Bile salt synthesis
Hepatic Transporter Proteins involved in Bile Formation

**Basolateral membrane transporter proteins fx:**
- NTCP uptake of bile salts
- OATP bulky organic anions

**Canalicular membrane transporter proteins fx:**
- BSEP ATP dependent transport of bile salts
- MRP2 transport hydrophilic conjugates with glutathione
- MDR3 phospholipid transporter
- ATP8B1 translocation of phospholipids
- ABCG5/G8 cholesterol secretion
Regulation of Bile Secretion
Background

• UDCA 13-15mg/kg/day – the only therapy for PBC approved by FDA

• Improves biliary enzymes and IgM, slow histologic progression to cirrhosis

• Anticholestatic and antiinflammatory effects
  – replace hydrophobic bile acids
  – activation of
    1. canalicular bile salt export pump (BSEP)
    2. canalicular multidrug resistance protein 3 (MDR3)
    3. basolateral multidrug resistance associated protein 4 (MRP4)

• About 1/3 patients not sufficiently controlled with UDCA monotherapy

[Lindor et al, AADSL 2009]
• Other drugs have been tested, but none as single agent to be beneficial: chlorambucil, penicillamine, cyclosporine, corticosteroid, azathioprine, mycophenolate mofetil, thalidomide, methotrexate, malotilate and colchicine
  [Lindor et al, AADSL 2009]

• Negative studies for combination therapy using UDCA plus colchicine/ MTX/ silymarin

• Budesonide effects controversial

• Fibrates are being evaluated:

  Small uncontrolled studies and case reports
  
Bezafibrate

- 2-(4-{2-[((4-chlorobenzoyl)amino)ethyl]phenoxy})-2-methylpropanoic acid

- bezafibrate is an agonist of PPARα

- peroxisome proliferator-activated receptorα (PPARα)

  nuclear hormone receptor protein functions as transcription factors regulating expression of genes involved in lipid metabolism
Bezafibrate in PBC

Previous studies:

• Iwasaki et al, Bezafibrate may have a beneficial effect in pre-cirrhotic primary biliary cirrhosis. Hepatology Res 1999;16:12-18.


Original Article

The efficacy of ursodeoxycholic acid and bezafibrate combination therapy for primary biliary cirrhosis:
A prospective, multicenter study

Shinji Iwasaki,1 Hiromasa Ohira,2 Shuhei Nishiguchi,3 Mikio Zeniya,4 Shuichi Kaneko,5 Morikazu Onji,6 Hiromi Ishibashi,7 Isao Sakaida,8 Shigeki Kuriyama,9 Takafumi Ichida,10 Saburo Onishi,1 Gotaro Toda11 and Study Group of Intractable Liver Diseases for Research on a Specific Disease, Health Science Research Grant, Ministry of Health, Labour and Welfare of Japan

• 2 prospective studies:
  a) UDCA vs BF   (n= 45)
  b) UDCA + BF vs UDCA in patients refractory to UDCA monotherapy   (n= 21)

• UDCA + BF improved biliary enzymes in non-cirrhotic Japanese patients with PBC refractory to UDCA monotherapy
Figure 2  Biochemical changes in patients treated by additional bezafibrate (BF) plus ursodeoxycholic acid (UDCA) and by UDCA alone. Data are presented as box plots representing the 10th, 25th, 50th (median), 75th and 90th percentiles. *P < 0.05; **P < 0.01 versus values at baseline. ALP, alkaline phosphatase; ALT, alanine aminotransferase.
Anti-cholestatic action by BF proposed mechanism:

- Fibrate class agents are ligands of peroxisome proliferator-activated receptor α (PPARα)
- PPARα- nuclear hormone receptor protein functions as transcription factors regulating expression of genes involved in lipid metabolism
- ? Induction of MDR3 through activation of the PPARα
- MDR3 – translocating phospholipids through canalicular membrane
- MDR3 activated by UDCA monotherapy and combination therapy of UDCA and BF, role of BF in combination therapy remains unknown
Anticholestatic Effects of Bezafibrate in Patients with Primary Biliary Cirrhosis Treated with Ursodeoxycholic Acid

Akira Honda, 1,2 Tadashi Ikegami, 1 Makoto Nakamuta, 3 Teruo Miyazaki, 2 Junichi Iwamoto, 1 Takeshi Hirayama, 1 Yoshifumi Saito, 1 Hajime Takikawa, 4 Michio Imawari, 5 and Yasushi Matsuzaki 1
Aim

• Explore the mechanisms of remission of cholestasis by bezafibrate in PBC patients who failed to response to UDCA monotherapy

• *in vivo* and *in vitro* studies
Study Methods

• Inclusion:
  1. asymptomatic and untreated early stage PBC patients (4M, 27F)
  2. PBC dx by lab and histology (Scheuer’s classification I or II)

