Characterization of hepatobiliary transport of a bile acid *in vitro* to identify its rate-determining process; Use of Opti-expression technology

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Abstract

[Purpose] For evaluating drug transport kinetic, various kinds of *in vitro* model have been utilized extensively. Yet, it is time-consuming to construct such model and most of them tends to be far from the actual physiologically relevant expression level. The alternative technique was surfaced to overcome such issues. The present study demonstrated that the validity of a novel transfection system, Opti-expression technology (http://optiviabio.com/opti-expression-transient-expression/), can perform as an experimental system mimicking *in vivo* expression level with easy access of manufacturing and utilizing.

[Results and Discussion] The basolateral uptake intrinsic clearance and biliary efflux intrinsic clearance obtained in SCHH were 12.2 and 1.54 µL/min/mg protein, respectively. These values were comparable with NTCP-mediated initial uptake clearance and BSEP-mediated apical intrinsic clearance (PS_{apical}) in MDCK II cells, which implies that expression level of these 2 transporters was comparable between human hepatocytes and transiently transfected MDCK II cells. As BSEP DNA amount increases in MDCK II cells, the basal to apical transcellular transport clearance showed a plateau value; this indicates that the rate-limiting step was NTCP-mediated uptake process. PS_{apical} exhibited linear correlation with amount of plasmid DNA used for transfections, suggesting its utility to assess a transporter function quantitatively.

[Conclusions] Opti-expression technology is able to adjust the expression level of transporter by changing plasmid DNA amount. These double transfected cells can mimic physiological expression level, which in term reflects hepatocytes function.

Methods

[^{3}H]-taurocholate (TCA) was used as a probe substrate for NTCP and BSEP in all the assay. MDCK II cells cultured on porous membrane inserts were transiently transfected with plasmids encoding transporters using Opti-Expression technology. The intrinsic clearance of basal uptake was assessed by initial uptake in sandwich cultured human hepatocytes (In Vitro ADMET Laboratories, lot# HH1027) (SCHH) and MDCK II cells transfected with NTCP plasmid (10 ng/µL). The intrinsic clearance of biliary excretion was measured in SCHH by integration plot.

$$CL_{int,bile} = \frac{X_{t,bile}\ (pmol/mg)}{AUC_{0-t,hepatocytes}(\mu M \cdot min)}$$

Transcellular transport from basal to apical side and intracellular accumulation of [^{3}H]-taurocholate were determined in MDCK II cells.

$$\mathrm{PS}_{\mathrm{trans}} = \frac{V_{apical}\ (pmol/mg)}{C_{dosing}(\mu M)} \qquad \mathrm{PS}_{apical} = \frac{V_{apical}\ (pmol/mg)}{C_{cell}(\mu M)}$$

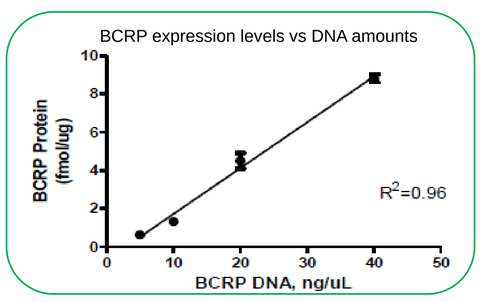
Introduction

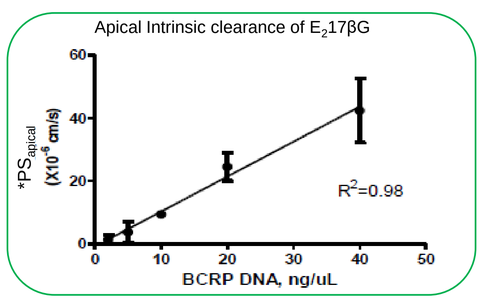
Opti-expression™ technology

・Opti-Expression™ technology, Optivia's (A Bio IVT company) patented methods, is an electroporation based transient expression system in polarized cells.

・With Opti-Expression transfection technology, BCRP protein expression levels are linear with transfected DNA amounts.

・Apical intrinsic clearance also exhibited linear correlation with BCRP expression levels.

