Regulation of the cell surface expression and transport capacity of BSEP by small chemical molecules

Hisamitsu Hayashi and Yuichi Sugiyama

Dept. of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan
Schematic diagram illustrating the enterohepatic circulation of bile acids

Liver

Biliary excretion via BSEP

Hepatic uptake via NTCP and OATPs

Bile Acids

Blood

NTCP

OATPs

Hepatocyte

Cholestasis

Enterohepatic Circulation

Canalicular Membrane

Bile flow

Sinusoidal Membrane

Colon

Jejunum

Ileum

Excretion of bile acids
Molecular changes of biliary transport system in patients with PFIC2

PFIC2 (Progressive familial intrahepatic cholestasis type 2) develops in childhood and leads to end stage liver disease by second decade of life.

- No medical therapy has been established yet.
Predicted topology and frequently found mutations of BSEP

- E297G
- D482G

**ATP-binding domain**

*Strautnieks SS et al., Nature Genet 20: 233-238 (1998)*
*Jansen PL et al., Gastroenterology 117: 1370-1390 (1999)*
*Strautnieks SS et al., Gastroenterology 134: 1203-14 (2008)*
Both E297G and D482G mutations reduce the cell surface expression of BSEP due to the impaired trafficking.

Hayashi et al., Hepatology. 2005 41(4):916-24
Analysis of taurocholate transport activity by wild type and mutated BSEP (HEK293 cells).

- Problem in PFIC2 patients with E297G and D482G
  - Reduced cell surface expression of BSEP due to the impaired trafficking.

- Therapeutic goal
  - The restoration of BSEP expression at the cell surface.

Hayashi et al., Hepatology. 2005 41(4):916-24
Development of the therapeutic method for PFIC2 patients with E297G and D482G

☆ Therapy by small chemical molecules
  • Search candidates from the approved drugs for other diseases and low toxic compounds.

→ Immediately applied at the clinical place.

☆ 4-Phenylbutyrate (4PBA)
  • FDA approved drug for urea cycle disorder.
  • Increase the cell surface expression of mutated membrane protein. (Rubenstein et al. J Clin Invest. 1997 15;100(10):2457-65.)
Establishment of screening system for the therapeutic compounds

The screening system using transcellular transport can evaluate the change of BSEP function by candidates without complex procedure, compared to the methods to directly evaluate it.
Identification of 4PBA effect using screening system


**Transcellular transport (pmol/mg protein)**

- **GFP**
  - Time (min): 0, 50, 100, 150
  - Symbols: ○: b→a_Control, ●: b→a_4PBA, □: a→b_Control, ▼: a→b_4PBA

- **WT**
  - Time (min): 0, 50, 100, 150
  - Symbols: ○: Control, ●: + 4PBA

- **E297G**
  - Time (min): 0, 50, 100, 150
  - Symbols: ○: Control, ●: + 4PBA

- **D482G**
  - Time (min): 0, 50, 100, 150
  - Symbols: ○: Control, ●: + 4PBA

**Key:**
- **b→a:** basal to apical
- **a→b:** apical to basal
Examination of 4PBA effect on BSEP-mediated transport and BSEP expression

• **Calculation of PS\textsubscript{apical}**

- **Immunoblotting**
  --- cell surface biotinylation

4PBA treatment induces cell surface expression and transport capacity of WT and mutated BSEP in MDCK II cells.
**4PBA-mediated prolongation of the half-life of cell surface-resident BSEP**

- **Biotin-labeling chase study**

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4PBA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The prolonged half-life of cell surface-resident BSEP is responsible for the increased BSEP expression.

Evaluation of 4PBA effectiveness in vivo

- Male, SD rat, 8 weeks
- Dose: 600 mg/kg per day in three divided doses by gavage (10 days)

**Immunoblotting**
---Canalicular membrane vesicles

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>4PBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bsep</td>
<td>1.0</td>
<td>3.2</td>
</tr>
<tr>
<td>P-gp</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>DPPIV</td>
<td>1.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>


4PBA treatment can increase Bsep expression at the canalicular membrane, and consequently, enhance bile acid transport via the canalicular membrane in SD rats.
Conclusion

☆ 4-phenylbutyrate treatment induces cell surface expression and transport capacity of WT and PFIC2-type mutated BSEP.

☆ The prolonged half-life of cell surface-resident BSEP is responsible for the increased BSEP expression.