Efflux pumps inhibitors: the long journey from procaryotes to eucaryotes

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Transport across membranes
Summer School, Jacobs University (Bremen), July 2007
Efflux pumps and their clinical relevance

Efflux pump inhibitor’s properties

EPI DISCOVERY

- By rational design
- By screening
  - bacterial EPI
  - eucaryotic EPI
Efflux pumps

⇒ UBIQUITOUS

**Eucaryotes**: ABC-pump mediated resistance

**Bacteria**: mainly RND (Gram -) and MFS (Gram +) pumps

Efflux pumps spectra

Mesaros et al., La lettre de l’Infectiologue 2005, 4: 117
Efflux pumps clinical relevance

Physiological function = protective role

P-gp membrane localisation
Efflux pumps clinical relevance

Physiological function = protective role

But efflux pumps are able to expel a wide range of molecules, including a lot of drugs

**Bacteria:** Antibiotics, detergents, dyes, bile salts….

**Eucaryotes:** Anti-cancer drugs (P-gp, MRP, BCRP…)
Antifongic drugs (CDR1, *Candida albica*)
Antiparasital drugs (Pgh1, *Plasmodium falciparum*)
Antibiotics…
Efflux pumps clinical relevance

**Bacteria:**
- → of the MCI of antibiotics (within the same class, or broad range of ATB)
- Cause intrinsic resistance (*Pseudomonas*,…)
- Several pumps can be expressed at the same time

**Eucaryotes:**
- Related to therapeutic failures (40% of tumors develop resistance to anti-cancer drugs)
- Related to negative prognosis or poor outcome for chemotherapy
- Modulate drugs pharmacokinetic: ↔ bioavailability, → excretion
Efflux pumps clinical relevance

For both bacteria and eucaryotes:

- Efflux pumps can confer MDR resistance
- They can add themselves to other resistance mechanisms

Efflux pumps can be seen as a « new » target:
Efflux pump inhibitors (EPI), by blocking the pumps, will restore drugs activity
Efflux pump inhibitors

An Efflux Pump Inhibitor will:

- restore the activity of the drug in resistant cells (intrinsic or acquired resistance)
- be devoid of effect in wild type cells
- decrease the frequency of apparition of resistant mutants
Efflux pump inhibitors

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An EPI would act by:

- binding to the pump with an increased affinity (competitive inhibition)
- impairing access to the binding site for the drug (non competitive inhibition)
- dissipating the energy source used by the pump (not clinically relevant)
MDR pumps binding sites

AcrB periplasmic drug binding pocket

MDR pumps binding sites

Modeling of the binding of two P-gp substrates

Longitudinal view across the membrane

rhodamine

verapamil

Vandevuer et al., *Proteins* 2006, 63: 466
EPIs criteria

To be used *in vivo*, an EPI should:

- be stable and *not toxic*
- be *selective* of a family of pumps:
  - eukaryotic pumps: selective of one sub-family (P-gp, MRP, BCRP)
  - bacterial pumps: a wide inhibitor (Gram +/Gram -) would be advantageous, devoided of activity against human pumps
- not cause side effects by perturbing efflux pumps physiological role
- be co-administrable with the drug

Otherwise, EPIs can be used *in vitro* as tools to detect the presence of efflux pumps (diagnostic) and to study them (affinity, binding sites…).
Rational design

Case of specific pumps: Tetracycline pumps

Chemical modification of the substrate:

⇒ 6-fold increase of the affinity of 13-CPTC for Tet(B)

⇒ Competitive inhibitor → Useful in addition to tetracyclines

Rational design

Case of specific pumps: Tetracycline pumps

![Chemical structures of Doxycycline and 13-cyclopentylthio-tetracycline](image)

- Different classes of Tet efflux pumps
- Other resistance mechanisms (ribosomal protection)
- Development of new tetracyclines not recognised by these pumps
  - **by-pass the efflux pump**
Rational design

