Use of *in vitro* cell assays and noninvasive imaging techniques to reduce animal experiments in drug development

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absorption

distribution

metabolization

elimination

Bioavailability

Drug efficacy and side effect

portal vein

intestine

Liver

fecal elimination

intestinal elimination

hepatic elimination

Renale elimination
Membrane transporters

- can be major determinants of the pharmacokinetic, safety and efficacy profiles of drugs

- two major superfamilies — ATP-binding cassette (ABC) and solute carrier (SLC)
Expression of membrane transporters

International Transporter Consortium, Nat Rev Drug Discov. 2010
Uptake transporters

Intestine
- OCT1
- OCTN1
- OCTN2
- OATP1A2
- OATP2B1
- MRP1
- MRP2
- MDR1
- DEPT1
- ASBT
- MCT1

Blood-brain-barrier
- OCTN1
- OCTN2
- OAT3
- OAT1A2
- OATP2B1
- MDR1
- BCRP
- MRP1
- MRP2
- MRP4
- MRP5

Liver
- OCT1
- OCT3
- OATP1B1
- OATP1B3
- OATP2B1
- MRP3
- MRP4
- MRP6
- MRP2
- BSEP
- MDR1
- MDR3
- MATE1
- NTCP
- OCTN2
- OAT2
- OAT7

Hepatocyte

Brain capillary endothelial cells

International Transporter Consortium, Nat Rev Drug Discov. 2010
Efflux transporters

Intestine
- OCT1
- OCTN1
- OCTN2
- OATP1A2
- OATP2B1
- MRP1
- MRP3
- OSTα
- OSTβ
- PEPT1
- ASBT
- MCT1

Blood-bRAIN-barrier
- OCTN1
- OCTN2
- OAT3
- OAT1A2
- OATP2B1
- MDR1
- BCRP
- MRP1
- MRP2
- MRP4
- MRP5

Liver
- MRP3
- MRP4
- MRP6
- OCT1
- OCT3
- OATP1B1
- OATP1B3
- OATP2B1
- MDR1
- MDR3
- OAT2
- OAT7
- BSEP
- MATE1
- NTCP
- OCTN2
- BCRP
- MRPL

Hepatocyte

International Transporter Consortium, Nat Rev Drug Discov. 2010
The function of drug transporters can be explained by the use of probe drugs.

<table>
<thead>
<tr>
<th>Transporterprotein</th>
<th>Probe drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-glycoprotein</td>
<td>Verapamil, Talinolol, Digoxin</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>Pravastatin</td>
</tr>
</tbody>
</table>

Associated with the organ removal from experimental animals.
Magnetic resonance imaging (MRI)
Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA, Primovist®)

- Gadolinium-based MRI- contrast agent
- significantly improves detection and characterization of focal liver lesion
- selektivly taken up in the liver cells
Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA, Primovist®)

- Gadolinium-based MRI- contrast agent
- significantly improves detection and characterization of focal liver lesion
- selektivly taken up in the liver cells
Evidences for Gd-EOB-DTPA (Primovist®) to be a substrate of hepatic transporters

- substrate of rat Oatp1a1 in Xenopus laevis oocytes
- known inhibitors of Oatps (BSP, rifampicin) compete with the hepatic enhancement in rodents (van Montfoort et al. 1999)
- enhancement in hepatocellular carcinoma tissue is predicted by expression of human OATP1B3 (Narita et al. 2009)
Hypothesis

- Gd-EOB-DTPA (Primovist®) as a new probe drug

- To visualize and characterise the function of transporter proteins and the drug absorption
  - cellular uptake and elimination via the same transporters like many drugs
Purpose

- **In vitro:**
  - Identify the transporters of Gd-EOB-DTPA (Primovist®) for the hepatic and intestinal uptake and elimination

- **In vivo:**
  - Pharmacokinetics (i.v. und oral) and MRI analysis with wild-type and Mrp2-deficient rats
  - Reduce the number of experimental animals
  - Gd-EOB-DTPA (Primovist®) in liver can be quantified using MRI without removal of tissue samples from experimental animals
*In vitro* method to analyze the substrate affinity to uptake transporters