• Control group: 49 healthy Japanese volunteer (11M 38F; ages 22-79 years old)
UDCA 10-13mg/kg/d x 3/12 until ALP and GGT stabilised (max 6/12 tx) (n= 31)

Complete response (n = 12)

Incomplete response: ALP or GGT > ULN (n=19)

BF (400mg/d) + UDCA x 3/12

• Blood tests before and after UDCA monotherapy and after addition of BF
• in vivo and in vitro studies
• *in vivo*
• Serum markers for cholesterol and bile acid metabolism
  - sterol concentration (lathosterol, sitosterol, campesterol)
  - serum bile acid profile
    *7α-hydroxy-4-cholesten-3-one (C4): an intermediate in the biochemical synthesis of bile acids from cholesterol - markers of bile acid synthesis
    *4β-hydroxycholesterol - marker of CYP3A4/5 activity
  - serum fibroblast growth factor 19 (FGF 19) - markers of bile acid trans-intestinal flux
• *in vitro*
• Cell culture

- Human hepatoma cell line (HepaRG)
- D0 HepaRG cell/ Thawing and Seeding medium 670
- D3 medium replaced with 500uL/well of Induction Medium 640 containing BF, rifampicin, carbamazepine or GW 4064 dissolved in 1% acetonitrile

• Assays of cell **CYP3A4** activity and **Pregnane X receptor (PXR)** activation

• RNA extracted from HepaRG cells measured by reverse transcription and PCR
# Results

## Characteristics of Patients

<table>
<thead>
<tr>
<th>Laboratory Data</th>
<th>Control (n=49)</th>
<th>Before UDCA Treatment (n=31)</th>
<th>Before BF Treatment (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>57.8±1.6 [22-79]</td>
<td>60.3±1.8 [37-81]</td>
<td>58.8±1.6 [45-73]</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>11/38</td>
<td>4/27</td>
<td>1/18</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>21±1 [11-34]</td>
<td>64±18† [19-120]</td>
<td>45±5† [20-101]</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>17±1 [7-30]</td>
<td>82±34† [12-138]</td>
<td>51±9† [18-152]</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>25±2 [7-58]</td>
<td>196±27† [30-757]</td>
<td>178±59† [47-445]</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>230±9 [126-336]</td>
<td>517±43† [229-1163]</td>
<td>597±51† [266-952]</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.7±0.1 [0.3-1.2]</td>
<td>0.7±0.2 [0.3-1.3]</td>
<td>0.6±0.1 [0.3-1.1]</td>
</tr>
<tr>
<td>IgM (mg/dL)</td>
<td>97±12 [56-161]</td>
<td>288±27† [90-637]</td>
<td>306±60† [130-466]</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>199±4 [130-257]</td>
<td>213±9 [120-356]</td>
<td>228±18 [118-343]</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>115±4 [46-194]</td>
<td>138±7* [91-254]</td>
<td>149±18 [54-228]</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>65±2 [33-111]</td>
<td>53±4* [13-95]</td>
<td>55±5 [13-89]</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>91±6 [33-214]</td>
<td>107±7* [47-199]</td>
<td>113±11 [40-243]</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM [range].

Before UDCA treatment, all PBC patients before treatment with UDCA; Before BF treatment, PBC patients who exhibited an incomplete biochemical response to the UDCA monotherapy (600 mg/day) and before additional treatment with bezafibrate.

*P < 0.05, significantly different from control.

†P < 0.005, significantly different from control.

‡P < 0.0001, significantly different from control.
Results

Baseline Biomarker Levels for Cholesterol Metabolism

<table>
<thead>
<tr>
<th>Serum Biomarkers</th>
<th>Control (n=49)</th>
<th>Before UDCA Treatment (n=31)</th>
<th>Before BF Treatment (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bile acid metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4 (ng/mg CHOL)</td>
<td>15.7±2.9 [2.3-118]</td>
<td>12.1±1.8 [0.8-49]</td>
<td>11.8±2.1 [1.5-38]</td>
</tr>
<tr>
<td><strong>Cholesterol metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lathosterol (µg/mg CHOL)</td>
<td>2.8±0.3 [0.9-11.7]</td>
<td>2.2±0.2 [0.7-5.8]</td>
<td>2.2±0.3 [0.8-6.1]</td>
</tr>
<tr>
<td>Sitosterol (µg/mg CHOL)</td>
<td>1.6±0.1 [0.4-3.8]</td>
<td>2.0±0.2* [0.8-3.9]</td>
<td>2.4±0.2† [1.1-4.3]</td>
</tr>
<tr>
<td>Campesterol (µg/mg CHOL)</td>
<td>1.8±0.1 [0.4-5.1]</td>
<td>2.0±0.1 [0.7-3.7]</td>
<td>1.9±0.2 [0.7-3.3]</td>
</tr>
<tr>
<td><strong>Oxysterol metabolism</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4β-HC (ng/mg CHOL)</td>
<td>29±3 [11-135]</td>
<td>44±4† [24-140]</td>
<td>51±5† [20-92]</td>
</tr>
<tr>
<td>24S-HC (ng/mg CHOL)</td>
<td>31±2 [17-74]</td>
<td>34±2 [22-69]</td>
<td>41±2† [20-64]</td>
</tr>
<tr>
<td>27-HC (ng/mg CHOL)</td>
<td>77±3 [35-140]</td>
<td>75±4 [48-124]</td>
<td>75±4 [39-102]</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM [range].

