Treatment of Pulmonary Arterial Hypertension
Role of RhoA/Rho-kinase Pathway

S. Duong-Quy¹,²,³, Y. Bei¹,², Z. Liu², A.T. Dinh-Xuan¹

(Presented by S. Duong-Quy)

¹University Paris Descartes, Cochin Hospital-APHP, Paris, France
²University Tongji, Shanghai East Hospital, Shanghai, China
³Lam Dong Medical College, Viet Nam

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1. Overview
   Physiopathology of PAH

2. Overview
   Classification/Treatment of PAH

3. Rho-kinase
   Molecular structure/Mechanism

4. Rho-kinase inhibitors
   Role in PAH and Perspective
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Overview: Physiopathology of PAH

- **Hypoxic vasoconstriction**
- Imbalanced release of potent vasoactive mediators
  - NO, Prostacyclin
  - TxA2, ET-1

- **Respiratory disorders:**
  - High-altitude residence
  - COPD
  - Sleep apnea

Physiopathology of PAH

- Hypoxic vasoconstriction

Overview: Physiopathology of PAH

Overview: Physiopathology of PAH

Endothelial Cell

Smoth Muscle Cell

Hyperpolarisation

Relaxation

S. Duong-Quy, et al. Rev Mal Respir 2010 (souspress)
Overview: Physiopathology of PAH (5)

S. Duong-Quy, et al. Rev Mal Respir 2010 (souspress)

GMPc: cyclin guanosine monophosphate
Overview: Physiopathology of PAH

- Decrease of a cross-sectional area of pulmonary vascular bed
  
- Lung parenchyma disease: systemic sclerosis

- Thromboembolic obstruction of proximal or distal PA

- Volume and pressure overload from cardio-vascular disorders
  
- Atrial or ventricular septal defect

- Mitral valvulopathy, cardiomyopathie, PVO disease


Overview: Vascular changes in PAH

- Hypertrophy of media
- Arteriopathy plexiform
- Arteriopathy thrombotic

Overview: Vascular changes in PAH

Hypertrophy of media
Arteriopathy plexiform
Arteriopathy thrombotic
PAH in COPD

1. Overview
   Pathophysiology of PAH

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Overview: New Classification of PAH

Table 4  Updated clinical classification of pulmonary hypertension (Dana Point, 2008¹)

1  Pulmonary arterial hypertension (PAH)
   1.1  Idiopathic
   1.2  Heritable
      1.2.1  BMPR2
      1.2.2  ALK1, endoglin (with or without hereditary haemorrhagic telangiectasia)
      1.2.3  Unknown
   1.3  Drugs and toxins induced
   1.4  Associated with (APAH)
      1.4.1  Connective tissue diseases
      1.4.2  HIV infection
      1.4.3  Portal hypertension
      1.4.4  Congenital heart disease
      1.4.5  Schistosomiasis
      1.4.6  Chronic haemolytic anaemia
   1.5  Persistent pulmonary hypertension of the newborn

1' Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis

2  Pulmonary hypertension due to left heart disease
   2.1  Systolic dysfunction
   2.2  Diastolic dysfunction
   2.3  Valvular disease

3  Pulmonary hypertension due to lung diseases and/or hypoxia
   3.1  Chronic obstructive pulmonary disease
   3.2  Interstitial lung disease
   3.3  Other pulmonary diseases with mixed restrictive and obstructive pattern
   3.4  Sleep-disordered breathing
   3.5  Alveolar hypoventilation disorders
   3.6  Chronic exposure to high altitude
   3.7  Developmental abnormalities

4  Chronic thromboembolic pulmonary hypertension

5  PH with unclear and/or multifactorial mechanisms
   5.1  Haematological disorders: myeloproliferative disorders, splenectomy.
   5.2  Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis
   5.3  Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
   5.4  Others: tumoural obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

ALK-1 = activin receptor-like kinase 1 gene; APAH = associated pulmonary arterial hypertension; BMPR2 = bone morphogenetic protein receptor, type 2; HIV = human immunodeficiency virus; PAH = pulmonary arterial hypertension.
Overview: Treatment of PAH

Plan

Overview: Treatment of PAH

Avoid pregnancy (I-C)
- Influenza and pneumococcal immunization (I-C)
- Supervised rehabilitation (IIa-B)
- Psycho-social support (IIa-C)
- Avoid excessive physical activity (III-C)

General measures and supportive therapy
- Expert Referral (I-C)

Diuretics (I-C)
- Oxygen* (I-C)
- Oral anticoagulants:
  - IPAH, heritable PAH and PAH due to anorexigens (IIa-C)
  - APAH (IIb-C)
  - Digoxin (IIb-C)

Acute vasoreactivity test
- (I-C for IPAH)
- (IIb-C for APAH)

Vasoreactive

Nonvasoreactive

Who-FC I-III
- CCB (I-C)

Sustained response (Who-FC I-II)

