Disposition and Elimination of Organic Anions and Cations: Transporters and Decision Trees

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Overview

- How did we get from observing physiological phenomena to studying cloned genes?

- What are the advantages of having cloned transporters?
  - mechanistic characterization in heterologous expression systems
  - membrane targeting/polarization
  - knockout mouse models

- Do organic anion and cation transporters (OATs/OCTs) impact ADME and human disease states?
Renal Transport Then and Now

BLOOD

K^+

Na^+

α-KG^=

PAH^-

EPITHELIUM

K^+

Na^+

α-KG^=

PAH^-

MITOCHONDRIA

PAH^-

PAH^-

PAH^-

- 60 mV

URINE

circa 1996

blood (basal)

OCTN1

OCTN2

URAT1

URAT2

OCT2

OCT2A

OCT3

MRP2

MRP4

PEPT1&2

proximal tubule cell

2010
“Renal” Transporters = Barrier Membrane Transporters

a. Intestinal epithelia

[Diagram showing blood to intestine with transporters such as OATP, PEPT1, ASBT, MCT1, MRP2, BCRP, and P-gp]

b. Hepatocytes

[Diagram showing blood to bile with transporters such as OCT1, OAT7, OATP1B1, OATP2B1, NTCP, MRP3, MRP4, MRP6, BSEP, P-gp, MRP2, MATE1, and OSTα, OSTβ]

c. Kidney proximal tubules

[Diagram showing blood to urine with transporters such as OATP4C1, OCT2, OAT1, OAT2, OAT3, OAT4, URAT1, PEPT1, PEPT2, MRP2, MRP4, MATE1, MATE2-K, and P-gp, OCTN1, OCTN2]

d. Blood–brain barrier

[Diagram showing blood to brain capillary endothelial cells with transporters such as P-gp, BCRP, MRP4, MRPS, OATP1A2, OATP2B1, and OCTN1, OCTN2]

ITC Whitepaper, Nat Rev Drug Discov 9, 2010
Transport Proteins

Hediger et al., Pflug Arch 447, 2004
Expression Cloning Strategy

1. Plate Bacteria Containing cDNA Library
2. Purify Plasmid DNA
3. in vitro Transcription [cRNA]
4. Inject Xenopus oocytes
5. Expression Assay
6. Subsection Positive Filter

- Filter Plate Bacteria Containing cDNA Library
- Purify Plasmid DNA
- in vitro Transcription [cRNA]
- Inject Xenopus oocytes
- Expression Assay
- Subsection Positive Filter

[Diagram showing the steps of the expression cloning strategy]
Where Does the Cloned Transporter go?

RENAL PROXIMAL TUBULE CELL

BLOOD

EPITHELIUM

URINE

\( \text{Na}^+ \)

\( \text{K}^+ \)

\( \text{H}^+ \)

\( \text{ATP} \)

\( \text{TEA}^+ \)

-70 mV

RENAL PROXIMAL TUBULE CELL
Oct2-GFP Targeting in MDCK Cells

Sweet et al., Am J Physiol Renal Physiol 279, 2000
Oct2-GFP Targeting in Killifish Renal Tubules

Sweet et al., *Am J Physiol Renal Physiol* 279, 2000
Oct1, Oct2, and Oct1/2 Knockout Mice

Renal Transport Pathway Mediating Metformin/Cimetidine DDI

hMATE1/hOCT2 cells

MET (11 µM)

CIM (1 µM)

CIM (1 mM)

Ki for Cimetidine:
hOCT2 \cong 150 \mu M
hMATE1 \cong 1 \mu M

Tsuda, et al. JPET 329, 2009
OCT3 Expression in Renal Cell Carcinoma

OCT3 Function and Renal Cell Carcinoma

OCT3 Function and Renal Cell Carcinoma

Organic Anion Transporter (SLC22) Family

- rOat10 (Slc22a13)
- mOat10 (Slc22a13)
- rOat6 (Slc22a20)
- mOat6 (Slc22a20)
- rUrat1 (Slc22a12)
- mUrat1 (Slc22a12)
- hURAT1 (SLC22A12)
- hOAT4 (SLC22A11)
- rOat5 (Slc22a19)
- mOat5 (Slc22a19)
- rOat8 (Slc22a19)
- hOAT7 (SLC22A9)
- rOat1 (Slc22a6)
- mOat1 (Slc22a6)
- rbOat1 (Slc22a6)
- pOat1 (Slc22a6)
- rOat3 (Slc22a8)
- mOat3 (Slc22a8)
- rbOat3 (Slc22a8)
- hOAT3 (SLC22A8)
- mOat2 (Slc22a7)
- hOAT2 (SLC22A7)
- rOat2 (Slc22a7)
Uptake in Renal Slices From Oat3\(^{-/-}\) Mice

