Emerging Importance of Nutrient Transporter-Mediated DDIs
-- focusing on the thiamine transporters

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Thiamine

- **Vitamin B1**
  - Essential nutrient
    - Recommended daily intake of 1.1 to 1.2 mg thiamine for adults
  - Present in three forms: thiamine, thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP)
  - TPP is the coenzyme for cellular metabolism
  - Pentose phosphate pathway and energy production
Thiamine Deficiency

• Poor dietary intake
• Increased metabolic requirement
• Reduced GI absorption
• Chronic medical conditions
• Chronic alcohol use
  - reduction of the THTR expression
  - inhibition of thiamine absorption through intestine and the reabsorption by kidney

• Clinical symptoms
  - Beriberi – cardiovascular
  - Wernicke’s encephalopathy (WE) – neurological
Wernicke’s encephalopathy (WE)

• An acute neurological disorder
  - characterized by the triad ophthalmoplegia, ataxia, and mental confusion
  - the most important encephalopathy due to a single vitamin deficiency

• The main cause of WE is due to an inadequate supply of thiamine to the brain
  - Absorption in GI epithelium - active and saturable transport at the intestine: THTR-1 and THTR-2
  - Export into the blood – basolaterol transporters
  - Distribution to extracellular fluid of brain- Cross the blood-brain barrier to reach the neurons
  - Enter into mitochondria of neurons

• WE normally treated with high doses thiamine through parenteral route (IV/IM)
Transporters that are Responsible for Thiamine Transport

Enterocyte
- THTR1
- THTR2

Hepatocytes
- THTR1
- THTR2
- OCT1/2
- MATE1/2K
- SLC25A19
- RFC?

Brain Endothelial

Kidney proximal tubule cells
- THTR
- MATE1/2K
- other?

Liang et al, Mol Pharmaceu 12, 4301 – 4310, 2015
Giacomini M et al, Drug Metab Disp 45, 76 – 85, 2017
Zhao and Goldman, Mol Asp Med 34, 373 – 385, 2013
Chen et al, PNAS 111, 9983 – 9988, 2014
Interplay between Folate and Thiamine Transporters

- Thiamine (T) is transported by THTR1/2
- TMP and TPP are transported by RFC
- Transport of TMP and TPP can be inhibited by folate
Thiamine Transporters

• THTR-1 (SLC19A2) and THTR-2 (SLC19A3)
  - THTR-1 is widely expressed while THTR-2 is expressed at the highest level in the duodenum
  - THTR-1 localized on both the BBM and BLM of enterocytes, while THTR-2 restricted to the BBM localization.
  - Share considerable similarity to one another (48%) and to RFC (~40%)
    o THTR-1/2 do not transport folate and RFC does not transport thiamine
  - THTR-2 may be more important in intestinal thiamine uptake
    o Uptake was significantly reduced in THTR-2 knockout mice while normal uptake was observed in THTR-1 KO mice
  - Intestinal thiamine uptake is adaptively regulated by the thiamine level in the diet
    o Expression of THTR-2 was upregulated in transgenic mice fed with a thiamine deficient diet
Fedratinib is a Janus kinase (JAK) inhibitor

Terminated in 2013 due to the observations of WE in several patients in Phase III study

-WE reported with fedratinib use is likely due to thiamine deficiency
Fedratinib shares a common moiety, 4-aminopyrimidine, to thiamine, which is important for binding to thiamine transporters.

Fedratinib interferes with the oral absorption of thiamine via inhibition of thiamine transporter.

Summary from Fedratinib/THTR Study

- The uptake of thiamine in Caco-2 and THTR1/2 cells is saturable (Km ~ 2 to 3 uM);
- Fedratinib is a potent inhibitor of thiamine uptake

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (uM)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caco-2</td>
<td>HEK-THTR1</td>
</tr>
<tr>
<td>Amprolium</td>
<td>0.8</td>
<td>ND</td>
</tr>
<tr>
<td>Fedratinib</td>
<td>2.1</td>
<td>NA</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tofacitinib</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Oxythiamine</td>
<td>&gt; 200</td>
<td>ND</td>
</tr>
</tbody>
</table>

- No significant impact on thiamine metabolism pathway
- The oral absorption and brain uptake of thiamine may be compromised
Pharmacophore

