D-PREX® (Disposition Profile Exploration)

“Single systematic procedure to evaluate total hepatocellular disposition profiles simultaneously, and to see the overall picture of metabolism & transport interplay.”

Katsuhiro KANDA, Ph.D.
Hitachi High-Tech Corporation

※ Patents are registered in US, Europe, China, and Japan (e.g. US9880153).
※ “D-PREX” is a registered trademark of Hitachi High-Tech Corporation in the US and Japan.
How could we improve drug discovery efficiency?

Currently...

- Low correlation between human and animals

Approach

- Use of human-relevant models

<table>
<thead>
<tr>
<th>Conventional methodology</th>
<th>D-PREX® methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake assay</td>
<td>Uptake (1 + 2 + 3 + 4)</td>
</tr>
<tr>
<td>Cell suspension, Membrane vesicle etc.</td>
<td>Metabolism</td>
</tr>
<tr>
<td>Difficult to see the overall picture</td>
<td>3 Biliary excretion</td>
</tr>
<tr>
<td>Respective assay for each individual endpoint</td>
<td>4 Cellular residue</td>
</tr>
<tr>
<td>Metabolism assay</td>
<td>Additional endpoints</td>
</tr>
<tr>
<td>Microsomes, Sandwich culture etc.</td>
<td></td>
</tr>
<tr>
<td>Transport assay</td>
<td>Diffusion</td>
</tr>
<tr>
<td>Sandwich culture, Membrane vesicle etc.</td>
<td>2 Transporter-mediated</td>
</tr>
<tr>
<td>Basolateral efflux (1 + 2)</td>
<td></td>
</tr>
</tbody>
</table>
**D-PREX® (Disposition Profile Exploration)**

**Endpoints**

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>Abbrev.</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated drug uptake</td>
<td>-</td>
<td>$1 + 2 + 3 + 4$ av. CE# + EA# + IA#</td>
</tr>
<tr>
<td>Intracellular drug prior to EA</td>
<td>-</td>
<td>$1 + 2 + 3 + 4$ av. EA# + IA#</td>
</tr>
<tr>
<td>Conditioning efflux</td>
<td>CE</td>
<td>$1 + 2$ in CE av. CE#</td>
</tr>
<tr>
<td>Biliary excretion</td>
<td>BilEx</td>
<td>$3$</td>
</tr>
<tr>
<td>Basolateral efflux</td>
<td></td>
<td>$1 + 2$ in EA EA2</td>
</tr>
<tr>
<td>Total</td>
<td>BasEf</td>
<td>$1 + 2$ in EA EA2</td>
</tr>
<tr>
<td>Passive</td>
<td>BasEf-PD</td>
<td>$1$</td>
</tr>
<tr>
<td>Transporter</td>
<td>BasEf-TP</td>
<td>$2$</td>
</tr>
<tr>
<td>Intracellular residues</td>
<td>IcRes</td>
<td>$4$</td>
</tr>
</tbody>
</table>

**D-PREX®** is a systematic *in vitro* assay procedure consisting of multiple sequential sampling processes to simultaneously evaluate overall hepatocellular disposition profiles, resulting from complex interplay of hepatic transport and metabolism.

Sequential supernatant sampling in **D-PREX®** prior to cell lysis enables high-throughput evaluation.
Pitfalls in Predicting Hepatobiliary Drug Transport Using Human Sandwich-Cultured Hepatocytes

Vineet Kumar, Cindy Yanfei Li, Kazuya Ishida, Emese Kis, Zsuzsanna Gáborik & Jashvant D. Unadkat

The AAPS Journal 22:110 (2020)

D-PREX® only requires intact cell culture (Ca+) in the drug uptake process, such pitfalls don’t have much influence.

[Tricky conventional methodology]
Sandwich-cultured hepatocytes
↓ Preincubation under Ca−/+ buffer
↓ Drug uptake both under Ca+ buffer (Ca− influence cellular conditions)

Comparison between Ca−/+ intracellular drug amounts

Pitfalls
① Tight-junction reformation by Ca repletion during uptake process
② NTCP (uptake transporter) inhibition under Ca− condition
【Aim】Evaluate the performance of D-PREX® system

【Method】
- **Material**: SW of rat fresh hepatocytes (Day 4)
- **Reagent**: 10μM CDF & 10μM Rh123
- **Method**: Sandwich cultured cells
  - Exposure reagents for 30min
  - Fractionation by D-PREX®
  - Fluorescence detection
- Calculate disposition rates (graphs)

