clinical applications
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Beta-lactam activity against intracellular MRSA
MRSA vs. MSSA: intracellular activity of β-lactams

MRSA are as susceptible as MSSA to β-lactams when intracellular!

*Lemaire et al., AAC (2007) 51:1627-32*
MRSA vs. MSSA: extracellular activity of β-lactams

MRSA are as susceptible as MSSA in broth at acidic pH

Lemaire et al., AAC (2007) 51:1627-32
MRSA vs. MSSA: extracellular activity of β-lactams

Neutralization of lysosomes makes intracellular MRSA resistant to β-lactams!

MRSA are inside [acidic] vacuoles

Lemaire et al., AAC (2007) 51:1627-32
PBP2a conformation is modified by acidic pH

FIGURE 4. Circular dichroic spectra of PBP 2a at pH 7.0 (left panel) and pH 5.5 (right panel) in the absence (open symbols) and in the presence (closed symbols) of oxacillin (30 µM) for 30 min at 25 °C. The thin dotted lines in each graph represent minima of PBP 2a molar ellipticity at 222 nm (vertical arrow on the abscissa) for each condition. The spectrum of oxacillin has been subtracted from all data points.

_Lemaire et al., JBC (2008) 283:12769-76_
Impact of intraphagosomal acid pH on the expression of methicillin–resistance in S. aureus

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Rates of hydrolysis by purified β-lactamases

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class A</th>
</tr>
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<tbody>
<tr>
<td>Staphylococcus aureus PC 1</td>
<td></td>
</tr>
<tr>
<td>Ro 63-9141</td>
<td>0.93</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>19</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>200</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10,000</td>
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</table>

Affinity for PBPs

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} for competition with fluorescein-labeled ampicillin (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis PBP 2'</td>
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</tr>
<tr>
<td>Ro 63-9141</td>
<td>0.87</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>115</td>
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<tr>
<td>Imipenem</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Methicillin</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

Model of the active site of SaPBP2' complexed with ceftobiprole.

Lovering et al., ECCMID (2006) P1586
Hebeisen et al., AAC (2001) 45:825-31
Ceftobiprole MIC is not markedly influenced by pH

Ceftobiprole is as active against intracellular MSSA and MRSA

*Ceftriaxone*

*Ceftobiprole*

*Ceftriaxone*

*Ceftobiprole*

Lemaire et al. AAC (2009) 53:2289-2297
Conclusion of the MRSA story and links with the clinics ....

- Intracellular medium can modulate the expression of resistance mechanisms
- Conformation of PBP2a is critical for activity
- Design of new beta-lactams able to « open » PBP2a
- Ceftobiprole rejected but ceftaroline accepted by the FDA in 2010
Nephrotoxicity of aminoglycosides:
from molecular mechanisms
to clinical implications

• Pharmacologie cellulaire et moléculaire, UCL, Bruxelles
  H. Servais, S. Denamur
  supervision: M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke
• Unité de biologie cellulaire, UCL
  D. Tyteca, P. Vandersmissen
  supervision: P. Courtoy
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Antibiotics: from molecules to man

Nephrotoxicity of aminoglycosides
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Nephrotoxicity of aminoglycosides
nephrotoxicity cascade

Tulkens, Am. J. Med. (1986) 80(6B): 105-114
Aminglycoside–induced apoptosis

Molecular mechanisms of apoptosis

Gentamicin allows for a partial relocalisation of acridine orange in the cytosol

Gentamicin induces ROS production in lysosomes

Molecular mechanisms of apoptosis

Denamur et al, FRMB, under revision
Molecular mechanisms of apoptosis

Gentamicin allows for a partial relocalisation of cytochrome C from mitochondria to the cytosol

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Antibiotics: from molecules to man

Nephrotoxicity of aminoglycosides
Relationship between accumulation and apoptosis

Introducing gentamicin in the cytosol by electroporation markedly increases toxicity

Servais et al. AAC (2006) 50:1213-1221
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Nephrotoxicity of aminoglycosides
Once a day administration as a way to reduce toxicity

* Giuliano et al., J. Pharm. Exp. Ther., 1986
Once a day administration as a way to reduce toxicity

![Graph showing serum creatinine levels in rats after different dosing regimens](image-url)
Conclusion of the aminoglycoside story and links with the clinics ....

• aminoglycoside apoptosis is mediated by lysosomal destabilization
  → Lysosomal phospholipidosis as a protecting factor ?

• electroporation as a way to screen toxic potential of new molecules

• once-a-day administration reduces toxicity and at the same time improves activity ....
Happy researchers in our cellular and molecular pharmacology group ...
Happy researchers in our cellular and molecular pharmacology group …

Another post-doc from Vietnam trained in Molecular Biology
Hope it may help you to be as successful as Hoang Anh was in Europe …