Proposed Change to Transport Model

Oat3 localized to basal membrane; mechanism should be reevaluated
Like Oat1, Oat3-mediated uptake is energetically coupled to the Na\(^+\) gradient through the Na\(^+\)/dicarboxylate cotransporter.
Oat3-mediated renal basolateral ES uptake is energetically coupled to the Na\(^+\) gradient through the Na\(^+\)/dicarboxylate cotransporter.
PAH Elimination is Not Altered in Oat3\(^{\text{-/-}}\) Mice

Impaired ES Elimination in Female Oat3\(^{-/-}\) Mice

Markedly Impaired PCN G Elimination in Oat3^{(-/-)} Mice

Pharmacokinetic Changes in \textit{Oat3}(-/-) Mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>Compound</th>
<th>Genotype</th>
<th>( V_1 )</th>
<th>( V_{ss} )</th>
<th>AUC PAH, ES, &amp; PCNG</th>
<th>AUC Inulin</th>
<th>Total Clearance</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(ml/kg)</td>
<td>(ml/kg)</td>
<td>(min*ng/ml)</td>
<td>(min*ug/ml)</td>
<td>(ml/min/kg)</td>
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<tr>
<td>Male</td>
<td>PAH</td>
<td>wild-type (n = 3)</td>
<td>249 ± 12</td>
<td>516 ± 33</td>
<td>113 ± 14</td>
<td></td>
<td>13 ± 1.5</td>
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<tr>
<td></td>
<td></td>
<td>Oat3(-/-) (n = 3)</td>
<td>231 ± 28</td>
<td>477 ± 46</td>
<td>98 ± 7.0</td>
<td></td>
<td>15 ± 1.1</td>
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<tr>
<td></td>
<td>ES</td>
<td>wild-type (n = 4)</td>
<td>147 ± 22</td>
<td>786 ± 78</td>
<td>14 ± 1.8</td>
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<td>39 ± 4.8</td>
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<tr>
<td></td>
<td></td>
<td>Oat3(-/-) (n = 4)</td>
<td>113 ± 20</td>
<td>682 ± 104</td>
<td>14 ± 1.7</td>
<td></td>
<td>39 ± 4.6</td>
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<td></td>
<td>PCN G</td>
<td>wild-type (n = 4)</td>
<td>213 ± 14</td>
<td>406 ± 20</td>
<td>65 ± 8</td>
<td></td>
<td>29 ± 3.6</td>
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<tr>
<td></td>
<td></td>
<td>Oat3(-/-) (n = 4)</td>
<td>163 ± 8</td>
<td>270 ± 31</td>
<td>118 ± 11</td>
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<td>16 ± 1.6</td>
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<td>Female</td>
<td>Inulin</td>
<td>wild-type (n = 7)</td>
<td>97 ± 9</td>
<td>194 ± 16</td>
<td>447 ± 51</td>
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<td>14 ± 1.5</td>
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<tr>
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<td>Oat3(-/-) (n = 6)</td>
<td>92 ± 7</td>
<td>178 ± 17</td>
<td>449 ± 37</td>
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<td>PAH</td>
<td>wild-type (n = 4)</td>
<td>247 ± 11</td>
<td>503 ± 17</td>
<td>95 ± 5.0</td>
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<td>15 ± 0.9</td>
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<td>Oat3(-/-) (n = 5)</td>
<td>241 ± 9.0</td>
<td>477 ± 17</td>
<td>109 ± 5.0</td>
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<td>13 ± 0.7</td>
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<tr>
<td></td>
<td>ES</td>
<td>wild-type (n = 4)</td>
<td>45 ± 26</td>
<td>469 ± 85</td>
<td>21 ± 2.0</td>
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<td>25 ± 2.0</td>
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<td>Oat3(-/-) (n = 4)</td>
<td>61 ± 17</td>
<td>324 ± 44</td>
<td>27 ± 2.0</td>
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<td>20 ± 1.0</td>
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<tr>
<td></td>
<td>PCN G</td>
<td>wild-type (n = 4)</td>
<td>184 ± 23</td>
<td>349 ± 25</td>
<td>55 ± 2.8</td>
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<td>34 ± 1.7</td>
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<tr>
<td></td>
<td></td>
<td>Oat3(-/-) (n = 4)</td>
<td>91 ± 23</td>
<td>209 ± 13</td>
<td>167 ± 23</td>
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<td>11 ± 1.4</td>
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<tr>
<td></td>
<td>Inulin</td>
<td>wild-type (n = 8)</td>
<td>100 ± 9.0</td>
<td>199 ± 18</td>
<td>370 ± 39</td>
<td></td>
<td>16 ± 2.0</td>
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<tr>
<td></td>
<td></td>
<td>Oat3(-/-) (n = 9)</td>
<td>100 ± 4.0</td>
<td>207 ± 11</td>
<td>434 ± 40</td>
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<td>14 ± 1.0</td>
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</table>
Reduced Diuretic Efficacy in Oat1 Knockout Mice