【Comparison】Reflection of the reagent properties

CDF likely to excrete extracellularly
- High rate of “Sup”
- High rate of “ExEfx-Dif + ExEfx-TP”

Rh123 likely to remain intracellularly
- High rate of “Cell”

CDF shows relatively high biliary excretion rate
- Higher rate of “BCEfx”
RSV Disposition & TP Inhibition (Ko143)

**Major efflux transporters**

- MRPs
- ENTs
- BSEP
- ABCGs
- MRP2
- MATE1
- P-gp
- BCRP
- MDR3

**Hepatocyte**

**Ko143**

- [Chemical structure of Ko143]

**RSV uptake evaluation**

- RSV uptake is not inhibited by Ko143

<table>
<thead>
<tr>
<th>Ko143 (μM)</th>
<th>0.0 µM</th>
<th>0.1 µM</th>
<th>1.0 µM</th>
<th>10.0 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RSV (%)</strong></td>
<td>Basal</td>
<td>Basal</td>
<td>Basal</td>
<td>Basal</td>
</tr>
<tr>
<td>Disposition Profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell</td>
<td>46.2</td>
<td>52.4</td>
<td>65.5</td>
<td>70.8</td>
</tr>
<tr>
<td>BC</td>
<td>15.0</td>
<td>11.5</td>
<td>7.0</td>
<td>2.8</td>
</tr>
</tbody>
</table>

- Biliary excretion decrease
- Basolateral efflux decrease

⇒ Intracellular residue increase
Disposition fractions under Ko143 treatment

Incubation time for CE-sampling: 10 min
Data point: Mean ± SD (N = 4)
* : p < 0.05 (t-test)

Significant inhibitory effect of RSV transport to BasEf by Ko143... Why?

<table>
<thead>
<tr>
<th>Probe</th>
<th>Biliary excretion</th>
<th>Basolateral efflux</th>
<th>Intracellular residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
</tr>
<tr>
<td>PTV</td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
</tr>
</tbody>
</table>

**Calculation of disposition fraction (%)**

- **BilEx**: Biliary excretion
  \[ \frac{(EA1 - EA2)}{(EA2 + IA2)} \times 100 \]
- **BasEf**: Basolateral efflux
  \[ \frac{EA2}{(EA2 + IA2)} \times 100 \]
- **IcRes**: Intracellular residue
  \[ \frac{IA1}{(EA2 + IA2)} \times 100 \]

BilEx : Biliary excretion
BasEf : Basolateral efflux
IcRes : Intracellular residue
Membrane vesicle experiments

- To evaluate which transporter(s) is involved in the efflux of RSV and PTV

## Preparation of membrane vesicles

<table>
<thead>
<tr>
<th>Target transporters</th>
<th>Expressing cells</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BilEx</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>human BCRP</td>
<td>HEK293</td>
<td>1 μM E3S</td>
</tr>
<tr>
<td>human MRP2</td>
<td></td>
<td>1 μM Ko143</td>
</tr>
<tr>
<td><strong>BasEf</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>human MRP3</td>
<td></td>
<td>50 μM E₂17βG</td>
</tr>
<tr>
<td>human MRP4</td>
<td></td>
<td>100 μM BB</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Probe</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₃S</td>
<td>Estrone 3-sulfate</td>
<td></td>
</tr>
<tr>
<td>E₂17βG</td>
<td>β-estradiol-17-β-D-glucuronide</td>
<td></td>
</tr>
<tr>
<td>DHEAS</td>
<td>Dehydroepiandrosterone sulfate</td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>Benzbromarone</td>
<td></td>
</tr>
<tr>
<td>SSZ</td>
<td>Sulfasalazine</td>
<td></td>
</tr>
</tbody>
</table>

## Experimental procedure

Reaction mixture (Membrane vesicles, probe, +/- inhibitor)

- pre-warm at 37°C for 15 min
- add 37°C pre-warmed Starting reagent
- incubate at 37°C
- terminate by adding ice-cold washing mixture
- filtrate and rinse

LC-MS/MS quantitation of incorporated probe

## Experimental conditions

<table>
<thead>
<tr>
<th>Probes</th>
<th>1 μM RSV</th>
<th>1 μM PTV</th>
<th>due to lower limits of quantitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor</td>
<td>1 μM Ko143</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Starting reagent**

- AMP (background)
- ATP

**Incubation time**

- 1 min for BCRP
- 10 min for MRPs
Net flux ratio of probe accumulation

ATP-dependent accumulation:
Calculated by subtracting the accumulation with AMP from that with ATP in each experimental condition (Mean ± SD (N = 3))