Yes
- Continue CCB

No

Initial Therapy

<table>
<thead>
<tr>
<th>Recommendation-Evidence</th>
<th>WHO-FC I</th>
<th>WHO-FC II</th>
<th>WHO-FC III</th>
<th>WHO-FC IV</th>
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<tbody>
<tr>
<td>I-A</td>
<td>Ambrisentan, Bosentan, Sildenafil</td>
<td>Ambrisentan, Bosentan, Sitaxentan, Sildenafil, Epoprostenol i.v., Iloprost inhaled</td>
<td>Iloprost i.v., Teprostatin i.v.</td>
<td>Epoprostenol i.v.</td>
</tr>
<tr>
<td>I-B</td>
<td>Tadalafil†</td>
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<td>IIa-C</td>
<td>Sitaxentan</td>
<td>Iloprost i.v., Teprostatin i.v.</td>
<td>Ambrisentan, Bosentan, Sitaxentan, Sildenafil, Tadalafil†, Iloprost inhaled, and i.v. Treprostatin s.c., i.v., Inhaled†, Initial Combination Therapy</td>
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doi:10.1093/eurheartj/ehp297
Overview: Treatment of PAH (2)

**INITIAL THERAPY**

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<td>IIb-B</td>
<td>Beraprost</td>
<td></td>
<td></td>
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</table>

**INADEQUATE CLINICAL RESPONSE**

- Sequential combination therapy (IIa-B) §
  - ERA
  - Prostanoids +
  - PDE-5 I

**BAS (I-C) and/or Lung transplantation (I-C)**

*European Heart Journal (2009) 30, 2493–2537*
Overview: Treatment of PAH (3)

Overview: New Treatment for PAH

EGF : epidermal growth factor
ERK : extracellular signal regulated kinase

S. Duong-Quy, et al. RMR 2008

Long-term Use of Imatinib in Patients with Severe PH

Phase III Clinical Trials : TRIUMPH

EGF : epidermal growth factor
ERK : extracellular signal regulated kinase

S. Duong-Quy, et al. RMR 2008
Overview: New Treatment for PAH (5)

- Ach

Endothelial Cell

Donnor of NO

SNP

Oxydant Stress

Superoxyde Anion

Peroxynitrite

Stimulators and activators of sGC

BAY 41-2272
Stimulators

BAY 58-2667
Activators

Donnor of NO

Ach

eNOS

Fe²⁺

Fe³⁺

sGC Reduced

sGC Oxydized

cGMP

Relaxation

GTP

ODQ (5 µM)

Oxydant Stress

(Diabetes, Hypercholesterolemia,…)
Overview: New Treatment for PAH (5)


**Stimulators and activators of sGC**

- **BAY 41-2272**
  - Stimulators
- **BAY 58-2667**
  - Activators

**Oxydant Stress**
- (Diabetes, Hypercholesterolemia, ...?)

**Relaxation**

**Endothelial Cell**
- *Ach*
- eNOS
- Superoxide Anion
- Peroxynitrite
- Oxydant Stress

**Smooth Muscle Cell**
- sGC Reduced
- cGK
- Relaxation
- sGC Oxydized
- GTP

**Donnor of NO**
- Ach
- SNP
- ODQ (5 µM)
- Oxydant Stress

**Activators**
- BAY 58-2667
- BAY 41-2272

**Stimulators**
- Ach
- SNP
- ODQ (5 µM)
Overview: New Treatment for PAH

MLCK: myosin light chaine kinase; MLCP: myosne light chaine phosphatase

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Rho-kinase: Molecular Structure

ROCK-1
1 76 338
Kinase
RBD
Phosphorylation Domain (PH)
Coiled-coil region
CRD
Cystein-rich Domain (CRD)

ROCK-2
1 92 354
Kinase
RBD
Phosphorylation Domain (PH)
Coiled-coil region
CRD
Cystein-rich Domain (CRD)

RBD: Rho-binding domain; PH: Pleckstrin-homology domain; CRD: Cystein-rich domain.
**Rho-kinase: Autoinhibition (2)**

RBD: Rho-binding domain; PH: Pleckstrin-homology domain; CRD: cysteine-rich domain

Kinase

ROCKs

RBD  PH  CRD

Coiled-coil region

RBD: Rho-binding domain; PH: Pleckstrin-homology domain; CRD: cysteine-rich domain
Rho-kinase: Positive regulation

RBD: Rho-binding domain; PH: Pleckstrin-homology; CRD: cystein-rich domain

ROCKs

RhoA

GTP

Coiled-coil region

AA: Arachidonic acid; SP: Sphingosine phosphoryl choline

RBD: Rho-binding domain; PH: Pleckstrin-homology; CRD: cystein-rich domain
Rho-kinase: Vascular pathology (4)

Agonistes (Ang II, ET-1, 5-HT, PDGF, Thrombine, NE, Uro II...)