Case of specific pumps: Tetracycline pumps

毽 By-passing efflux pumps

Tetracycline

Minocycline
Substitution that impairs efflux

Tigecycline

Screening for EPI

- Chemical libraries
- Natural extracts
- Synthetic products
Screening for EPI

Chemical libraries

natural extracts  synthetic products

Screen:
- Against bacterial strains over-expressing efflux pumps (S. aureus NorA, P. aeruginosa MexAB-OprM…) or against cancer cells
- First screen: ↗ potency of the drug?
- Second screen: ↗ intracellular accumulation of the drug?
- And then confirmation of an interaction between the efflux pump and the EPI (interference in photoaffinity labelling, co-crystallisation…)
Bacterial EPI

Reserpine (indole alkaloid)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pump</th>
<th>Family</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>Bmr</td>
<td>MFS</td>
<td>FQ</td>
</tr>
<tr>
<td>S. aureus</td>
<td>NorA</td>
<td>MFS</td>
<td>FQ</td>
</tr>
<tr>
<td>MRSA</td>
<td>Tet(K)</td>
<td>MFS</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>PmrA</td>
<td>MFS</td>
<td>NOR</td>
</tr>
<tr>
<td></td>
<td>PatA and PatB</td>
<td>ABC</td>
<td>FQ</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>Lde</td>
<td>MFS</td>
<td>FQ</td>
</tr>
</tbody>
</table>

⇒ Inhibition of Gram + efflux pumps, mainly involved in FQ efflux

⇒ As resistance to reserpine has been reported, this suggests a direct binding to the pump (at least to Bmr*)

Neurotoxic at the concentrations required to inhibit pumps in vivo
⇒ reserpine can not be used in humans

*Klyachko et al., J. Bacteriol. 1997, 179: 2189
Bacterial EPI

Screening of natural compounds

Porphyrin

Flavonolignan

- NorA pump inhibitors (MFS family), isolated from Berberis plants
- Restore berberine activity, a weak antibiotic produced by the plant
- Plants may have evolve so that they produce weak antibiotic associated with EPI

Bacterial EPI

Screening of natural compounds

- Most identified EPI are provided by plant extracts
- Most are active against Gram + pumps (MFS family), and not against Gram - pumps
- Some are also active against eukaryotic pumps

⇒ All these molecules have a large size and are lipophilic (alkaloids, flavonolignans, flavones/isoflavones, catechin gallates, diterpenes….)

⇒ Starting point for further lead optimization
⇒ Need to prove the EPI/pump interaction
⇒ Check for in vivo toxicity…
Results from a screen against NorA

Among ~ 4 000 molecules ⇒ 180 were able to restore CIP activity

Distribution of active molecules according to their MIC against S. aureus NorA in presence of CIP at ¼ of its MIC

Extract from J. elliptica

benzothiophene

benzofurane

(3)

indoles
Results from a screen against NorA

EtBr efflux from *S. aureus* NorA
Results from a screen against NorA

EtBr efflux from a *B. subtilis* over-expressing NorA

EtBr efflux from *S. aureus* NorA

Fournier dit Chabert et al., *Bioorg. Med. Chem.* 2007, **15**: 4482
# ABC families within eucaryotes

## Table 1: List of human ABC genes, chromosomal location, and function

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Alias</th>
<th>Location</th>
<th>Function</th>
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<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>ABC1</td>
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<td>Cholesterol efflux onto HDL</td>
<td>ABCC1</td>
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<td>ABC2</td>
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<td>Drug resistance</td>
<td>ABCC2</td>
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<td>ABC3</td>
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<td>ABCR</td>
<td>1p22.1-p21</td>
<td>N-retinylidene-PE efflux</td>
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<td>7p11-q11</td>
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<td>PGY1, MDR</td>
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<td>ABCB2</td>
<td>TAP1</td>
<td>6p21</td>
<td>Peptide transport</td>
<td>ABCD1</td>
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<td>ABCB3</td>
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<td>ABCB8</td>
<td>MABC1</td>
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<td>ABCF1</td>
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<td>ABC8, White</td>
<td>21q22.3</td>
<td>Cholesterol transport</td>
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<td>ABCP, MXR, BCRP</td>
<td>4q22</td>
<td>Toxin efflux, drug resistance</td>
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<td>White3</td>
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<td>Sterol transport</td>
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P-gp inhibitors