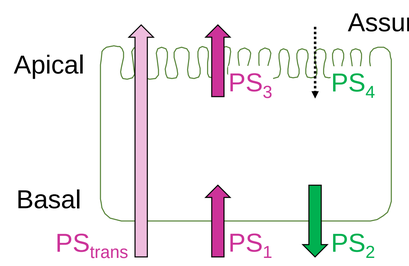




$PS_{apical} = J_{apical}/C_{cell,u}$

* Figure adapted from Fig. 4C in Ref. Li C *et al. 19th North American ISSX/29th JSSX Meeting.* Poster 447. 2014

Hepatobiliary transport of taurocholate (TCA) -Extended clearance concept-

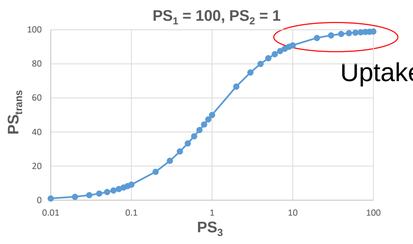


PS: permeability-surface area products
$PS_1 = PS_{basal\_passive} + PS_{NTCP}$
$PS_3 = PS_{apical\_passive} + PS_{BSEP}$

$$PS_{trans} = PS_1 \times \frac{PS_3}{PS_2+PS_3} = (PS_{basal\_passive} + PS_{NTCP}) \times \frac{PS_{apical\_passive}+PS_{BSEP}}{PS_2+(PS_{apical\_passive}+PS_{BSEP})}$$

$$Cell/Medium\ ratio = \frac{PS_1}{PS_2+PS_3} = \frac{PS_{basal\_passive}+PS_{NTCP}}{PS_2+(PS_{apical\_passive}+PS_{BSEP})}$$

When PS_3 is significantly greater than PS_2, PS_1 becomes the rate-determining step.



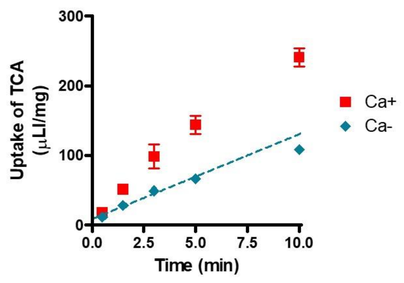
Uptake limited

Results

Fig.1. Comparison of NTCP-mediated TCA uptake between SCHH and MDCK II cells

1. Sandwich Cultured Human Hepatocytes (SCHH)

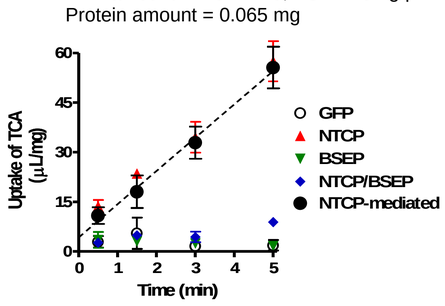
Substrate: 48.0 nM [^{3}H]-TCA



$PS_{inf\ (0.5\text{-}5)}$ = 12.2 µL/min/mg

2. MDCK II

Substrate: 82.9 nM [^{3}H]-TCA
DNA amount: NTCP = 10, BSEP =5 ng/µL
Protein amount = 0.065 mg



$PS_{inf\ (0.5\text{-}5)}$ = 10.1 µL/min/mg
$PS_{inf\ (0.5\ min)}$ = 21.7 µL/min/mg

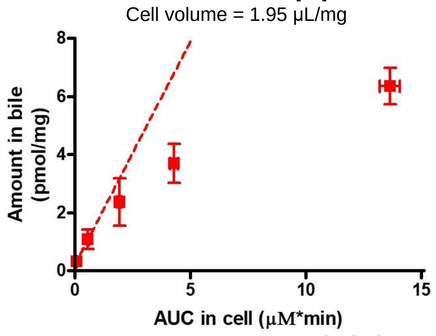
MDCK II cells transfected with NTCP DNA (10 ng/µL) yielded the basal uptake clearance similar to human hepatocytes (SCHH).

➡ Suggesting the NTCP expression levels were comparable between the two systems at the physiologically relevant levels.

Fig.2. Comparison of BSEP-mediated TCA efflux between SCHH and MDCK II cells

1. SCHH

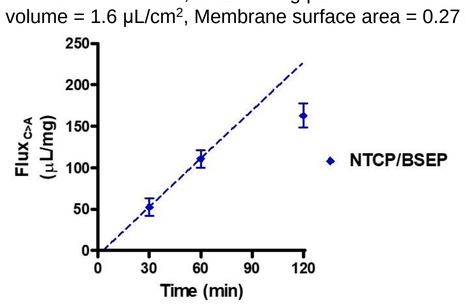
Substrate: 48.0 nM [^{3}H]-TCA
Cell volume = 1.95 µL/mg



