Bile acids and the brain. Suggested pathogenetic mechanism in connection with formation of brain xanthomas in patients with CTX

Ingemar Björkhem, Marjan Shafaati, Ulla Andersson, Ute Panzenboeck, Magnus Hansson, Shoshana Shpitzen, Vardiella Meiner, Wolfgang Slatter, Eran Leitersdorf
CYP27A1

Bile acids

CYP27A1
Cerebrotendinous Xanthomatosis (CTX)

- CTX is caused by mutations in the CYP27A1 gene.
- The clinical picture includes xanthomas of the brain, skin, and tendons. The xanthomas in the brain may cause neurological disturbances (ataxia) and early dementia.
- Biochemically the patients have markedly reduced levels of 27-hydroxycholesterol in the circulation, normal or low cholesterol levels, increased levels of cholestanol, markedly increased levels of abnormal 25-hydroxylated C27-bile alcohols.
Lack of CYP27A1 activity in humans leads to cerebrotendinous xanthomatosis
The sterol 27-hydroxylase is antiatherogenic
• Treatment of CTX with chenodeoxycholic acid is effective, even xanthomas in the brain may reduced in size.

• This means that the lack of CYP27A1 mediated flux of 27-oxygenated metabolites of cholesterol can not be the key mechanism behind the formation of xanthomas.

• A mouse model with a disrupted Cyp27 gene does not develop xanthomas. In contrast to patients with CTX, Cyp27-/- mice do not have increased levels of cholestanol.
Rat \( T^{14C} = 1.25 \) \( T^{14C} = 1.02 \)
Healthy man \( T^{14C} = 1.74 \) \( T^{14C} = 1.25 \)
CTX- patient \( T^{14C} = 1.54 \) \( T^{14C} = 0.35 \)

75 (1985) 448-455
Comparison CTX with Cyp27-/- mice

<table>
<thead>
<tr>
<th></th>
<th>CTX-patients</th>
<th>Cyp27 -/- mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>7α-Hydroxy-cholesterol</td>
<td>19-50x</td>
<td>4.4x</td>
</tr>
<tr>
<td>7α-Hydroxy-4-cholesten-3-one</td>
<td>90-160x</td>
<td></td>
</tr>
<tr>
<td>Cholesta-4,6-dien-3-one</td>
<td>17-24x</td>
<td></td>
</tr>
<tr>
<td>4-Cholestren-3-one</td>
<td>60-250x</td>
<td>5-9x</td>
</tr>
<tr>
<td>Cholestanol</td>
<td></td>
<td>1.8x</td>
</tr>
</tbody>
</table>
Inhibition of CYP7A1 by bile acids in the normal situation

Feed-back inhibition

7α-Hydroxy-4-cholesten-3-one

Cholesterol \xrightarrow{\text{CYP7}} \text{Bile acids}
Metabolic consequences of a defective CYP27A1 enzyme
Metabolic consequences of a defective CYP27A1 enzyme

- 7α-Hydroxy-4-cholesten-3-one
- Bile acids

25-Hydroxylated bile alcohol
Metabolic consequences of a defective CYP27A1 enzyme
Cholesterol

7-alpha-OH-4-cholesten-3-one

Cholestanol
Blood brain barrier model experiments

- pBCECs on filter insert
- apical (‘blood’)
- basolateral (‘brain’)

% Transfer of Steroid (Mean +/- SD; n=3)

- $7\alpha$OH-Cholestenone
- Cholestanol
- Cholesterol
- $27\alpha$OH-Cholesterol
- $7\alpha$OH-Cholesterol

Time (h)
Production of Cholestanol from 7α-hydroxy-4-cholesten-3-one
27-hydroxylation

27-hydroxylation

27-hydroxylation

27-hydroxylation

27-hydroxylation

27-hydroxylation
Why is the accumulation of cholestanol accompanied by accumulation of cholesterol?

- Cholestanol is less effective as a HMG CoA reductase inhibitor than cholesterol. Dilution of the cholesterol pool with cholestanol may thus increase cholesterol synthesis.

- There may be some direct conversion of cholestanol into cholesterol, due to a relatively low substrate specificity of the lathosterol 5alpha reductase.