**Uptake assay**

**control cells**

**transfected cells**

![Diagram showing uptake assay with substrate uptake in transfected cells compared to control cells.](image-url)
Stable transfected cell lines in the C_DAT

<table>
<thead>
<tr>
<th></th>
<th>HEK293-cells (human embrionic kidney)</th>
<th>MDCK2-cells (Madin-Darby canine kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1A2</td>
<td>*1 &lt;br&gt;*2 &lt;br&gt;*3</td>
<td>OATP1A2 &lt;br&gt;*1 &lt;br&gt;*2 &lt;br&gt;*3</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>*1a &lt;br&gt;*1b &lt;br&gt;*5 &lt;br&gt;*15</td>
<td>OATP1B1 &lt;br&gt;*1a &lt;br&gt;*1b &lt;br&gt;*5 &lt;br&gt;*15</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>WT &lt;br&gt;c.334T&gt;G &lt;br&gt;c.699G&gt;A &lt;br&gt;c.1564G&gt;T &lt;br&gt;c.334T&gt;G + 699G&gt;A</td>
<td>OATP1B3</td>
</tr>
<tr>
<td>OATP2B1</td>
<td>WT &lt;br&gt;c.601G&gt;A &lt;br&gt;c.995G&gt;A &lt;br&gt;c.1457C&gt;T</td>
<td>OATP2B1 &lt;br&gt;c.601G&gt;A &lt;br&gt;c.995G&gt;A &lt;br&gt;c.1457C&gt;T</td>
</tr>
<tr>
<td>OATP1C1 OATP3A1 OATP4A1 OATP4C1</td>
<td></td>
<td>OATP3A1 OATP4A1 OATP4C1</td>
</tr>
<tr>
<td>OCT1 OCT2 OCT3 OCTN2</td>
<td></td>
<td>OCT1 OCT2 OCT3 OCTN2</td>
</tr>
<tr>
<td>NTCP ASBT</td>
<td></td>
<td>NTCP ASBT</td>
</tr>
<tr>
<td>ABCB1 ABCC2</td>
<td></td>
<td>ABCB1 ABCC2 ABCC3</td>
</tr>
</tbody>
</table>
Characterization of stable transfected cell lines

HEK-OATP1A2

Substrate 1

$K_m = 23.1 \, \mu\text{mol/l}$

$V_{max} = 87.8 \, \text{pmol/mg} \times \text{min}$

Substrate 2

$K_m = 80.5 \, \mu\text{mol/l}$

$V_{max} = 20.5 \, \text{pmol/mg} \times \text{min}$

Substrate 1 + Inhibitor 1

$IC_{50} = 77.9 \, \mu\text{mol/l}$

Substrate 1 + Inhibitor 2

$IC_{50} = 21.3 \, \mu\text{mol/l}$
Affinity of Gd-EOB-DTPA (Primovist®) to uptake transporters

OATP1B1

OATP1B3

NTCP

Leonhardt et al., Drug Metab Disp 2010

Jia et al., Invest Radiol. 2013; accepted manuscript
### Affinity of Gd-EOB-DTPA (Primovist®) to uptake transporters

<table>
<thead>
<tr>
<th>Transporter</th>
<th>$K_m$ (mmol/l)</th>
<th>$V_{max}$ (pmol/mg x min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B1</td>
<td>1,2</td>
<td>6,3</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>0,5</td>
<td>7,4</td>
</tr>
<tr>
<td>NTCP</td>
<td>0,04</td>
<td>1,4</td>
</tr>
<tr>
<td>OATP2B1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ASBT</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OCT3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Jia et al., Invest Radiol. 2013; accepted manuscript
inside-out vesicles

Cell lysis and crushing of the Plasmamembran

build the vesicle

Plasmamembran
MRP2/MRP3
Uptake of Gd-EOB-DTPA (Primovist®) using *inside-out* vesicle

*Graph and data*:

