Pathophysiological role of prostaglandin transporter OATP2A1/SLCO2A1 in pulmonary fibrosis

Takeo Nakanishi, Ph. D. Kanazawa University
Local Disposition of Prostaglandin (PG) E₂

Little is known about the role of transporters in inflammatory diseases.

AA; Arachidonic acid  
COX; Cyclooxygenase, PLA; Phopholipase, PGDH; 15-ketohydrorxyprostaglandin dehydrogenase, PTGES; PGE Synthase,
PG Metabolisms and Lung Diseases

- **PGE$_2$** is anti-fibrotic and has beneficial actions to down-regulate fibroblast metabolic functions in the lungs. [Cancheri et al, Trends Immunol 25:40, 2006]

- Digital clubbing is noted in idiopathic pulmonary fibrosis (IPF) and lung cancer, which are associated with serum levels of transforming growth factor (TGF-β1). [Schwartz et al, Textbook of respiratory medicine, 1994, Hirakata et al, Eur J Clin Invest 26:820, 1996]

- Loss-of-function mutations in **SLCO2A1** causes primary hypertrophic osteoarthropathy (HPO) and digital clubbing, associated with aberrant PG metabolism. [Seifert et al, Hum Mutat 33: 660, 2012]

**IPD; interphalangeal depth, DPD; distal phalangeal depth**
OATP2A1 is a PGE₂ Uptake Carrier

- Known as a member of organic anion transporting polypeptide family *(OATP2A1)* encoded by *SLCO2A1*.  

- Has been characterized an **influx transporter** for prostanoids (e.g. PGE₂, PGF₂α, and PGD₂) with a relatively **high affinity** for PGE₂ (e.g. Km = 20 ~ 90 nM).  
  [Schuster Annu. Rev. Physiol 60:221, 1998]

- Facilitates PGE₂ metabolism by cellular uptake of prostanoids.

- Exchanges a PG with an organic anion such as lactate.  
Expression of OATP2A1 in the Lungs

Objectives

- To clarify expression of functional OATP2A1 in the lungs
- To understand its pathophysiological significance in inflammation and pulmonary fibrosis.

These study may provide us with a clue to treat a refractory pulmonary fibrosis.
Contents

- Expression of Functional OATP2A1 in the Lungs (Physiological Condition)
- In Bleomycin (BLM)-induced Fibrosis (on Day 14)
- Under Acute Inflammatory Condition induced by BLM (on Day 5)
Expression of Oapt2a1 in Mouse Lungs (DAB Staining/Light Microscopic Analysis)

EC; Endothelial Cells. AT1/2: Type1/2 Alveolar Epithelial Cells

AT1 or AT2?

PLOS ONE, 10: e0123895, 2015
Expression of Oatp2a1 in Mouse Lungs (DAB Stain/Electron Microscopic Analysis)
Transdifferentiation of AT2 to AT1-like Cells

Rat Lung

On Day6

AT2

AT1-like

Type 2 (AT2) (Day 2)

Type 1 (AT1) (Day 6)

Type 1

Type 2

Pro-SPC; pro-surfactant protein C, a marker for AT2 cells.

[3H]PGE2 Uptake by Rat AT1-like Cells

37°C, pH 7.4
Time: 0.5, 1, 5, 10, 20 min

Mean ± S.E.M. (n = 3) *, p < 0.05 (vs. AEC Type 2)
mRNA Expression of Transporters That Recognize PGE$_2$ in Mouse Lungs

RT-PCR

Mrp4  Oct1  Oct2  Oat1  Oatp2a1  Oatp2b1  Oatp3a1  Oatp1a4  Oatp1a5
Establishment of Slco2a1 Global Knockout

**Conditional Slco2a1 KO Construct**

- **WT allele**
  - *Not1*
  - E1
  - *Sal1*
  - 4903
  - 15015

- **Flox allele**
  - E1
  - x CAG-Cre

- **KO allele**

Chang HY. et al., Circulation, (2010)

**Genotype (PCR)**

- 2a1<sup>-/-</sup>, Cre
- 2a1<sup>+/+</sup>, Cre
- 2a1<sup>Flox/-</sup>
- 2a1<sup>Flox/+</sup>

**Phenotype**

(Western Blot, Lung)

WT vs 2a1<sup>-/-</sup>

- Gapdh
  - 37 kDa
  - 25 kDa
PGE$_2$ Uptake by AT1-like Cells Derived from Slco2a1$^{-/-}$ Mice

Oatp2a1 in AT1-like Cells

WT

Slco2a1$^{-/-}$

PGE$_2$ Uptake

[³H]PGE$_2$: 1.5 nM (0.25 μCi/mL)
Time: 1 min
37°C, pH7.4

Mean ± S.E.M. (n=5, 4) *, p < 0.05 (vs. WT)

PLOS ONE, 10: e0123895, 2015
Expression of Microsomal PGE Synthase-1 (PTGES) in Mouse Lungs

Negative Control

Airway

Respiratory Zone

Alveolus

EC

AT2

B. Vessel

Alveolus

PLOS ONE, 10: e0123895, 2015
Expression of Prostaglandin Dehydrogenase (15-Pgdh) in Mouse Lungs

Negative Control

[Diagram showing the expression of Prostaglandin Dehydrogenase (15-Pgdh) in mouse lungs with labels for Alveolus, AT1, AT2, and EC.]