Reduced Excretion in Oat1 Knockout Mice

Role of URAT1 in Hereditary Renal Hypouricemia

Ichida et al., JASN 15, 2004
Choroid Plexus Morphology

GFP Constructs Expressed in Rat Choroid Plexus

Fluorescein Transport in Murine Choroid Plexus

Wild-type  Oat3−/−
FL Transport by Wild-type and Oat3\(^{(-/-)}\) CP

![Graph showing fluorescence intensity for different conditions: Na Free, 0.2 mM Prob, Control, High K, Na Free, Low Na, 0.1 mM ES, 0.2 mM Prob, Control.]

- **Oat3\(^{(-/-)}\)**: ~ 80% Reduction
- **Wild-type**: ~ 33% Reduction

Variables:
- Cells
- Vessels

Fluorescence Intensity

0 50 100 150 200 250
Cultured Kidney Model

Relative Gene Expression in Cultured Kidneys

Two major patterns of gene expression:

- Group A: progressive increase in expression over the seven days of culture
- Group B: expression did not substantially change over the duration of culture

Organic Anion Accumulation in Murine Cultured Kidney

mOat1 Functional Pattern Using mOat3\textsuperscript{-/-} WEK

WEK = [cultured] whole embryonic kidney

mOat3 Functional Pattern Using mOat1−/− WEK

WEK = [cultured] whole embryonic kidney

mOat1 function using mOat3−/− WEK

mOat3 function using mOat1−/− WEK

Need to Test if an OAT/OCT Substrate?

- **Is renal elimination an important route of elimination of NME?**
  - **Criteria:** $\text{CL}_r \geq 0.5 \text{ CL}_{Total}$
  - **Yes**
    - **Is secretory clearance an important route of NME elimination?**
      - **Criteria:** $\text{CL}_r > 1.5 \text{ fu} \times \text{GFR}$
      - **Yes**
        - Is NME a substrate of OCT2, OAT1 or OAT3? **Criteria:** uptake in the transporter-overexpressing cells greater than in empty vector cells (see footnote)
      - **No**
        - Renal secretory transporters are not important in the elimination of the drug
  - **No**
    - Renal clearance is not a sufficiently important determinant of drug levels

- Clinical DDI study with cimetidine for OCT2 and with probenecid for OAT1, OAT3 as inhibitor drugs
DDI Study for OAT/OCT Inhibition?

Is the NME an inhibitor of OCT2, OAT1 or OAT3?
Criteria: determine the IC\textsubscript{50} of NME against MPP\textsuperscript{+}, for OCT2; PAH for OAT1 or OS for OAT3 or other model substrates

- Yes
  - Unbound $C_{\text{max}}$/IC\textsubscript{50} of the NME $\geq 0.1$
    - Clinical DDI study with a sensitive substrate (see footnote)
  - Unbound $C_{\text{max}}$/IC\textsubscript{50} of the NME $< 0.1$
    - DDI study is not needed

- No
  - Poor or not an inhibitor of OCT2, OAT1 or OAT3
Summary

OATs and OCTs:

- Impact the disposition and elimination of drugs, endogenous compounds, and xenobiotics

- This impact on ADME is the combined result of their tissue expression profile, membrane targeting, specificity, and affinity

- Are a key site for drug-drug interactions

- Contribute to some disease states

- May contribute to interindividual variation observed in patient populations
Oat3 knockout mice revealed:

- Oat3 is targeted to the basolateral membrane in renal proximal tubules

- \textit{In vivo} Oat3 does not play a major role in PAH elimination

- Gender differences in steroid conjugate elimination (M > F)

- Oat3 is pivotal for penicillin G drug elimination \textit{in vivo}

- Oat3 is expressed and functional in choroid plexus

- Oat3 is targeted to the apical membrane and positioned to mediate the efflux of organic anions from CSF to blood
Ex vivo organ culture model allows:

- quantitation of OAT/OCT function in intact, polarized tissue with native expression
- monitoring of uptake and efflux pathways
- functional assay in transgenic and knockout animals; model SNPs
- access to organs/tissues at any developmental stage
Contributors

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