RENAL DRUG TRANSPORTERS (esp. OAT1, OAT3) IN PHYSIOLOGY, UREMIA AND DRUG ELIMINATION

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Support: NIDDK, NICHD, NIEHS, Nancy Kaehr in Research
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Defenses Against Drug/Xenobiotic Toxicity

**Metabolism**
- Phase I, e.g., Cytochrome P450s
- Phase II, e.g., Epoxide hydrolase, conjugation reactions

**Excretion**
- Hepatic -- larger (> 500 Da.), more lipid soluble
- Renal -- smaller, more hydrophilic
The importance of certain “drug” transporters to Pharma and FDA

• The FDA guideline: “investigational drugs should be evaluated in vitro to determine whether they are a substrate of Organic Anion Transporter 1 (OAT1) (plus OAT3 and 5 others*)... when renal active secretion is important” (FDA Administration. 2012)

*OCT1, OATP1B1, OATP1B3, MDR/Pgp, ABCG2/BCRP
SLC and ABC “drug” transporters that have been implicated in the handling of drugs, toxins and metabolites.
Multispecific “Drug” Transporters in Different Organs Throughout the Body

Nature Reviews | Drug Discovery
Nigam SK, 14: 29-44 (2015)
Dynamic changes in EXPRESSION and FUNCTION (PAH transport) of SLC22 family drug, toxin and metabolite transporters during late prenatal and postnatal kidney development

A

SL22 transporters

Normalized Intensity Values

-2.0
0.0
2.0
4.0
6.0
Early Embryonic Intermediate Embryonic Late Embryonic Postnatal Mature

Stages of Kidney Development

B

Weeks of age

0 5 10 15 20 25

1 2 3

clearance, PAH (μl/min x g)

C

SL22 transporters regulated at least 2 fold between at least 1 pair of consecutive stages of kidney development

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Title</th>
<th>Fold change between consecutive stages</th>
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<tr>
<td></td>
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<td>[interm emb vs early emb]</td>
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The “classical” pathway of organic anion secretion in the proximal tubule (DC, dicarboxylates; OA, organic anions):

- **Avid**: some substrates cleared in the “first pass”
- **P-aminohippurate (PAH)** is the prototypic substrate of this pathway

**Substrates:**
- **Exogenous**: β-lactams, NSAIDs, diuretics, MTX, nucleoside analog antivirals, many others
- **Endogenous**: neurotransmitter metabolites (5HIAA, HVA), cyclic nucleotides, prostaglandins, urate
SLC22 Family of Transporters Has Over 30 Mammalian Members—Conserved Evolutionarily (e.g. fly, worm orthologs)—At least 6 subfamilies—Focus on OAT subclade

Loss of OAT1 RNA and protein in KO mice

Northern analysis

Imunostaining and β-gal staining

OAT1 knockout mice
(Satish Eraly, JBC 2006)
Loss of furosemide responsiveness in OAT1 (SLC22A6), OAT3 (SLC22A8) but not URAT1 (SLC22A12) KO mice

**OAT1**

- UNaV, µmol/min/g
- ED50 (mg/kg)
- WT
- KO
- 0.70±0.06
- 3.1±0.5**

**OAT3**

- ED50 (mg/kg)
- 0.50±0.03
- 1.7±0.2*

**RST/URAT**

- ED50 (mg/kg)
- rst +/+ (n=5) 0.81±0.27
- rst1 -/- (n=5) 0.71±0.16

Loss of furosemide responsiveness in OAT1 (SLC22A6), OAT3 (SLC22A8) but not URAT1 (SLC22A12) KO mice

Vallon et al. AJP 2008
Mercury conjugates are effectively organic anions and transported into the proximal tubule by Oat1

(Control)  

(HgCl₂ 4mg/kg, i.p., 18h)

(Torres et al. J. Biol. Chem. 2011)
Oat1 knockout is largely protected from organic mercurial toxicity to the kidney

Torres et al. J. Biol. Chem. 2011
Metabolomic profiling of Oat1 knockout plasma and urine samples.

