Endogenous biomarkers for Renal Transporters

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Organic anion and cation transport systems in the kidney

Wright SH, et al. Physiol Rev. 2004

Hydrophilic organic anions with low MW
Endogenous substrates: cyclic nucleotides, prostaglandins, uric acid and dicarboxylates
Drugs: PAH, β-lactam antibiotics, antiviral drugs (adefovir, tenofovir), NSAIDs, and diuretics (furosemide)
Inhibitor: probenecid

Bulky organic anions
Endogenous substrates: uremic toxins, steroid conjugates (DHEAS, E₂17βG and E1S)
Drugs: β-lactam antibiotics (PCG), metotrexate, HMG-CoA reductase inhibitors (pravastatin) and H₂ receptor antagonists (famotidine), Ro64-0802, fexofenadine
Inhibitor: probenecid

Hydrophilic organic anions with low MW
Endogenous substrates: creatinine, NMN
Drugs: metformin, nadolol, atenolol, sumatriptan, frovatriptan
Inhibitor: DX-619

Various organic cations with low MW
Endogenous substrates: creatinine, NMN, thiamine, carnitine
Drugs: metformin, cephalexin, MIBG, ASP, Rhodamine123
Inhibitor: cimetidine, pyrimethamine

Bulky organic cations
Endogenous substrates: creatinine, NMN, thiamine, carnitine
Drugs: metformin, cephalexin, MIBG, ASP, Rhodamine123
Inhibitor: cimetidine, pyrimethamine

Tubular secretion

Wright SH, et al. Physiol Rev. 2004
Selected human transport proteins for drugs and endogenous substances.


DDI (CysA, rifampicin)

DDI with GFJ constituents

DDI (GF120918, curcumin, eltrombopag)

SNP&DDI (rifampicin, ritonavir)

DDI (rifampicin, probenecid)

DDI (quinidine, tariquidar, elacridar)

DDI (probenecid)

DDI (quinidine)

DDI (pyrimethamine, cimetidine, trimethoprim)
Importance of evaluation of DDI in the efflux process

Transporters mediate tissue uptake and subsequent efflux.

Inhibition of uptake process (OCT2)

Inhibition of efflux process (MATE)

Calm on the surface, but big fire in the core (内輪は火の車)
Pyrimethamine

- Anti-malarial and anti-toxoplastic drug
- Inhibit the dihydrofolate reductase
- Protein binding: 87% (human) 92% (mouse)
- Unbound plasma concentration: 200 nM (at the clinical dose 50 mg, po)
- \( t_{1/2} \): 96 hr (human) 4 hr (mouse)
- Decrease renal secretion of creatinine

Inhibition constant (\textit{in vitro})

<table>
<thead>
<tr>
<th></th>
<th>MATE1</th>
<th>MATE2-K</th>
<th>OCTN1</th>
<th>OCTN2</th>
<th>OCT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_i ) Mouse</td>
<td>145 ± 36 nM</td>
<td>—</td>
<td>&gt; 10 ( \mu )M</td>
<td>&gt; 30 ( \mu )M</td>
<td>6.0 ± 1.5 ( \mu )M</td>
</tr>
<tr>
<td>human</td>
<td>77 ± 13 nM</td>
<td>46 ± 6 nM</td>
<td>?</td>
<td>?</td>
<td>10 ± 1 ( \mu )M</td>
</tr>
</tbody>
</table>


\textit{Effect of metformin on the plasma concentration time profile and urinary excretion of metformin in healthy subjects}

<table>
<thead>
<tr>
<th></th>
<th>MD</th>
<th>MD+PYR</th>
<th>ThD</th>
<th>ThD+PYR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC/dose (h/mL*10^{-6})</td>
<td>21 ± 2</td>
<td>23 ± 2 (110%)</td>
<td>22 ± 4</td>
<td>30 ± 3 (139%) *,#</td>
</tr>
<tr>
<td>( f_{e0-24} (%) )</td>
<td>78 ± 6</td>
<td>65 ± 5 (84%) *</td>
<td>55 ± 2 #</td>
<td>50 ± 4 (91%) #</td>
</tr>
<tr>
<td>CL\textsubscript{r} (mL/min)</td>
<td>623 ± 97</td>
<td>451 ± 55 (77%) *</td>
<td>395 ± 31 #</td>
<td>255 ± 27 (66%) *,#</td>
</tr>
</tbody>
</table>

\*\( p < 0.05 \); compared with control. \#\( p < 0.05 \); compared with MD.
Time profiles of the radioactivities of $^{11}$C-metformin in the liver and kidney in mice

(A) Blood (Heart)  
(B) Liver  
(C) Whole kidney  
(D) Corticomedulla region  
(E) Renal pelvis

Coronal plane

Frame #6  [30sec]
Frame #20  [500sec]
Drug interactions involving the efflux transporter may enhance the drug response of victim drugs with greater magnitude than expected from the plasma concentrations.

