Modelos in vitro de barreras celulares utilizados para el desarrollo de nuevos fármacos.

BioInVitro Research Area

Maya Vilà, PhD 5 Abril 2011
Development of new pharmaceutical compounds

Long and costly process

Pre-clinical

Discovery
HTS
In Vitro Screens
In Vivo Screens
Phase I
Phase II
Phase III

Clinical

Lead Identification
Lead Optimization
IND
NDA

Investigational New Drug (IND). New Drug Application (NDA)
ADME/T Properties

- Absorption
- Distribution
- Metabolism
- Excretion
- Toxicity
The barriers to target

The knowledge of the drug behavior in front of these barriers can help us to know which are the properties we have to improve in the selected candidates

IN VITRO MODELS
ADME/T Properties

- Solubility (A)
- Acidic Stability (A)
- Mucosa permeability (A, D)
- Efflux transporters (A, D)
- Intestinal Metabolism (M)
- Formulated Pill
- Target neuron cell
- BBB
- Excretion Inhibition (E)
- Gallbladder
- Biliary clearance (E)
- Phase I and II metabolism (M)
- CYP Inhibition (M)
- CYP Induction (M)
- CYP Phenotyping (M)
- Reactive Metabolite (M)
- Systemic Circulation
- Renal Clearance (E)
- Urine
- Artery
- Vein
- Tissue protein binding (D)
- Cell permeability (D)
- Efflux Transporters (D)
- Tissue metabolisms (M)
- Toxic Metabolite (M)
- Plasma protein binding (D)
- Plasma Stability (M)
The Gastrointestinal Barrier

Oral absorption

- Solubility
- Ionization (pKa)
- Lipophilicity (log D, Log P)

Permeation in the GI tract

Small Intestine (absorptive)

Large Intestine (mucosecretor)
Pathways for intestinal absorption of a compound

- **Lumen**
  - Transcellular Passive Permeability
  - Carrier-mediated Transport
  - Paracellular Passive Permeability

- **Blood**
  - Lipophilic compounds
  - Certain Nutrients
  - Hydrophilic compounds
  - Peptides
  - Substrates of ABC Transporters

**Efflux Transporters**

**Limit intestinal absorption**
Caco-2 Characteristics

- Grown on polycarbonate filters during 21 days
- Spontaneous differentiation
- Polarized intestinal barrier (absorptive phenotype)
- Barrier integrity parameters
  - TEER
  - LY

![Intestinal Lumen Diagram](attachment:intestinal_lumen_diagram.png)
Caco-2 Applications

- Oral Absorption efficiency
- Oral bioavailability
- Oral toxicity

- Evaluation of
- Study of mechanisms involved in oral and intestinal absorption
- Study the effects of transporters on permeability
- Evaluation of substrates and inhibitors of Pgp
- Accepted by EMEA and FDA as a predictive model of human *in vivo* intestinal permeability
Ready-to-use Reagents

READY-TO-USE

- Solid Shipping Medium
- Packaging system

Patented ADVANCELL’s Technology

- Storage and Transportation at RT
- Cell functional properties maintained
- Easy-to-use and handle
- Avoiding in house cell culture
- Standard internal quality controls
- Available on demand
**CacoReady™**

**BENEFITS**

- Validated system under quality standards
- Full cell functionality after transportation

**Papp of Reference Compounds**

\[
P_{\text{app}} = \frac{dQ}{Dt \cdot A \cdot C_0}
\]
**BENEFITS**

<table>
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<tr>
<th>MON</th>
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<th>WED</th>
<th>THUR</th>
<th>FRID</th>
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<td>D15</td>
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- **Shipment**
- **Removal of SM**
- **TEER control**
- **Medium change**
- **Permeability Assay**

**Flexibility:** up to 5 days to perform the experiment

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**TEER Values**

<table>
<thead>
<tr>
<th>Timepoint</th>
<th>TEER (Ω·cm²)</th>
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<tbody>
<tr>
<td>Before reception</td>
<td>3000±100</td>
</tr>
<tr>
<td>1 day</td>
<td>3100±100</td>
</tr>
<tr>
<td>3 days</td>
<td>3000±100</td>
</tr>
<tr>
<td>5 days (day 21 of cell culture)</td>
<td>3100±100</td>
</tr>
<tr>
<td>9 days</td>
<td>3200±100</td>
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</tbody>
</table>

