New developments in Urea Cycle Disorders
and its impact on patients

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SFEIM 2015, Lille, France
The urea cycle

Häberle, Arch Biochem Biophys, 2013
Urea synthesis depends on bicarbonate availability

Experiments (n=24) in isolated perfused rat liver (pH 7.4)

Urea synthesis depends on carbonic anhydrase

Experiments in isolated perfused rat liver

Häussinger D & Gerok W, Eur J Biochem, 1985
Mitochondrial Carbonic Anhydrase VA Deficiency Resulting from CASA Alterations Presents with Hyperammonemia in Early Childhood

Clara D. van Karnebeek,1,2,3,4,* William S. Sly,9 Colin J. Ross,2,3,4 Ramona Salvarinova,1,2,3 Joy Yaplito-Lee,10 Saikat Santra,11 Casper Shyr,3,4,5 Gabriella A. Horvath,1,2,3 Patrice Eydoux,3,5,8 Anna M. Lehman,3,5 Virginie Bernard,3,4,5 Theresa Newlove,3,6 Henry Ukpeh,2 Anupam Chakrapani,11 Mary Anne Preece,12 Sarah Ball,12 James Pitt,10,13 Hilary D. Vallance,3,7,8 Marion Coulter-Mackie,2,3 Hien Nguyen,9 Lin-Hua Zhang,2,3,4 Amit P. Bhavsar,3,4,5 Graham Sinclair,3,7,8 Abdul Waheed,9 Wyeth W. Wasserman,3,4,5 and Sylvia Stockler-Ipsiroglu1,2,3

The American Journal of Human Genetics 94, 453–461, March 6, 2014
Requirement of HCO$_3^-$ for urea and citric acid cycle

Enzymes affected by lack of bicarbonate

- CPS1 ureagenesis
- PC gluconeogenesis
- 3MCC branched chain amino acid degradation
- PCC branched chain amino acid degradation
CA Va deficiency – clinical summary

- 4 patients described, 10 patients unpublished
- 9/14 patients from Indian subcontinent
- Characteristics:
  - Onset in neonatal period or infancy
  - Hyperammonemia
  - Lactate elevated
  - Metabolic acidosis and ketosis
  - Hypoglycemia
- Positive response to carglumic acid
CA Va deficiency – our experience

96 PATIENTS with neonatal hyperammonemia:
  negative for NAGS and CPS1 mutations

CA5A mutation analysis: 10 patients identified
Data from 2004 - 2014

NAGS gene requests, n=248

CPS1 gene requests, n=218

Total:
> 30% late-onset
Country-specific statistics

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Samples</th>
<th>Positive Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>88</td>
<td>30%</td>
</tr>
<tr>
<td>Turkey</td>
<td>67</td>
<td>32%</td>
</tr>
<tr>
<td>Germany</td>
<td>41</td>
<td>21%</td>
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<tr>
<td>Great Britain</td>
<td>38</td>
<td>19%</td>
</tr>
<tr>
<td>Italy</td>
<td>23</td>
<td>29%</td>
</tr>
</tbody>
</table>

Data from 2004 - 2014
Treatment

Several new drug formulations

Taste-masked granules of sodium phenylbutyrate
approved by the EMA
switch-over trial, safety & efficacy confirmed and palatability superior


Glycerol phenylbutyrate
chemical esterified form of PBA, FDA approved
avoids sodium intake and it is a tasteless liquid
may exhibit better pharmacokinetics than NaPB


Flavor-masked forms of sodium benzoate

Combescot et al, Pharm Dev Technol, 2015
Outcome depending on **peak ammonia** at the first crisis

Retrospective survey in Japan on all UCDs; n=216; 1978 – 1995; 92 neonatal onset

> only patients with ammonia < 180 µmol/L are neurologically intact

Outcome depending on **peak ammonia** at the first crisis

Retrospective survey in Japan; all UCDs; n=177; 1999 – 2009; 77 neonatal onset

- ammonia > 360 µmol/L: only 18/153 patients with normal development
- ammonia < 180 µmol/L: marker for good prognosis

1998 paper


2012 paper

Cross-sectional, observational study

Criteria for non-classical UCD patients

- Manifestation outside neonatal period
- Mild course
- Unusual manifestation
- Asymptomatic with just biochemical phenotype

Data on 208 patients from 20 European metabolic centers

Rüegger C et al, J Inher Metab Dis, 2014
Diagnoses & frequency of symptoms

OTDC most common
69% ♂ and 31% ♀

65% had symptoms
(ASSD 35% symptoms)

Time to diagnosis and outcome

→ Average delay of 1.6 years to diagnosis
→ Delay > 1 year: 75 % cognitive impairment
< 1 year: 46 % cognitive impairment

Rüegger C et al, J Inher Metab Dis, 2014
Improving surveillance for hyperammonemia in the newborn

Retrospective evaluation newborns (n=25) in 10 years ammonia $> 400 \mu$mol/L

Question time and level of 1st ammonia?