Fold accumulation:
Ratio of ATP-dependent accumulation in transporter-expressing vesicles compared with that in control vesicles
(Ratio ≥ 2 was defined as a positive signal according to the FDA guidelines)

* : \[ p < 0.05 \] (t-test)
Ko143 Inhibitory Effect to RSV&PTV Disposition

### Vesicular transport analyses

- **MRP3 and 4 mediated the BasEf of RSV**
- **Ko143 inhibited MRP4-mediated transport of RSV**
  - Partial inhibition of BasEf by Ko143 supported the above D-PREX® disposition profiling results

- **MRP4 mediated the BasEf of PTV, but low affinity**
  - (Fold accumulation of 2.5)
  - Further investigation is required to confirm the mode of action in BasEf of PTV

### D-PREX® disposition profiling

- **Ko143 conc-dependent decrease of BilEx fraction**
- **Ko143 conc-dependent decrease of BasEf fraction**

- Similar tendency to RSV in BilEx
- BasEf decrease was insignificant

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**RSV**

<table>
<thead>
<tr>
<th>Ko143</th>
<th>0 µM</th>
<th>0.1 µM</th>
<th>1.0 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP3</td>
<td>48.5%</td>
<td>36.7%</td>
<td>27.3%</td>
</tr>
<tr>
<td>MRP4</td>
<td>42.1%</td>
<td>52.6%</td>
<td>69.7%</td>
</tr>
<tr>
<td>MRP2</td>
<td>12.4%</td>
<td>12.4%</td>
<td>8.0%</td>
</tr>
</tbody>
</table>

**PTV**

<table>
<thead>
<tr>
<th>Ko143</th>
<th>0 µM</th>
<th>0.1 µM</th>
<th>1.0 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP3</td>
<td>32.5%</td>
<td>32.5%</td>
<td>27.3%</td>
</tr>
<tr>
<td>MRP4</td>
<td>30.0%</td>
<td>30.0%</td>
<td>61.9%</td>
</tr>
<tr>
<td>MRP2</td>
<td>10.2%</td>
<td>10.2%</td>
<td>6.2%</td>
</tr>
</tbody>
</table>
Metabolite disposition profiling

【APAP & Major metabolites → Urinary excreted】

- Biliary excretion (1-2%)
- Excreted in urine (1-4%)
- Cys conjugate (3%)

APAP-glucuronide (40-67%)

APAP-sulfate (20-46%)

APAP glucuronide

APAP-sulfate

NAPQI (5-15%)


Sandwich cultured PXB-cells (SCPC)

PXB-cells are fresh human hepatocytes isolated from humanized PXB-mouse

Metabolism in intact SCH

Simultaneous disposition profiling of parent compound and metabolites

SCPC Gene Expression Levels on Day 8
Metabolite disposition profiling

**Accumulation of APAP and metabolites**

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Proportion (%)</th>
<th>Known proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP-Sulf</td>
<td>30.8</td>
<td>20 - 46</td>
</tr>
<tr>
<td>APAP-Gluc</td>
<td>40.3</td>
<td>40 - 67</td>
</tr>
</tbody>
</table>

*Within a range of known metabolite proportion from human clinical studies.*

BasEf-TP fraction of APAP & metabolites (-Sulf & -Gluc) decreased by 5 μM MK571 treatment, which may suggest the contribution of MRPs-mediated transport.

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Sekisui Medical

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D-PREX® technology

Hitachi High-Tech

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- Drug Metabolism
- Enzyme Inhibition & Induction
- Protein Binding
- Metabolite Identification
- ADME Screening

In Vivo ADME/PK & Distribution
- QWBA
- Microautoradiography
- Excretion / Mass Balance
- Tissue Distribution
- Blood / Plasma & Lymphatic Partition Rate

Bioanalytical Pharmacology
- In Vitro Ligand Binding & Radioreceptor Assays
- Immunoassays

Chemical Synthesis
- Radiolabeled Synthesis
- Metabolite Synthesis
- Peptide Synthesis

Cellular Products
- Hepatocytes (Cryol/Fresh, Genotyped...)
- Non-Parenchymal Cells (Kupffer Cells)

Subcellular Fractions
- Liver Microsomes
- S9 Fractions
- Cytosol
- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
- Extrahepatic Fractions

Custom Products
- Various Species, Tissues & Preparations

Research Biobank
- Normal & Diseased Tissue Samples

Recombinant Enzymes

Substrates & Metabolites

JCRB Cell Lines...