Looking forward: Enzyme replacement therapy for homocystinuria

Tomas Majtan and Jan P. Kraus

Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA
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• Consulting for Orphan Technologies, Ltd. (TM)
• Holding patent(s) for a product referred to in the presentation (TM, JPK)
CBS-deficient homocystinuria (CBSDH, HCU)

- HCU is chiefly caused by the missense mutations in the cystathionine beta-synthase (CBS) gene

<table>
<thead>
<tr>
<th>Biochemical marker</th>
<th>HCU</th>
<th>Control</th>
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<tbody>
<tr>
<td>Homocysteine (Hcy)</td>
<td>100-500 µM</td>
<td>~10 µM</td>
</tr>
<tr>
<td>Methionine (Met)</td>
<td>300-2000 µM</td>
<td>~30 µM</td>
</tr>
<tr>
<td>S-AdenosylHcy (SAH)</td>
<td>140-2000 nM</td>
<td>~15 µM</td>
</tr>
<tr>
<td>Cystathionine (Cth)</td>
<td>0-8 nM</td>
<td>50-350 nM</td>
</tr>
<tr>
<td>Cysteine (Cys)</td>
<td>40-140 µM</td>
<td>200-400 µM</td>
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</tbody>
</table>

Clinical manifestations

- **Ocular** (myopia, ectopia lentis)
- **Skeletal & connective tissue** (osteoporosis, various deformities, scoliosis, Marfanoid habitus)
- **Vascular** (thromboembolism, stroke)
- **Central nervous system** (seizures, mental retardation)
Sulfur amino acid metabolism & current therapeutic options

- **B-vitamin therapy** \( (B_6, B_{12}, \text{folate}) \)
  - \( \sim 40\% \) of patients show response

- **Low Met diet**
  - Effective, delays symptoms
  - Very poor compliance

- **Betaine**:
  - Partial response
  - No removal of sulfur from the cycle
  - Build up of Met

⇒ **UNMET NEED FOR NOVEL THERAPEUTIC OPTIONS:**
- Enzyme replacement therapy (ERT)
Mouse models of HCU

- **KO mouse** – total knock-out of mouse CBS gene
  phenotype: Severe growth retardation, 100% neonatal lethality, liver damage

- **I278T mouse** – human CBS I278T mutant cDNA
  phenotype: decreased mean survival, growth retardation, facial alopecia, moderate liver steatosis

- **HO mouse** – human CBS WT gene
  phenotype: normal survival, mild hepahpathy & steatosis
Enzyme replacement therapy with human CBS

- Enzyme replacement “sink” hypothesis:
  CBS activity in circulation will lead to a degradation of Hcy in blood, which would trigger a flux of Hcy from tissues and thus results in normalization of metabolic balance.

Full-length human CBS
tetrameric, lower yield, lower activity, aggregation, allosteric regulation by SAM

Truncated human CBS (htCBS)
dimeric, high yield, high activity, no aggregation, no allosteric regulation
Modification with a polyethylene glycol (PEG) was needed to:

- increase half-life
- maintain efficacy
- decrease its potential immunogenicity

**htCBS**

$\text{t}_{1/2} = 2.7 \text{ h}$

**PEG-htCBS**

$\text{t}_{1/2} = 30.4 \text{ h}$
ERT improved or normalized plasma & tissue metabolites in KO mice

⇒ The “sink” hypothesis works!
ERT rescued neonatal death of KO mice
ERT prevented liver disease in KO mice – light microscopy

Buffer-injected KO
MODERATE TO SEVERE HEPATOPATHY

Micro-vesicular steatosis (red arrows)
Macro-vesicular steatosis (orange arrow)
Focal hepatocellular necroses with resorptive inflammatory reaction (yellow arrow)

PEG-htCBS-treated KO
MINIMAL TO MILD CHANGES

Regular cellular architecture
No to minimal steatosis
No death of hepatocytes and no inflammation

Buffer-injected control
MINIMAL CHANGES
ERT prevented liver disease in KO mice – electron microscopy

**Buffer-injected KO**

Cytoplasmic rich in various vesicles and cisternae
Lipid droplets (black asterisk)
Disorganized ER cisternae (black arrows)
Swollen mitochondria with disorganized cristae (white arrows)

**PEG-htCBS-treated KO**

Normal composition of cytoplasm
Cytoplasmic glycogen (black asterisks)
Normal ER (black arrows)
Regular mitochondria (white arrows)

**Buffer-injected control**
ERT prevented loss of bone mass & fat content in KO mice

Majtan et al. FASEB J (2017)
How patients can get involved in the OT-58 program

- Patients can participate in a Natural History Study:
  - To monitor the natural progress of the disease in patients on current therapies
  - Ongoing in 3 countries (6 sites total) – www.ClinicalTrials.gov ID# NCT02998710

- Patients can participate in Treatment Clinical Trials:
  - To establish initial safety and efficacy of OT-58 in adult homocystinuric patients
  - Plans to start a Phase I/II trial in 2018

- For more information, please visit www.orphantechnologies.com
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