- There may be an upregulation of cholesterol synthesis caused by 7 alpha hydroxy-4-cholesten-3-one.
Incubation of Astrocytes with 7-alpha-hydroxy-4-cholesten-3-one

- Ratio: Cholestanol/Cholesterol
- Ratio: Lathosterol/Cholesterol
Incubation of MDM with 7-alpha-hydroxy-4-cholesten-3-one.
Effects on mRNA levels of key genes

[Graph showing mRNA levels of key genes in response to different concentrations of 7-alpha-hydroxy-4-cholesten-3-one. The x-axis represents concentrations of 7-alpha-hydroxy-4-cholesten-3-one (Control, 0.5ug/mL, 1ug/mL, 2ug/mL), and the y-axis represents mRNA levels ranging from 0.1 to 1000. The graph includes lines for huSREBP1c, hSREBP2, h-HMGCoAR, and HuACAT1.]
Conclusions

• Accumulation of cholestanol in patients with CTX is likely to be of critical importance for the formation of xanthomas

• Most probably this accumulation is a consequence of the marked accumulation of 7 alpha hydroxylated bile acid intermediates which are precursors for cholestanol
• One of the bile acid intermediates, 7 alpha hydroxy-4-cholesten-3-one passes the blood-brain barrier very efficiently and is converted into cholestanol in the brain

• It seems likely that also the accumulation of cholesterol in the brain xanthomas is secondary to the flux of 7 alpha hydroxy-4-cholesten-3-one into the brain
SREBP regulation of cholesterol synthesis

- SCAP
- Insig
- CN
- ER
- Golgi
- Nucleus
- Sterols
- Activates transcription

Mature SREBP

N Junker
Sterol structure and effect on HMG CoA reductase in mouse

Accessory systems that may be affected in the CTX patient

**Substrate transport**

- StarD5?
- Cholesterol

**Electron transport**

- Adrenodoxin?
- Adrenodoxin reductase?
- Mitochondria

- CYP27A1
- e⁻
\[ ^{[\text{H}_6]}\text{7}\alpha\text{-hydroxy-4-cholesten-3-one} \]
\[ m/z \, 388 \]

\[ ^{[\text{H}_6]}\text{cholestanol} \]
\[ m/z \, 466 \]

\[ ^{[\text{H}_6]}\text{4-cholesten-3-one} \]
\[ m/z \, 390 \]
Testing the hypothesis that the increased accumulation of cholestanol in the brain of patients with CTX is due to a flux of 7alpha-hydroxy-4-cholesten-3-one into the brain

Daily intraperitoneal injections of 7alpha-hydroxy-4-cholesten-3-one, 200 ug, in a wild type mice and a Cyp27-/- mice for 10 weeks

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp27 -/- mouse</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Cyp27 +/- mouse</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Untreated Cyp27+/-</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Incubation of MDM with Cholestanol

- Lathosterol x 100
- Cholestanol

Ratio vs. Cholestanol:

- X-axis: Cholestanol (0 ug, 2 ug, 5 ug, 10 ug, 20 ug)
- Y-axis: Ratio (0, 5, 10, 15, 20, 25)

Graph shows the change in ratio of Lathosterol x 100 and Cholestanol with different concentrations of Cholestanol.
Production of 4-cholesten-3-one from 7α-hydroxy-4-cholesten-3-one

![Graph showing time in hours on the x-axis and production of 4-cholesten-3-one/Cholesterol on the y-axis. The graph compares Neuroblastoma, Astrocytoma, and Microglia.](image)
Increase in plasma levels of intermediates in cholestanol synthesis in patients with CTX

CYP27A1 is a widely expressed mitochondrial enzyme

- It belongs to the cytochrome p450 enzyme family.
- CYP27A1 is expressed in most tissues. In the liver it has an important role for bile acid synthesis.
- Requires NADPH, O₂, adrenodoxin, and adrenodoxin reductase for enzymatic activity.
- Transfer of substrate into the mitochondria may be a limiting factor for enzymatic activity.
$7\alpha$-Hydroxy-4-cholesten-3-one Added to Medium (µg/mL)

Cholestanol:Cholesterol
Incubation of Astrocytes with Cholestanol

- Ratio latho x 100/Cholesterol
- Cholestanol/Cholesterol

Graph showing the ratio of lathosterol to cholesterol and cholestanol to cholesterol over different concentrations of cholestanol (0, 10, 20, 40 ug).