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<td>–</td>
<td>Cyclic oligopeptide immunosuppressant</td>
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<td></td>
<td>Tamoxifen</td>
<td>–</td>
<td>Nonsteroidal anti-estrogen</td>
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1st generation: not developed for MDR pumps inhibition, low affinity for pumps, side effects at the concentrations required to inhibit P-gp in vivo

McDevitt et al., Pharmacol. Ther. 2007, 113: 429
P-gp inhibitors

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<tr>
<td>2nd Generation</td>
<td>PSC833</td>
<td>Valspodar</td>
<td>Non-immunosuppressive derivative of cyclosporine A</td>
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<td></td>
<td>VX-710</td>
<td>Biricodar</td>
<td>Derivative of FK-506 - macrocyclic antibiotic</td>
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<td></td>
<td>S9788</td>
<td>–</td>
<td>Triazine</td>
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<tr>
<td></td>
<td>SR33557</td>
<td>–</td>
<td>Indolizin sulfone</td>
</tr>
</tbody>
</table>

2nd generation: more potent, toxicity reduced, but anti-cancer drugs pharmacokinetic impaired (metabolisme & elimination, via interactions with cytochrome P450)

McDevitt et al., Pharmacol. Ther. 2007, 113: 429
# P-gp inhibitors

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<td>3rd Generation</td>
<td>GF120918</td>
<td>Elaeridar</td>
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<td>LY335979</td>
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<td></td>
<td>OC144-093</td>
<td>Ontogen</td>
<td>Diarylimidazole</td>
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</tbody>
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3rd generation: more specific and powerful, under clinical trials

*In vivo* assays to assess P-gp inhibition:

- Drug efflux assay with CD65+ cells
- Use of $^{99}$Tc-marker substrates of P-gp

McDevitt *et al.*, *Pharmacol. Ther.* 2007, **113**: 429
P-gp inhibitors

elacridar

Tariquidar
Non competitive I

3rd generation

zosuquidar
MRP inhibitors

Most P-gp inhibitors are inactive against MRP1 (P-gp substrates are hydrophobic, MRP1 substrates are hydrophilic, conjugated to glutathione)

Known inhibitors:
- Agosterol (competitive inhibitor, binds to the C-terminal half of MRP1)
- Natural flavonoids
- Raloxifene analogs...

Agosterol A

Raloxifene
MRP modulators

MRP1 transports drugs either conjugated to glutathione or co-transport them with free glutathione

**Glutathione-S-transferase** (GST), which catalyses formation of GSH-conjugates, is a target to modulate MRP1 activity

Development of compounds able to mimic GSH (competitive inhibitors) or GSH-conjugates → PEPTIDOMIMETICS

Selective for MRP1 versus P-gp but may interfere with the physiological role of GSH

BCRP inhibitors

Elacridar (GF-120918) is a reference inhibitor for BCRP (also known to inhibit P-gp)

Reserpine

Fumitremorgin (mycotoxin from *Aspergillus fumigatus*) inhibits drug transport and ATPse activity, but neurotoxic → derivatives

Acridone derivatives

...
EPI discovery:
Successful...
But no clinical EPI yet available!...
Perspectives in EPI development

Different « targets » can be considered:

- The drug binding site
- The NBD of ABC transporters, required for the function of the pump (but highly conserved!)
- Residues involved in communication between several parts of the efflux system (TMD and NBD for ABC pumps, monomers of tripartite pumps…)

But it will remain difficult to get a specific EPI that does not alter the physiological functions of the efflux pump…. 
Perspectives in EPI development

Others possibilities to tackle efflux resistance:

- Interference with gene expression → Downregulation of MDR transporters (with antisense oligonucleotides, via antagonists of nuclear regulators…)

- Interference with efflux pump assembly (tripartite efflux pumps in Gram-bacteria)

- Bypass MDR efflux by developing drugs which are poor substrates of efflux pumps

  (glycylcyclines vs tetracyclines, ketolides versus macrolides, new fluoroquinolones versus older ones, new anthracyclines…)
Perspectives

For the patient:
- Detect accurately efflux pump(s) over-expression
- Take it into account to propose and adapt an efficient treatment

Development of new molecules:
- Consider efflux pumps in the early stages of conception of new drugs
- Pursue efforts to develop safe and selective EPI

*This might be accelerated with the structure elucidation of more efflux pumps*