**SM Application**
High reproducibility among different batches

<table>
<thead>
<tr>
<th>Substance</th>
<th>Papp A-&gt;B (10⁻⁶ cm/s)</th>
<th>STD (AB)</th>
<th>Papp B-&gt;A (10⁻⁶ cm/s)</th>
<th>STD (BA)</th>
<th>Efflux Ratio</th>
<th>Permeability</th>
<th>Efflux</th>
<th>Recovery A-&gt;B</th>
<th>Recovery B-&gt;A</th>
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<tbody>
<tr>
<td>Atenolol</td>
<td>0,1</td>
<td>0,04</td>
<td>0,2</td>
<td>0,04</td>
<td>1,4</td>
<td>LOW</td>
<td>NO</td>
<td>105</td>
<td>100</td>
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<tr>
<td>Atenolol + CsA</td>
<td>0,1</td>
<td>0,1</td>
<td>0,1</td>
<td>0</td>
<td>0,8</td>
<td>LOW</td>
<td>NO</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>Pindolol</td>
<td>12,3</td>
<td>3,9</td>
<td>13,3</td>
<td>3,7</td>
<td>1,1</td>
<td>HIGH</td>
<td>NO</td>
<td>103</td>
<td>103</td>
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<tr>
<td>Pindolol + CsA</td>
<td>12,8</td>
<td>4,9</td>
<td>11,3</td>
<td>3,6</td>
<td>0,9</td>
<td>HIGH</td>
<td>NO</td>
<td>104</td>
<td>102</td>
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<tr>
<td>Propanolol</td>
<td>34,3</td>
<td>6</td>
<td>28,6</td>
<td>7,7</td>
<td>0,8</td>
<td>HIGH</td>
<td>NO</td>
<td>96</td>
<td>96</td>
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<tr>
<td>Propanolol + CsA</td>
<td>31,9</td>
<td>6,7</td>
<td>27,6</td>
<td>9,9</td>
<td>0,9</td>
<td>HIGH</td>
<td>NO</td>
<td>94</td>
<td>96</td>
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<tr>
<td>Indinavir</td>
<td>1</td>
<td>0,5</td>
<td>29,7</td>
<td>8,7</td>
<td>28,7</td>
<td>LOW</td>
<td>YES</td>
<td>105</td>
<td>91</td>
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<tr>
<td>Indinavir + CsA</td>
<td>7,1</td>
<td>2,2</td>
<td>9,7</td>
<td>3,7</td>
<td>1,4</td>
<td>Medium</td>
<td>NO</td>
<td>101</td>
<td>98</td>
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Data obtained from n=13 independent experiments
- *In vivo vs. In vitro* absorption of compounds that cross the barrier in a paracellular passive diffusion way: 20-80% lower in Caco-2

- Colonic epithelia is characterized by the presence of mucus

- ADVANCECELL has developed a more physiological barrier that mimics the intestinal epithelium based on the co-culture of Caco-2 and Goblet cells
CacoGoblet system leads to a more permissive epithelium which is more similar to physiological conditions.
Comparison of std compounds

Papp coefficient of standard compounds
Intestinal absorption

Distribution
- CNS (BBB, blood – CSF barrier)
- Fetus (B-placenta barrier in syncytiotrophoblast)
- Testis (Btestis barrier)

Modulation of metabolism

Drug-drug interaction

Excretion
- Intestinal
- Liver
- Kidney

Toxicity
- Interference with transport and metabolism of endogenous substrates
Role of Transporters in physiological barriers

**PREADYPORT™**

In collaboration with SOLVO Biotechnology

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**PREADYPORT™-MDR1**

- MDR1
  - Intestine
  - Liver
  - BBB
  - Kidney

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**PREADYPORT™-BCRP**

- BCRP
  - Intestine
  - Liver
  - BBB
  - Testis

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**BCRP/OATP2B1**

- Liver
- Placenta

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can be used for regulatory submission studies

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suitable for studying low permeability substrates
The kit can be used up to 7 days after reception

<table>
<thead>
<tr>
<th>D1</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
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Seeding

TEER int control

SM Application

Transport Assay

Flexibility: up to 4 days for transport measurements
**TEER values**

![Graph showing TEER values for MDCKL WT and MDR1](image1)

- n=3 independent experiments

**Inhibition Curve**

![Graph showing inhibition curve for MDR1-96](image2)

- MDR1-96 All
- MDR1 96 BA

**Efflux ratio values for known P-gp/MDR1 Substrates and Inhibitors**

![Graph showing efflux ratios for P-gp/MDR1 substrates](image3)

- Digoxin
- D+PSC833

- n=3 independent experiments

![Graph showing efflux ratios for P-gp/MDR1 substrates](image4)

- Tallolol 20 μM
- Digoxin 10 μM

![Graph showing efflux ratios for P-gp/MDR1 substrates](image5)

- Quinidine
- Q + LY355796 0.5 μM
- Q + PSC833 5.0 μM
- Q + Verapamil 50 μM

- n=3 independent experiments
PreadyPort™-BCRP

Variability Study

Papp (cm/s)

<table>
<thead>
<tr>
<th>Condition</th>
<th>MDCKII-BCRP</th>
<th>MDCKII-WT</th>
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</thead>
<tbody>
<tr>
<td>Prazosin</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>Prazosin+Ko134</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Prazosin</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Efflux Ratio

<table>
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<tr>
<th>Condition</th>
<th>MDCKII-BCRP</th>
<th>MDCKII-WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Prazosin+Ko134</td>
<td>10</td>
<td>5</td>
</tr>
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</table>
Blood Brain Barrier (BBB)

**BBB in vitro model**

Coculture of endothelial cells with astrocytes
(12 days)
Thanks for your attention!