Nephrotoxic effect of cisplatin was enhanced by pyrimethamine treatment in mouse

Transporter Function and Metabolome

Drug-drug interaction

Genetic mutation  
Disease state

Alteration in the transporter function

PK profiles of drug  
(exogenous substance profiles in body fluids)

Inter-subject variation in drug response

Metabonome  
(endogenous substance profiles in body fluids)

Predictable?  
(Pharmaco-metabonomic Hypothesis*)

advantage: Multiple transporters’ activity can be evaluated without administration of exogenous probes

Diagnosis of variation of transporter function for personalized medicine

1. Kinetics of endogenous metabolites in the body

The impact of DDI caused by single- or multiple dose of inhibitors on the plasma concentrations of endogenous metabolites depends on not only the magnitude of reduction in the clearance, but also on the synthesis rate and plasm concentrations before inhibitor administration (amount produced during the study versus amount present in the body before inhibitor administration)

\[ V_d \frac{dC}{dt} = k_{in} - CL_{tot} \left( 1 - \frac{I_p}{K_i + I_p} \right) \cdot C \]
2. Other factors needs to be considered

- Diurnal variation
- Relatively large inter-individual difference
- (substrate-dependent inhibition constant)
- Lack of quantitative information on the clearance mechanisms and mass balance
- Continuous synthesis in the body

- Plural blood and urine sampling points (AUC evaluation)
- Clinical study in a crossover fashion (same subjects)
- In vitro inhibition study

Clinical studies to show the specificity/sensitivity of the endogenous compounds as probe of specific transporter/enzyme by PGx or DDI studies
OUR APPROACH

1. Conduct clinical DDI studies using probe drugs in healthy subjects with established inhibitors (the base line was defined in the same subjects)

2. Measure known endogenous or food-derived substrates in the plasma and urine, or comprehensive analysis to identify compounds the concentration of which is affected by drug administration using LC-MS/MS (so-called metabolome analysis)

3. Compare the impact of DDI between the probe drugs and endogenous compounds in the same subjects.
Role of MATEs in the tubular secretion of endogenous metabolites

★ Creatinine: a metabolite of creatine, and biomarker for kidney function

N-methylnicotinamide (NMN): a metabolite of nicotinamide which undergo extensive tubular secretion in humans. It has been used as biomarker for renal organic cation transport system.

Serum/plasma creatinine can be a biomarker of OCT2, MATE1 and MATE2-K.

★ N-methylnicotinamide (NMN): a metabolite of nicotinamide which undergo extensive tubular secretion in humans. It has been used as biomarker for renal organic cation transport system.

NMN is a substrate of hOCT2 and hMATE2-K. NMN may be an in vivo probe for renal organic cation transporters.

Effects of PYR on the plasma concentrations and urinary excretion of NMN in healthy subjects

☆N-methyl nicotinamide (NMN): a metabolite of nicotinamide which undergo extensive tubular secretion in humans. It has been used as biomarker for renal organic cation transport system.


NMN is a substrate of hOCT2 and hMATE2-K. NMN may be an in vivo probe for renal organic cation transporters.

Metformin and NMN share the H⁺ coupled transport in human brush border membrane vesicles.
Workflow of Metabolomic Approach

**Discovery the potential biomarkers for diagnosis of genetic polymorphism, drug side-effect, and disease by metabolomics**

**Group A**
- Wild type
- Drug administration
- Healthy subject

**Group B**
- Genetic deficient type
- Non-administration
- Patient

**Sample preparation**

**LC/MS* analysis**

**Comprehensive analysis of low molecular weight components in a biological sample**

**SIMCA-P+**

**OPLS-DA**

**S-plot**

**Peak extraction and alignment**

**Database**

**Ref:** Wiklund S, Anal Chem 80,115(2008)
Workflow of Untargeted Metabolomic Approach

**Urine Samples**
- With PYR 50 mg, p.o.
- W/o PYR

**LC/MS analysis**
- Column, Waters XBridge HILIC (4.6 x 50 mm)
- Mobile phase, 10 mM ammonium acetate (pH 5.0) and acetonitrile
- Positive ion-heated electrospray ionization
- Scanning range, $m/z$ 100 – 800
- Resolving power, 30,000

**Peak extraction and alignment**
- A total of ca. 2,000 peaks in human urine

**Selection of compounds of interest**
- Criteria:
  1) [peak response x urine volume] was decreased by more than half of the control in both humans and mice by PYR treatment.
  2) The decrease was seen in at least 7 of the 8 volunteers
  3) The peak intensity was strong enough for the product ion spectrum to be obtained.