Rho-kinase

Endothélium Intima
Dysfonction endothéliale
↓ production du NO
↑ Migration des cellules inflammatoires

 Média / CML
Changement du phénotype
↓ Apoptose
↑ Prolifération
↑ Migration
↑ sensibilisation au Ca++

Adventice
↑ Migration des cellules inflammatoires
↑ vasavasorum

Thrombose

Remodelage vasculaire

CML Hyperconstriction

↑ RVP, ↑ PAP, ↑ Lésions vasculaires

Hypertension pulmonaire
Rho-kinase: VSMC contractility

Active form GTPase-RhoA

Increased Rho-kinases expression and activity and pulmonary endothelial dysfunction in smokers with normal lung function

S. Duong-Quy, P. Dao, T. Hua-Huy, C. Guilley, Pierre Pascual and A. T. Dinh-Xuan
Rho-kinase Inhibitors and PAH (7)

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Role of Rho-kinase in PAH (1)

- **In Animal Model**
  - Principally studied in hypoxia-induced PAH
  - Monocrotaline-induced PAH
  - Severe occlusive PAH

- **In Human**
  - RhoA/Rho-kinase activation in human PAH:
    - Role of 5HT
Fasudil improves survival of rats with MCT-PAH

Abe K, et al. Cir Res 2004
Fasudil suppresses medial thickening in rats with MCT-induced PH

Abe K, et al. Cir Res 2004
Intravenous administration of fasudil decreased PAP (left panel) & increased CI (middle panel)

Fukumoto Y, et al. Heart 2005
Beneficial Acute Effects of Rho-Kinase Inhibitor in Patients With PAH

SAP: systolic systemic blood pressure, SVR: systemic vascular resistance, mPAP: mean pulmonary arterial pressure; TPR: total pulmonary resistance

Inhaled Rho Kinase Inhibitors are Potent and Selective Vasodilator in Rat with PAH

Nagaoka T, et al. Heart Vessels 2010
Acute vasodilator effects of inhaled Fasudil in patients with PAH

15 patients:

- 5 iPAH, 6 PAH/CID, 3 PAH/CHD, 1 PoPAH
- PAP: NO: $P < 0.01$, fasudil: $P < 0.05$
- PVR: NO: $P = 0.07$, fasudil: $P = 0.1$

- Inhaled NO and Fasudil: selectively affect lung tissues
- No correlation in vasodilator effects: NO-Fasudil
  - Inhaled Fasudil: effective as NO in PAH
  - Possibly through different mechanisms

Fujita H, et al. Heart Vessels 2010

iPAH: idiopathic PAH; CTD: connective tissue disease; CHD: congenital heart disease; PoPAH: portal PAH
Fasudil reduces MCT-PAH: Comparison with Bosentan and Sildenafil

Table 1 - Effects of fasudil (FAS) and its combinations with bosentan (BOS) and sildenafil (SIL) on cardiac function, haemodynamics, pulmonary arterial wall remodelling and right ventricular (RV) hypertrophy

<table>
<thead>
<tr>
<th></th>
<th>FAS</th>
<th>FAS+BOS</th>
<th>FAS+SIL</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_c$ beats·min⁻¹</td>
<td>400±31</td>
<td>352±10</td>
<td>371±8</td>
<td>0.392</td>
</tr>
<tr>
<td>Stroke volume mL</td>
<td>0.35±0.02</td>
<td>0.36±0.01</td>
<td>0.36±0.02</td>
<td>0.746</td>
</tr>
<tr>
<td>Cardiac output mL·min⁻¹</td>
<td>144±11</td>
<td>131±6</td>
<td>130±6</td>
<td>0.434</td>
</tr>
<tr>
<td>RVEDD mm</td>
<td>3.28±0.23</td>
<td>2.88±0.20</td>
<td>2.66±0.14</td>
<td>0.096</td>
</tr>
<tr>
<td>RVESD mm</td>
<td>1.96±0.25</td>
<td>1.48±0.22</td>
<td>1.28±0.19</td>
<td>0.168</td>
</tr>
<tr>
<td>RVFS %</td>
<td>42.4±4.2</td>
<td>49.8±4.7</td>
<td>45.1±6.5</td>
<td>0.576</td>
</tr>
<tr>
<td>TAPSE mm</td>
<td>3.03±0.13</td>
<td>3.38±0.19</td>
<td>3.47±0.14</td>
<td>0.126</td>
</tr>
<tr>
<td>RVSP mmHg</td>
<td>43±4</td>
<td>46±5</td>
<td>37±5</td>
<td>0.444</td>
</tr>
<tr>
<td>$P_{pa}$ mmHg</td>
<td>27.8±2.9</td>
<td>30.0±2.8</td>
<td>24.8±2.8</td>
<td>0.452</td>
</tr>
<tr>
<td>PVR mmHg·mL⁻¹·min⁻¹</td>
<td>0.29±0.15</td>
<td>0.32±0.03</td>
<td>0.29±0.05</td>
<td>0.890</td>
</tr>
<tr>
<td>PAWt ±</td>
<td>94±4</td>
<td>102±4</td>
<td>105±6</td>
<td>0.200</td>
</tr>
</tbody>
</table>

Conclusion and perspective

- Acute effect of Fasudil has been confirmed
- Long-term administration in patients with severe PAH is absolutely required
- Clinical trials suggested that oral Fasudil is well tolerated without any serious adverse reactions
- Large clinical studies in patients with PAH are expected to conclude on the effectiveness of Fasudil
Plan

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Thank You For Your Attention