- Alveolus
- AT1
- AT2
- EC
- B. Vessel
- Scale bar: 40 μm
Summary – Role of OATP2A1 in the Lung under Physiological Condition

PGE₂ secreted from epithelium into Alveolus

OATP2A1 is expressed in vascular endothelium and alveolar epithelium.
OATP2A1 contributed to PGE₂ uptake predominantly in AT1-like cells.

Question:
Does OATP2A1 play a role in inflammation and progression of fibrosis in the lung?
Bleomycin (BLM)-induced Lung Fibrosis

BLM was intratracheally (i.t.) injected at 1 mg/kg in PBS to;

- WT or BLM
- or Slco2a1^-/

Body weight loss

Histological inspection (H&E stain)

Collagen disposition (Sirius Red stain)

mRNA Expression in fibrosis-related genes

PGE_2 disposition

Fibrosis was examined on Day 14 and inflammation on Day 5 by assessing

Fibrosis was examined on Day 14 and inflammation on Day 5 by assessing
Alteration in Body Weight of BLM-injected Mice

**Slco2a1**

- Weight Relative to Day 0 (%)
- Day
- Dose of BLM (1 mg/kg)
  - (Maximum tolerated dose = 2.2 mg/kg for 21 days)
- Loss Relative to Day 0 (%)
- Loss of Body Weight (%)
  (on Day 13)

(Died of fibrosis)

*WT* 2a1−/−
Alveoli Structure in Mice Injected with BLM (Histological Inspection/H&E Stain)

BLM or PBS (vehicle) was intratracheally injected at 1 mg/kg in PBS; H&E Stain on Day14

WT+PBS

Slco2a1^{-/-}+PBS

Alveolar septum became thicker.
Alveoli were collapsed in more respiratory zone.
Sirius Red Stain for Collagen Deposition in BLM-induced Lung Fibrosis

Mean value of 19 stained images (at least 4 mice/group)

WT  Slco2a1−/−

* PLOS ONE, 10: e0123895, 2015
Alteration in mRNA Expression of Fibrosis-related Genes between WT and Slco2a1-/- Mice

Protein Expression of Cox-2 and Pgdh in the Lungs (on Day 14)
Amount of PGE$_2$ in the Lung and BAL Fluid of BLM-injected WT and Slco2a1$^{-/-}$ Mice

Lung Tissue

BAL Fluid

PGE$_2$ (pg/mg tissue)

WT

Slco2a1$^{-/-}$

PGE$_2$ (pg/mouse)

WT

Slco2a1$^{-/-}$

BLM

Fibrosis

*
## Analysis of 48-Eicosanoids in BAL Fluid

<table>
<thead>
<tr>
<th>Compounds</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
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<tr>
<td>2,3-Dinor-8-iso PGF2α</td>
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<td>15-KET-LTB4</td>
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<td>23</td>
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<td>N.D.</td>
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<tr>
<td>PGE₂</td>
<td>11-HETE</td>
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<td>12</td>
<td>7</td>
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<tr>
<td>LTD₄</td>
<td>12-HETE</td>
<td>87</td>
<td>77</td>
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</table>

**Slco2a1⁻/⁻**

N.D. = not detected

PG: Prostaglandin, LT: Leukotriene, DHET: Dihydroxyeicosatrienoic acid, HETE: Hydroxyeicosatetraenoic acid
Summary - Effect of the Absence of Slco2a1 on BLM-induced Fibrosis

- Fibrosis became more severe in Slco2a1−/− mice.
- Fibrosis-related gene expression was increased in the lung of Slco2a1−/− mice.
- Only PGE2 levels were increased in the alveolar lumen.
Hypothesized Mechanism for Aggravation of Pulmonary Fibrosis in Slco2a1−/− Mice

Activated Inflammatory Cell (e.g. Alveolar Mφ)

AT2

IL-1β

IL-1β

IL-1β

IL-1R1

PGE2

PGE2

PGE2

PGE2

PGE2

PGE2

PGE2

PGE2

OATP2A1

15-keto PGE2

15-PGDH

PDGF

EMT

TGF-β1

Question:

Why was more IL-1β released in alveolar inflammatory cells in Slco2a1−/−?
OATP2A1 in PGE$_2$ Secretion from Peritoneal Macrophages (PMφ)

OATP2A1 Expression in PMφ

**PGE$_2$ Production (Total)**

**PGE$_2$ Secretion**

*WT* 2a1$^{-/-}$

*Oatp2a1$^{-/-}$*

Biochemical Pharmacol, 98:629-638, 2015
Hypothesized Role of OATP2A1 in PGE$_2$ Secretion from Peritoneal Macrophages

- Oatp2a1 was localized in the cytoplasmic domains.
- PGE$_2$ uptake by subcellular fraction including light lysosome (e.g. acidic compartment) was inhibited with OATP2A1 inhibitors.
- PGE$_2$ was released in a Ca$^{2+}$-dependent manner.

Biochemical Pharmacol, 98:629-638, 2015
Conclusion

- Loss of function of OATP2A1 may cause drug-induced pulmonary fibrosis by altering distribution of PGE$_2$ and aggravating inflammation, suggesting OATP2A1 protecting the lungs, suggesting OATP2A1 as a site of drug-induced pulmonary fibrosis

- Loss of function of OATP2A1 may affect pro-inflammatory cytokine release from inflammatory cells (e.g. macrophages); however, we NEED future study.
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