**Structural identification**
- Online DB search
- Authentic standards
- W/o PYR vs. with PYR 0.5 mg/kg, i.v.
- W/o PYR vs. with PYR 50 mg, p.o.
## Summary of probe compounds for MATEs-mediated drug interaction studies

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urinary excretion mechanism</th>
<th>%Decrease of renal CL</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>secretion type (CLR&gt;fpGFR)</td>
<td>34%</td>
<td>• Straightforward approach</td>
<td>• Needs to be administered</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• No variation of intake</td>
<td>• Low impact of MATE inhibition on CL&lt;sub&gt;r&lt;/sub&gt;</td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td>20%</td>
<td>• High concentration</td>
<td>• Small change in renal CL</td>
</tr>
<tr>
<td>NMN</td>
<td></td>
<td>70%</td>
<td></td>
<td>• Need to determine the renal CL due to large diurnal variation of C&lt;sub&gt;plasma&lt;/sub&gt;</td>
</tr>
<tr>
<td>Thiamine</td>
<td>Reabsorption type (CLR&lt;fpGFR)</td>
<td>80%</td>
<td>• Plasma concentrations show small diurnal variation</td>
<td>• Low concentration in the plasma</td>
</tr>
<tr>
<td>Carnitine</td>
<td></td>
<td>90%</td>
<td></td>
<td>• Lack of in vitro support to be substrates of MATEs</td>
</tr>
<tr>
<td>Acetyl carnitine</td>
<td></td>
<td>90%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6β-hydroxycortisol</td>
<td>secretion type (CLR&gt;fpGFR)</td>
<td>Not-affected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

More than 80% inhibition of MATE could occur by pyrimethamine.
Selected human transport proteins for drugs and endogenous substances.


- DDI with GFJ constituents
- DDI (GF120918, curcumin, eltrombopag)
- SNP & DDI (rifampicin, ritonavir)
- DDI (rifampicin, probenecid)
- DDI (quinidine, tariquidar, elacridar)
- DDI (quinidine, cimetidine, trimethoprim)
- DDI (pyrimethamine, cimetidine, trimethoprim)
- DDI (CysA, rifampicin)
Outline of clinical DDI study in healthy subjects

**Inhibitors**
- (non, low, middle, high dose)
  - PAH (OAT1 inhibitor)
  - probenecid (OAT1 & OAT3 inhibitor)
  - probenecid (oral)
  - PAH (i.v. infusion)

**Test substrates**
- benzylpenicillin (OAT3 probe)
- adefovir (OAT1 probe)

Blood sampling
- adefovir or PCG benzathine (oral)

Urine sampling
- PAH (i.v. infusion)
- (hr)
- 0 0.5 1 2 3 4 6 8 12 24

* The protocol of this clinical study was approved by the ethics committees in the Faculty of Pharmaceutical Sciences, the University of Tokyo and the Kitasato University East Hospital.
6β-hydroxycortisol is found to be a OAT3 substrate

Renal clearance of 6β-OHF

Biomarker for estimating CYP3A4 activity

Conventional method

\[
R_{6β-OHF/F} = \frac{U_{6β-OHF}}{U_F}
\]

\(U_{6β-OHF}\) and \(U_F\) are amount of 6β-OHF and cortisol in urine for designated time interval

Current method

\[
CL_{6β-OHF} = \frac{X_{6β-OHF}}{AUC_F}
\]

\(X_{6β-OHF}\) and \(AUC_F\) are amount of 6β-OHF in urine and cortisol in plasma for designated time interval

[Diagram]

- Renal clearance of 6β-OHF involves tubular secretion (>fpGFR)
- 6β-OHF is a OAT3 and OCT2 substrate.

## Endogenous Compounds of Interest on Metabolome Analysis of Plasma and Urine Specimens from Subjects who Received Probenecid