Bill Wikoff, Wei Wu

Wikoff WR et. al., J Proteome Res, 10:2842-51(2011)
Untargeted (LC/MS) data: TCA cycle metabolites, Gut microbiome metabolites, uremic toxins, urate, vitamin cofactors, others

HAVE MEASURED AROUND 600 METABOLITES IN OAT1 KNOCKOUT PLASMA AND OAT3 KNOCKOUT PLASMA—AROUND 120 UNIQUE OAT1 METABOLITES AND 50 UNIQUE OAT3 METABOLITES
OAT1 and/or OAT3

**DRUGS?**
Antibiotics, NSAIDs, diuretics, antivirals

**METABOLITES?**
Krebs cycle intermediates (alpha KG), ketone bodies, uric acid

**SIGNALING MOLECULES?**
cAMP, prostaglandins, short chain fatty acids, bile acids

**EXOGENOUS TOXINS?**
Mercury-conjugates, aristolochic acid

**UREMIC TOXINS?**
Indoxyl sulfate, hippurate, polyamines, p-cresol sulfate

**GUT MICROBIOME PRODUCTS?**
Lots—including many uremic solutes

**NUTRIENTS?**
Pantothenic acid
Systems View: Reconstruction of metabolic networks from large-scale ‘omics’ data implicates drug transporters in metabolic pathways.
Increased and decreased intracellular reaction activities ($p < 0.05$) in OAT1-associated metabolic pathways based on knockout tissue microarray and metabolomics data (Ahn et al. J. Biol. Chem. 2011)
What do drug transporters really do?

Sanjay K. Nigam

MULTIPLE WAYS OF LOOKING AT TRANSPORTERS IN PHARMACOLOGY AND PHYSIOLOGY...
Microbiome/Nutrient-Gut-Liver-Kidney Axis Supported by Oat1 and Oat3 KO Metabolomics Data

Wikoff WR et. al., J Proteome Res, 10:2842-51(2011)
The role of the ABC/SLC transporters in the whole-body homeostasis ("The Remote Sensing and Signaling Hypothesis")

- Coordinate the transport of small molecules across cells/tissues.
- Participate in inter-organ and inter-organismal communication.
- Work in parallel with other well-studied regulatory systems.

Nigam SK, 14: 29-44 (2015)
“Remote Sensing and Signaling” by SLC and ABC transporters needs to be considered as one of several “canonical” homeostatic systems in the body.
REMOTE SENSING AND SIGNALING BETWEEN KIDNEY AND INTESTINE TO MAINTAIN URIC ACID HOMEOSTASIS IN CHRONIC KIDNEY DISEASE

SNPs in “intestinal” ABCG2 become $10^8$ times more significant than any other urate transporter in CKD (SLC2A9 next)

ISTHE UREMIC SYNDROME PARTLY DUE TO ALTERED UREMIC TOXIN REMOTE SENSING AND SIGNALING MEDIATED BY SLC AND ABC “DRUG” TRANSPORTERS?

Colleagues: Kevin Bush, Satish Eraly, Wei Wu, Carlos Lopez-Nieto, Sun-Young Ahn, Gregory Kaler, David Truong, Henry Liu, Gleb Martovetsky, Neema Jamshidi, Bernhard Palsson
SUMMARY 1: MULTISPECIFIC SLC (eg. OAT1) AND ABC (eg. ABCG2) DRUG TRANSPORTERS:

1. Transport small molecule drugs and environmental toxins

2. Transport metabolite “biomarkers” relating to levels and/or toxicity of drugs

3. Transport key metabolites (eg. alpha ketoglutarate), signaling molecules (eg. cyclic nucleotides, prostaglandins, odorants, fatty acids), antioxidants (eg urate) and microbiome-derived metabolites (eg. indoxyl sulfate)

4. Modulate complex metabolic pathways within cells (eg. kidney proximal tubule energy metabolism)
SUMMARY (2): MULTISPECIFIC SLC (eg. OAT1) AND ABC (eg. ABCG2) DRUG TRANSPORTERS:

5. Are involved in small molecule inter-organ “communication” (eg. gut, liver, kidney) and movement of metabolites/signaling molecules into and out of body fluids (eg. blood, bile, urine, CSF)

6. Are key for inter-organismal communication—microbiome products to host, mother-fetus (eg. fatty acids), mother-nursing infant (eg. carnitine), volatile odorant elimination in urine (eg. propionate)

7. Form a regulated homeostatic network mediating small molecule inter-organ and inter-organismal communication—loosely analogous to the endocrine, growth factor/cytokine and autonomic nervous system (the “remote sensing and signaling hypothesis”)

8. Dysregulation of Remote Sensing and Signaling in Uremia