New potential for treatment of Wilson`s disease

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Wilson´s disease

- Genetic autosomal recessive disorder of Cu transport
- Mutation in the gene ATPase 7B encoding the copper transporting protein
- ATP7B excretes copper into the bile at the canalicular side of hepatocytes (releasing Cu out of cells)
- When the amount of copper in the liver overwhelms the proteins that normally bind it, it causes oxidative damage (Fenton reaction: \( \text{Cu}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{3+} + \text{HO}^- + \text{OH}^- \), \( \text{Cu}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \text{HOO}^- + \text{H}^+ \))
- Damage eventually leads to chronic active hepatitis, fibrosis (deposition of connective tissue) and cirrhosis.
Clinical manifestation

- Disorder causes high accumulation of copper in organism, especially in:
  - Liver
  - Central nervous system

- Manifestation of Wilsons disease:
  - Liver disease (hepatic cirrhosis)
  - Neurological diseases (basal ganglia degeneration)
  - Psychiatric diseases
Current therapies

A. Low-copper diet, the average uptake of Cu 0.6 mg – 1.6 mg daily (high content of Cu is, e.g., in mushrooms, nuts, liver)

B. Low molecular weight copper-chelating agents p.o.
   - Lead to decreased uptake
   - Increase elimination of Cu into urine
     - Penicilamine
     - Triethylenetetraammine
     - Tetrathyliomolybdate

C. Additionally, high doses of Zn salts, p.o.
   - Maintenance therapy since blocks Cu uptake from gastrointestinal tract
   - Strong gastrointestinal adverse effects (Zn intake 1200 mg/day, normally 8 – 15 mg/day)
Effective sorbents

- should be able to adsorb copper released from food prior to uptake by the gastrointestinal tract

- exhibit an ability to scavenge copper secreted into the gastrointestinal tract, with subsequent elimination in the feces.
System concept

- Sorbent – polymeric beads
  - Poly(glycidyl methacrylate-co-ethylene dimethacrylate), 60:40 (w/w)
  - particle size 20 – 40 \( \mu \)m
  - Large specific surface
  - Non-resorbable from GIT
- Selectively chelating agents of Cu
Chelating groups

$N,N$-di(2-pyridylmethyl)amine (DPA)

Triethylenetetramine (TTA)

8-hydroxyquinoline (8HQ)
pH model study in GIT

- pH 2.0 (empty stomach), pH 4.0 (full stomach) – gastric environment model, chelating Cu

- pH 6.8 – small intestine model, stability of the chelated Cu against rechelation
Adsorption
DPA, TTA

- Adsorption: gastric environment simulation
  - Buffer pH 2, pH 4
  - 5 mg CuSO₄ + 500 mg of sorbents, t = 0 – 60 min

P2 – DPA = \(N,N\)-di(2-pyridylmethyl)amine

P3 – TTA = triethylenetetramine
Adsorption 8HQ

8HQ = 8-hydroxyquinoline
Desorption

- Desorption: intestinal environment simulation
  - pH 6.8

- MODEL - Cu chelating amino acids solution:
  (93 mg L-histidine, 133 mg L-cysteine, 6.67 g glycine, volume adjusted to 200 mL) – representing average concentration in GIT

- 500 mg of sorbents containing 5 mg of absorbed Cu
- 8HQ – just 0.4% of releasing Cu from intestine
Selectivity competition 
Cu$^{2+}$/Zn$^{2+}$

- Solution CuSO$_4$ 3.15 mmol.L$^{-1}$ + ZnSO$_4$ 315 mmol.L$^{-1}$; (8 – 15 mg of Zn per day)
- 2 hours in contact with sorbents

Selectivity = $[\text{Cu (sorbent)/Cu(solution)}] \times [\text{Zn(solution)/Zn(sorbent)}]$

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>pH</th>
<th>Selectivity for Cu$^{2+}$ compared to Zn$^{2+}$</th>
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</thead>
<tbody>
<tr>
<td>DPA</td>
<td>2.0</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>940</td>
</tr>
<tr>
<td>TTA</td>
<td>2.0</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>424</td>
</tr>
<tr>
<td>8HQ !</td>
<td>2.0</td>
<td>904</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>798</td>
</tr>
</tbody>
</table>
**In vivo experiments**

- $^{125}$I-polymeric beads – destiny of matrix in GIT
- $^{64}$Cu$^{2+}$ uptake – model of untreated patient (rat)
- $^{64}$Cu-polymer uptake – with polymeric beads:
  - 8HQ beads
  - DPA beads

- $^{64}$Cu – $T_{1/2} = 12.7$ hours, positron emission $e^+$, PET tomography
- Healthy Wistar rats (female), cca 220 g, 11 weeks old
[\textsuperscript{125}I]8HQ-polymer uptake from gastrointestinal tract

- Tracking destiny of polymer \textit{itself} in GIT
- Sorbent with 8HQ radiolabeled with \textsuperscript{125}I
Copper uptake using $^{64}$CuCl$_2$

- Untreated patient (model) with Wilson’s disease
- Three groups of animals (two experimental, one control):
  - 17 MBq pure $^{64}$CuCl$_2$, one control
  - 17 MBq, stock solution $^{64}$CuCl$_2$ + sorbent, two experimental
- Analysis after 24 hours
$^{64}\text{Cu}$ control group

PET/ slices; coronal plane, retention in liver even after 48 hours

15 min p.a.  2,5 h p.a.  5 h p.a.  8 h p.a.  24 h p.a.  48 h p.a.  72 h p.a.

Destiny of $^{64}\text{Cu}$ in rat organism, accumulation in organism
$^{64}$Cu uptake into liver

DPAB – N,N-di(2-pyridylmethyl)amine beads
8HQB – 8HQ beads
$^{64}\text{Cu}$-8HQ polymer PET/CT scan

Small retention of $^{64}\text{Cu}$ polymer in stomach wall (however, no Cu released)
$^{64}$Cu-8HQ polymer (through GIT)

PET/CT slices; coronal plane

15 min p.a.  2,5 h p.a.  5 h p.a.  8 h p.a.  24 h p.a.  48 h p.a.  72 h p.a.
Conclusion

- 8HQB significantly decreases $^{64}$Cu uptake
- $^{64}$Cu-8HQB is completely eliminated via feces
- 8HQ polymer is not uptaken into organism
- Simple system – potential approach for medicinal improvement like food supplement
- Potential to treated Wilson’s disease without side effects
Thank you for your attention

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