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Identity</th>
<th>Retention time (min)</th>
<th>Protonated molecule (m/z)</th>
<th>Fold change (probenecid-treated/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
</tr>
<tr>
<td><strong>1</strong></td>
<td><strong>Taurine</strong></td>
<td>7.32</td>
<td>124.0073</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td><strong>2</strong></td>
<td><strong>U305</strong></td>
<td>7.33</td>
<td>206.0100</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td><strong>U118</strong></td>
<td>7.29</td>
<td>297.9724</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td><strong>4</strong></td>
<td><strong>GCDCA-S</strong></td>
<td>4.28</td>
<td>528.2634</td>
<td>1.9±1.8</td>
</tr>
<tr>
<td><strong>5</strong></td>
<td><strong>Inosine</strong></td>
<td>5.03</td>
<td>267.0731</td>
<td>5.6±2.0</td>
</tr>
<tr>
<td><strong>6</strong></td>
<td><strong>2-Acetylthiazole</strong></td>
<td>7.28</td>
<td>126.0031</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td><strong>7</strong></td>
<td><strong>U67</strong></td>
<td>7.30</td>
<td>271.0036</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td><strong>8</strong></td>
<td><strong>4,4'-Sulfonyldiphenol</strong></td>
<td>7.30</td>
<td>249.0216</td>
<td>1.0±0.4</td>
</tr>
<tr>
<td><strong>9</strong></td>
<td><strong>U682</strong></td>
<td>7.28</td>
<td>286.9776</td>
<td>0.9±0.3</td>
</tr>
</tbody>
</table>
Correlations between the renal clearance of the endogenous substrates and probe drugs with or without probenecid treatment
Evaluation of DDI risk involving drug metabolizing enzymes and transporters during drug development

Phase I

- single dose

Phase II

- multiple dose

Plasma and urine specimens are subjected to the pharmacokinetic analysis of metabolites

Pharmacokinetic DDI risks caused by the investigational new drug can be evaluated using endogenous biomarkers even during Phase I

DDI risk using probe substrates

setting exclusion criteria of drugs to avoid DDI

helping decision of conducting clinical DDI study
EMA and MHLW also published draft DDI guidelines in 2013 and 2014, respectively.

**Guidance for Industry**

**Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations**

- Is the investigational drug an inhibitor of OCT2, OAT1, or OAT3?
  - **Criteria:** Uptake of model substrates (e.g., MPP+, for OCT2; PAH for OAT1, or ES for OAT3) decreases with increased concentrations of the investigational drug.
  - **Yes**
    - Determine the IC\textsubscript{50}
    - **Unbound C\textsubscript{max}/IC\textsubscript{50} of the investigational drug**
    - **In vivo DDI study with a sensitive substrate\textsuperscript{(a)}**
  - **No**
    - **Poor or not an inhibitor of OCT2, OAT1, or OAT3**

A proposed amendment of decision tree

- **drug-endogenous compound interaction study**
  - **strong**
    - In vivo DDI study with a sensitive substrate
  - **Moderate**
    - In vivo DDI study is recommended
  - **Weak or none**
    - not-needed

Cost and labor for conducting DDI study can be saved.
We need further studies

• Dose-dependence in the effect of inhibitors
• Ethnic difference in the impact on transporter endogenous biomarker
• Effect of other drug transporter inhibitors from weak to strong ones
Summary

- We could identify the endogenous biomarkers for kidney drug transporters:
  - OCT2: creatinine, N-methylnicotinamide
  - MATEs: creatinine, N-methylnicotinamide, thiamine
  - OAT1: taurine
  - OAT3: 6β-hydroxycortisol, glycochenodeoxycholate sulfate
  - OCT1: thiamine (needs investigation in humans)

Red colored compounds: Both plasma concentrations as well as renal clearance can be biomarkers for drug transporters.

- Using these endogenous biomarkers, DDI risks involving multiple renal transporters can be evaluated in the same subjects even during phase I studies without administration of probe substrates.

- Endogenous biomarkers for hepatic drug transporters remains to be determined (bilirubin, bile acids and their conjugated metabolites?)
Acknowledgement:
Dr. Yuichi Sugiyama (Professor emeritus, University of Tokyo, & Sugiyama laboratory, RIKEN)

**OCT2 and MATE study**
Sumito Ito, Yushun Kuroiwa, Chunyong Wu, Tomoko Kitoh (The University of Tokyo)
Yuichiro Imamura, Takashi Izumi (Daiichi Sankyo, Tokyo, Japan)
Yoshinori Moriyama, Ph.D. (PYR Okayama Univ)
Katsuhisa Inoue, Ph.D., Hiroaki Yuasa, Ph.D. (MATE1-HEK, Nagoya City Univ.)
Yuji Kumagai (Kitasato University East Hospital)
Tomoyoshi Shirosita (Sekisui Medical Co., Ltd.)

**Probenecid study**
Yuri Tsuruya, Kazuya Maeda (The University of Tokyo)
Yuji Kumagai (Kitasato University East Hospital)

**Rifampicin study**
Hanano Terashima, Takeshi Nakayama, Daiki Mori, Tadahaya Mizuno (University of Tokyo)
Issei Takehara, Nobuaki Watanabe (Daiichi Sankyo)

**Metabolome analysis**
Koji Kato (Taisho Pharmaceuticals)