$CL_{int,bile\ (0.5\text{-}1.5)}$ = 1.54 µL/min/mg

2. MDCK II

Substrate: 61.8 nM [^{3}H]-TCA, Protein amount = 0.065 mg
DNA amount: NTCP = 10, BSEP =5 ng/µL
Cell volume = 1.6 µL/cm^2, Membrane surface area = 0.27 cm^2

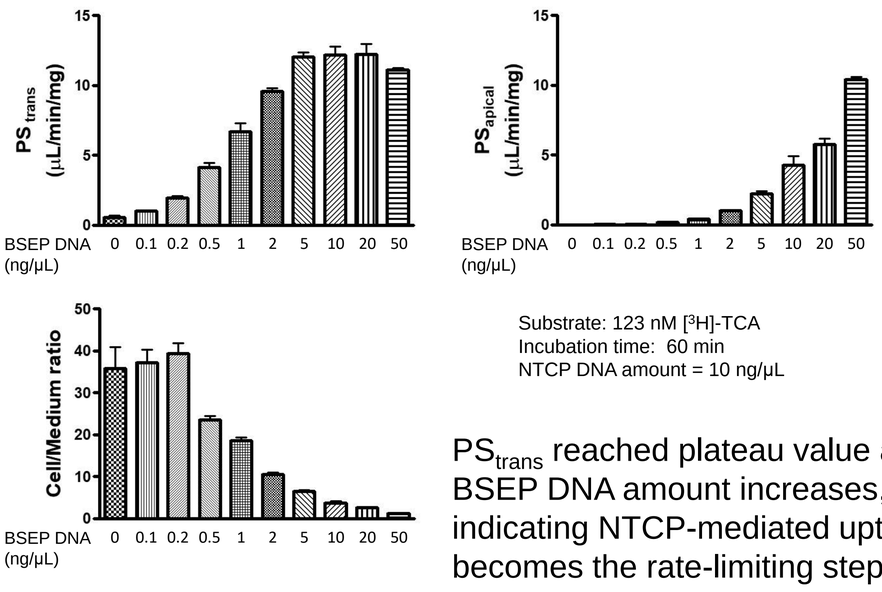


PS_{apical} = 1.94 µL/min/mg

MDCK II cells transfected with NTCP and BSEP DNA (10 and 5 ng/µL, respectively) yielded the apical intrinsic clearance similar to human hepatocytes (SCHH).

➡ Suggesting the BSEP expression levels were comparable between the two systems at the physiologically relevant levels.

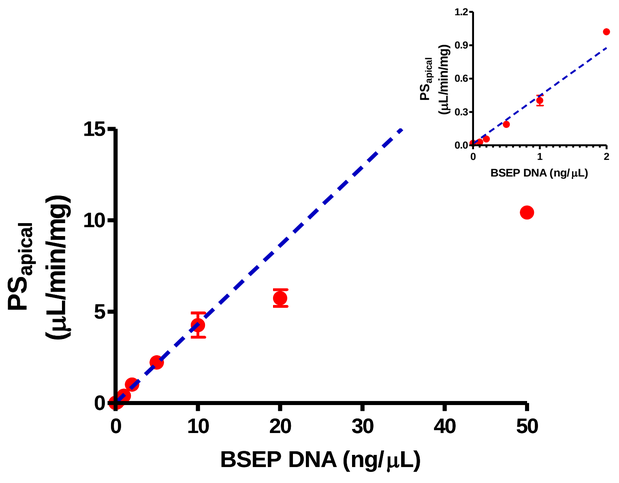
Fig.3. Effect of BSEP DNA change in the transcellular transport of TCA



Substrate: 123 nM [^{3}H]-TCA
Incubation time: 60 min
NTCP DNA amount = 10 ng/µL

PS_{trans} reached plateau value as BSEP DNA amount increases, indicating NTCP-mediated uptake becomes the rate-limiting step.

Fig.4. Correlation between the DNA amount used for the transfection and PS_{apical}



PS_{apical} exhibited linear correlation with amount of plasmid DNA used for transfections.

Conclusion

- With Opti-expression technology, the expression level of transporters is adjustable by changing plasmid DNA amount.
- Double transfected cells mimicking physiologically relevant expression level would reflect hepatocyte function. This model indicated that hepatic elimination of TCA should be determined by basolateral uptake intrinsic clearance.

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COI disclosure information

Authors: Sumito Ito, Masa Yasunaga, Yuichi Sugiyama

We have no financial relationship to disclose for our presentation contents.