2,4-Diaminopyrimididine

Nonaminopyrimididine

2 or 4-Aminopyrimididine

Inhibition of Thiamine Uptake by JAKi and Other Xenobiotics

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ Value for Thiamine Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caco-2</td>
</tr>
<tr>
<td></td>
<td>µM</td>
</tr>
<tr>
<td>Fedratinib</td>
<td>0.940 ± 0.080</td>
</tr>
<tr>
<td>AZD1480</td>
<td>183 ± 68.9</td>
</tr>
<tr>
<td>Cerdulatinib</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Momelotinib</td>
<td>&gt;30.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>154 ± 22.4</td>
</tr>
<tr>
<td>Oxythiamine</td>
<td>198 ± 16.0</td>
</tr>
<tr>
<td>Ampolium</td>
<td>9.40 ± 2.80</td>
</tr>
</tbody>
</table>

- 2,4-Diaminopyrimidine
- 3-Aminopyrimidine
- 2,4-Diaminopyrimidine
- 2-Aminopyrimidine

Are We Ready for a THTR2 Inhibition Decision Tree?

1. What is the clinical significance of THTR2 inhibition in toxicity and drug-vitamin interactions?
2. Is there an established SAR for the THTR2 inhibition?
3. What are the current in vitro and in vivo models?
4. Are there generally available selective substrates and/or inhibitors to evaluate drug interactions in humans?
5. Is there an IVIVE? Can we extrapolate data from one drug to another?
<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Indication</th>
<th>Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fedratinib</td>
<td><img src="image1" alt="Fedratinib Structure" /></td>
<td>cancer</td>
<td>THTR inhibition</td>
<td><a href="#">Zhang et al, DMD 2014; 42:1656-1662</a></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td><img src="image2" alt="5-Fluorouracil Structure" /></td>
<td>Cancer</td>
<td>inhibits the formation of TPP from Thiamine</td>
<td><a href="#">Cho et al, J Korean Med Sci 2009; 24: 747-50</a></td>
</tr>
<tr>
<td>Ifosfamide</td>
<td><img src="image3" alt="Ifosfamide Structure" /></td>
<td>Cancer</td>
<td>Interfere the conversion from thiamine to TPP</td>
<td><a href="#">Buesa et al, Clin Cancer Res 2003; 9: 4636-37</a></td>
</tr>
<tr>
<td>Tolazamide</td>
<td><img src="image4" alt="Tolazamide Structure" /></td>
<td>Type II diabetes</td>
<td>Lowering thiamine levels by increase glucose metabolism</td>
<td><a href="#">Kwee et al, NEJM 1983; 309: 599-600</a></td>
</tr>
<tr>
<td>nitroglycerine</td>
<td><img src="image5" alt="nitroglycerine Structure" /></td>
<td>Heart disease</td>
<td>Ethyl alcohol and propylene glycol on thiamine metabolism</td>
<td><a href="#">Shorey et al, Ann Intern Med 1984; 101:500</a></td>
</tr>
<tr>
<td>furosemide</td>
<td><img src="image6" alt="furosemide Structure" /></td>
<td>Heart disease</td>
<td>Interfere with magnesium absorption</td>
<td><a href="#">Jain 2011, Drug induced WE</a></td>
</tr>
<tr>
<td>Co-amilofruse (Amiloride/furosemide)</td>
<td><img src="image7" alt="Co-amilofruse Structure" /></td>
<td>Heart disease</td>
<td>Magnesium depletion And THTR inhibition??</td>
<td><a href="#">McLean and Manchip Lancet 1999; 353: 1768</a></td>
</tr>
</tbody>
</table>
In vitro and In vivo Models for THTR2

• In vitro models:
  - Caco-2 (expression and function of THTR1/2)
  - Transfected cell lines

• In vivo models:
  - Thiamine depleted chow
  - Long term injection with pyrithiamine
  - KO mouse model

Reprinted with permission from Hazell A et al, Neurosci Lett epub, 2017
Substrates & Inhibitors of THTR2

- **Substrates:**
  - Drugs
    - Metformin
    - Fedratinib
    - Famotidine
    - Trimethoprim
  - Endogenous compounds
    - Thiamine

- **Inhibitors:**
  - Fedratinib
  - Metformin
  - Trimethoprim
  - Amiloride
  - Amprolium
  - Pyrithiamine
  - Phenformin
  - Chloroquine
  - Verapamil
  - Famotidine

Extensive overlapping on substrates with MATE, OCT1, and OCT2.

Giacomini M et al, Drug Metab Disp 45, 76 – 85, 2017
Liang et al, Mol Pharmaceu 12, 4301 – 4310, 2015
Zhang Q et al, Drug Metab Disp 42, 1656 – 1662, 2014
Kato K et al, Pharm Res 32, 2192-2204, 2015
## What Is the Relevant in vitro Cut-off?