Before UDCA treatment, all PBC patients before treatment with UDCA; Before BF treatment, PBC patients who exhibited an incomplete biochemical response to the UDCA monotherapy (600 mg/day) and before additional treatment with bezafibrate; C4, 7α-hydroxy-4-cholesten-3-one; CHOL, cholesterol; FGF19, fibroblast growth factor 19; 4β-HC, 4β-hydroxycholesterol; 24S-HC, 24S-hydroxycholesterol; 27-HC, 27-hydroxycholesterol.

*P < 0.05, significantly different from control.
†P < 0.005, significantly different from control.
‡P < 0.0001, significantly different from control.
Results: Effects of UDCA+ BF on LFT

![Graph showing effects of UDCA+ BF on LFT levels of AST, ALT, GGT, ALP, and IgM.](image-url)
Results: Effects of UDCA+ BF on Lipids

T-CHOL

LDL

HDL

TG

Serum concentration (relative to pretreatment level)

UDCA (n = 31)  BF (n = 19)

P<0.005

P<0.05

P<0.001
Results: bile acid metabolism

- UDCA not change C4 or FGF19
- UDCA + BF significantly ↓ both C4 and FGF19
- UDCA + BF ↓ serum chenodeoxycholic acid and deoxycholic acid
Effects of BF on CYP3A4:
Induced CYP34A mRNA expression & activity (dose dependent)

Effects of BF on PXR activation:
Weak but significant activator of human PXR
• CYP3A4: member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are mono-oxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids.

• PXR: a nuclear receptor - activation leads to induction of CYP3A4
Results: *in vitro* BF on gene expression

- Control target genes of PPARα and PXR
- Down-regulate CYP7A1, CYP27A1, enzymes in cholesterol, bile acid and fatty acid synthesis
- Down-regulate sinusoidal NTCP (transport basolateral bile acids into hepatocytes)
- Up-regulate CYP3A4, canulicular MDR3, MDR1, MRP2
Discussion

- UDCA + BF significantly improved cholestasis in early stage PBC patients who were refractory to UDCA monotherapy
BF: Possible mechanisms of anti-cholestatic effects

1) MDR3 is target of PPARs, stimulation of biliary phospholipid secretion due to up-regulation of MDR3

✓ significant elevation of expression of MDR3 mRNA after addition of BF

✗ MDR3 also activated by UDCA

✗ MDR3 expression ↑ PBC patient
2) PPARα activation leads to down-regulation of NTCP (transport basolateral bile acids into hepatocytes) and CYP7A1, CYP27A1 (enzymes in classical and alternative bile acid synthesis pathways)

- ↓ hepatic bile acid concentration
  - protecting hepatocytes vs cytotoxic bile acids
  - ↓ FXR activity → ↑ MRP4 (basolateral transporter for bile acid eflux)
3) BF was ligand of PXR nuclear receptor

- Serum analysis
  - 4β-HC: a marker of CYP3A4/5 activity
  - C4: marker of CYP7A1 activity/ de novo bile acid synthesis

- Suggest BF upregulates CYP3A4/5 and downregulate CYP7A1

- in vitro, BF induced CYP3A4 mRNA expression and activity and inhibited expression of CYP7A1 mRNA in dose dependent manner

Expression of CYP3A4 mainly controlled by PXR, suggesting BF is a ligand of PXR
Limitation

• Small study population

• Definition of UDCA incomplete response patients: 90% improvement seen 6-9 months, but 20% normalized after 2 years [Jorgensen, Gut 1995]

• Did not study the anti-inflammatory effects which may contribute to the improvement of biomarkers

• Activation of PXR and PPARs reported to suppress inflammation through inhibition of proinflammatory genes (nuclear factor-κB, TNF-α and IL-1α)

Conclusion

• Bezafibrate is a dual PPARs/ PXR agonist

• Potent anticholestatic efficacy in early stage PBC patients with an incomplete biochemical response to UDCA monotherapy
Thank you