**MRP2**
- Graph showing the relationship between Gd-EOB-DTPA concentration (mmol/l) and uptake (pmol/mg x min).
- Table showing kinetic parameters:
  - $K_m$: 1.0 ± 0.5 (mmol/l)
  - $V_{max}$: 86.8 ± 31.3 (pmol/mg x min)

**MRP3**
- Graph showing the relationship between Gd-EOB-DTPA concentration (mmol/l) and uptake (pmol/mg x min).
- Table showing kinetic parameters:
  - $K_m$: 1.8 ± 0.3 (mmol/l)
  - $V_{max}$: 116 ± 15.9 (pmol/mg x min)

*Source*: Jia et al., Invest Radiol. 2013; accepted manuscript
In vivo study

- Animals: wild-type Lewis-rats
  Mrp2-deficient Lewis-rats

- Operation: Carotis catheter

- MRI: i.v.: bolus injection 0.025 mmol/kg
  p.o.: 0.025 mmol/kg

- Samples: Blood (i.v: 0-90 min; oral: 0-360 min)
  Urine (2d)
  Feces (5d)
MRI: after intravenous application

**Graph:**
- **X-axis:** Zeit (min)
- **Y-axis:** Liverenhancement (AU)
- **Legend:**
  - Wildtyp
  - Mrp2-defizient

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>MRP2-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>AUC₀₄ (AU x min)</td>
<td>14.8 ± 10.3</td>
<td>36.4 ± 8.5*</td>
</tr>
<tr>
<td>C_max (AU)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>T_max (min)</td>
<td>6.0 ± 3.1</td>
<td>48.6 ± 23.8*</td>
</tr>
</tbody>
</table>

Jia et al., Invest Radiol. 2013; accepted manuscript
Pharmacokinetics: after intravenous application

<table>
<thead>
<tr>
<th>Parameter</th>
<th>wild-type</th>
<th>Mrp2-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0-\infty}$ (µg x h/ml)</td>
<td>3.35</td>
<td>7.49*</td>
</tr>
<tr>
<td>$C_p$ (µg/ml)</td>
<td>10</td>
<td>10.4</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>2.12</td>
<td>1.95</td>
</tr>
<tr>
<td>$A_e$ _urin _ (µg)</td>
<td>62.5</td>
<td>666.0</td>
</tr>
<tr>
<td>$A_e$ _feces _ (µg)</td>
<td>1379.0</td>
<td>below LLQ*</td>
</tr>
</tbody>
</table>

* $p<0.05$ vs. wildtyp

Jia et al., Invest Radiol. 2013; accepted manuscript
Pharmacokinetics and MRI after oral administration

- Gd-EOB-DTPA (µg/ml)
  - Wildtyp
  - Mrp2-defizient

Zeit (min)

oral

native

nach 40 min

<table>
<thead>
<tr>
<th>p.o.</th>
<th>Wildtyp</th>
<th>Mrp2-defizient</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC $0\rightarrow\infty$ (µg x h/ml)</td>
<td>0,6</td>
<td>1,6</td>
</tr>
<tr>
<td>$C_{p0}$ (µg/ml)</td>
<td>0,2</td>
<td>0,5</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>1,3</td>
<td>0,9</td>
</tr>
<tr>
<td>Bioavailability (F)</td>
<td>17%</td>
<td>21%</td>
</tr>
<tr>
<td>$A_{e,\text{urin}}$ (µg)</td>
<td>29,7</td>
<td>194,0</td>
</tr>
<tr>
<td>$A_{e,\text{feses}}$ (µg)</td>
<td>3511,0</td>
<td>3775,0</td>
</tr>
</tbody>
</table>

Jia et al., Invest Radiol. 2013; accepted manuscript
The liver-specific uptake of Gd-EOB-DTPA (Primovist®) is realized by OATP1B1 and OATP1B3.

MRP2 is a major efflux transporter of the hepatobiliary elimination.

Cell-based in vitro assays have the potential to replace in vivo animal testing and provide reliable data.

Visualization by MRI can probably replace the quantitative determination of Gd-EOB-DTPA (Primovist®) in liver samples, thus reducing nearly 90% of the number of experimental animals.
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