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg)</th>
<th>Conc (uM)</th>
<th>IC$_{50}$ a (uM)</th>
<th>$[I_2]/IC_{50}$</th>
<th>$[I_1]/IC_{50}$</th>
<th>WE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fedratinib</td>
<td>400-500</td>
<td>&gt;3000</td>
<td>5-10</td>
<td>~2</td>
<td>&gt;1500</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td>Metformin</td>
<td>&gt;500</td>
<td>&gt;15000</td>
<td>2-4</td>
<td>~20</td>
<td>&lt;0.01</td>
<td>-</td>
</tr>
<tr>
<td>Momelotinib</td>
<td>150-300</td>
<td>1500-3000</td>
<td>~1</td>
<td>&gt;30 c</td>
<td>&gt;50</td>
<td>0.03</td>
</tr>
<tr>
<td>Amiloride</td>
<td>5-10</td>
<td>100</td>
<td>~0.1</td>
<td>200 d</td>
<td>&lt;0.001</td>
<td>Mg depletion</td>
</tr>
</tbody>
</table>

a, IC$_{50}$ values from Caco-2 or THTR2 cells  
b, Liang et al, Mol Pharmaceu 12, 4301 – 4310, 2015  
c, Giacomini M et al, Drug Metab Disp 45, 76 – 85, 2017  
d, Said et al, Am J Physio 277, C645-C651, 1999
THTR2 Summary

- THTR2 is important in the absorption and the distribution of thiamine, various drugs and xenobiotics.
- Further data are needed to establish a solid SAR between drugs/NCEs and THTR2.
- Additional probe substrates are needed to predict THTR2 inhibition.
  - No firm IVIVC data to support an establishment of $[I_2]/IC_{50}$ and $[I_1]/IC_{50}$ value.
  - Further studies to define thiamine as the appropriate probe substrate for THTR2.
- A combination of THTR2 transfected cell line (or Caco-2) and the THTR2 KO mouse model is helpful to define THTR2 substrates and inhibitors.
Acknowledgement

• Incyte
• ITCW3
Backup slide
Folate

• Vitamin B9
  - Essential nutrient
  - The major physiological folate is 5-methyltetrahydrofolate
  - Coenzyme for cellular one-carbon metabolism
  - Synthesis of thymidine and purine
  - Metabolism of amino acids

• Factors affecting folate absorption:
  - Congenital defect (gene mutation) in the uptake system
  - Intestinal disease
  - Chronic alcohol use
  - Drug interaction

• Folate deficiency:
  - Megaloblastic anemia
  - Growth retardation
  - Congenital neural tube defects
Folate Transporters -- RFC, PCFT, and FR

- **Reduced folate carrier (SLC19A1)**
  - Functions at neutral pH for folate absorption in distal GI
  - Substrates include 5-methyltetrahydrofolate, leucovorin, folic acid, and methotrexate

- **Proton-coupled folate transporter (SLC46A1)**
  - Functions at acidic pH for folate absorption in GI
  - High affinity with both folic acid and reduced folate

- **Folate receptor (FOLR1/2)**
  - Play in concert with PCFT in acidified endosomes

- **Efflux transporters**
  - MRP1, MRP3 and BCRP

Reprinted with permission from Zhao and et al, Expert Rev Mol Med. 11, E4, 2009
Drug Interactions involving Folate Transporter

**Methotrexate**

- Rheumatoid arthritis, Crohn’s disease, and cancer
- High affinity substrate of PCFT and RFC
- Potent inhibitor of PCFT
  - competitive inhibition of folic acid uptake
- Adverse effect: folate deficiency
- Supplementation with folate during methotrexate treatment

![Methotrexate (MTX)](image1)

![Folic acid](image2)
Encephalopathy

• Encephalopathy:
  - Disorder or disease of a brain.
  - The name is preceded by various terms that describe the reason, cause, or special conditions of the patient that leads to brain malfunction.
  - There are over 150 different terms that modify or precede "encephalopathy" in the medical literature

• Examples:
  - Anoxic encephalopathy (brain damage due to lack of oxygen)
  - Hepatic encephalopathy (brain malfunction due to liver disease)
  - Diabetic encephalopathy
  - Metabolic encephalopathy
  - Bovine spongiform encephalopathy (BSE) or "mad cow disease"
  - Drug-induced encephalopathy
  - Wernicke's encephalopathy (Wernicke's syndrome)