Result 1st ammonia $\bar{\Omega}$ 23 h after 1st blood gases

Consequence

Test run (1 year) all patients correctly diagnosed

Vergano et al., Mol Genet Metab, 2013
Monitoring

**Aim:** to find a useful marker for compliance and therapeutic monitoring

**Study:** 3000 urine & plasma data points (54 adult, 11 pediatric UCD patients (ages 6–17) treated with glycerol or sodium phenylbutyrate

Monitoring

**Results:** limited utility of plasma levels for therapeutic monitoring
BUT: morning spot and 24-h urinary phenylacetylglutamine correlate strongly with dose

➤ Biomarker for monitoring compliance & the need for dose adjustment
Valproate-induced hyperammononemia

**In vivo**
- Rats treated with VPA: quantification of NAG in liver by HPLC-MS/MS

**In vitro**
- NAG synthase activity in rat liver mitochondria under VPA or VP-CoA

→ NAG concentration significantly reduced in VPA-treated rats

→ NAGS activity *in vitro* severely impaired by VP-CoA >> VPA

→ Hyperammonemia under VPA caused by direct NAGS inhibition

N-carbamyl-L-glutamate can restore secondary NAG deficiency

Aires CCP et al, J Hepatol, 2011
Hyperammonemia after stem cell transplantation

- 2-year-old male patient
- High-dose chemotherapy & HSCT for neuroblastoma stage IV
- Severe hyperammonemia (maximum 475 µmol/L) few days after HSCT
- Plasma glutamine very high 1757 µmol/L
- Patient died from cerebral edema

Hyperammonemia after stem cell transplantation

- Liver biopsy: CPS1 activity and expression absent
- Mutation analysis: no mutation found in RNA

Chemotherapy-induced impairment of CPS1

Lämmle A et al, MGM, 2015
Future developments: ammonia adsorption

AST-120 (Spherical Carbon Adsorbent)
• oral adsorbent of engineered activated carbon microspheres
• surface areas exceeding 1600 m²/g
• sink for ammonia present in the gut

Future developments: bumetanide

Ammonia compromises astrocyte potassium buffering
- overactivation of $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter 1 (NKCC1) in neurons
- neurological dysfunction (depolarization of GABA reversal potential)
- prevention by NKCC1 inhibition with the diuretic bumetanide

Rangroo Thrane et al, Nat Med, 2013
Future developments: nanomedicine

Liposome mediated peritoneal dialysis

• Removal of excess metabolites or overdosed drugs into the peritoneal space
• Successful ammonia removal from rats with a greater extraction efficiency than traditional peritoneal dialysis

Future developments: nanomedicine


in vitro setups after 3 hours
Further reading

### Further reading

**Investigational treatments for HA as of January 2015**.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Route of administration</th>
<th>Mechanism of action</th>
<th>Status as of January 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(ethylene glycol)</td>
<td>Powder</td>
<td>O</td>
<td>Available; further clinical evidence needed to establish therapeutic benefit in HA</td>
</tr>
<tr>
<td>VSL#3®</td>
<td>Capsule</td>
<td>O</td>
<td>Phase III/IV; further clinical evidence needed to establish therapeutic benefit in HA</td>
</tr>
<tr>
<td>l-Ornithine l-aspartate</td>
<td>Solution</td>
<td>O, IV</td>
<td>Authorized in Germany; phase IV</td>
</tr>
<tr>
<td>l-Ornithine phenylacetate</td>
<td>Solution</td>
<td>IV</td>
<td>Phase II</td>
</tr>
<tr>
<td>Branched-chain amino acid</td>
<td>Powder, injectable solution</td>
<td>O, IV</td>
<td>Available; further clinical evidence needed to establish therapeutic benefit in HA</td>
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<tr>
<td>Zinc</td>
<td>Capsules</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>Acetyl-l-carnitine</td>
<td>Powder, injectable solution</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>MARS®</td>
<td>N/A</td>
<td>IV</td>
<td>Albumin-supported dialysis</td>
</tr>
<tr>
<td>Hepa Wash®</td>
<td>N/A</td>
<td>IV</td>
<td>Clinical</td>
</tr>
<tr>
<td>Prometheus®</td>
<td>N/A</td>
<td>IV</td>
<td>Clinical</td>
</tr>
<tr>
<td>ELAD®</td>
<td>N/A</td>
<td>IV</td>
<td>Phase III</td>
</tr>
<tr>
<td>Albumin</td>
<td>Suspension</td>
<td>IV</td>
<td>Albumin-supported dialysis; cell-supported bioartificial liver support system</td>
</tr>
<tr>
<td>AST-120</td>
<td>Suspension</td>
<td>O</td>
<td>Activated carbon adsorbent</td>
</tr>
<tr>
<td>Metformin®</td>
<td>Tablet</td>
<td>O</td>
<td>Glutaminase inhibitor</td>
</tr>
<tr>
<td>THDP-17</td>
<td>N/A</td>
<td>N/A</td>
<td>Glutaminase inhibitor</td>
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<tr>
<td>ALF-5755</td>
<td>Peptide solution</td>
<td>IV</td>
<td>Liver tissue regeneration</td>
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<td>Adeno-associated virus2/8 coding for OTC</td>
<td>Viral suspension</td>
<td>IV</td>
<td>Gene therapy</td>
</tr>
<tr>
<td>Baculovirus coding for GS</td>
<td>Viral suspension</td>
<td>IV</td>
<td>Gene therapy</td>
</tr>
<tr>
<td>HepaStem®</td>
<td>Cellular suspension</td>
<td>IV</td>
<td>Hepatocyte replacement therapy</td>
</tr>
</tbody>
</table>

GS glutamine synthetase, IP intraperitoneal, IV intravenous, N/A not available, O oral, OTC ornithine transcarbamoylase, UC urea cycle.

*Sources: ClinicalTrials.gov registry, Thomson Reuters Integrity® Database.