The Renaissance Seminar Series in the Graduate School of Medicine Educational Program

The 1st International Workshop of Pulmonary Hypertension in Tohoku University

PROGRAM
&
ABSTRACTS

March 19 (Wed), 2008

Ark Hotel Sendai

Sendai, Miyagi, Japan

Hiroaki Shimokawa, M.D., Ph.D.
President

Presidential Greeting

It is my great pleasure to organize the First Pulmonary Hypertension International Workshop of Tohoku University.

Tohoku University Graduate School of Medicine has been successfully granted by the Japanese Ministry of Education, Science, Sports, Culture and Technology for the advanced education program of the graduate school from 2007-2009. Our International Workshop is the first in The Renaissance Seminar Series in the Educational Program of Tohoku University Graduate School of Medicine.

Pulmonary hypertension still remains a fatal disorder in the world including Japan, for which effective treatments need to be developed based on the elucidation of the pathophysiology of the disorder. Tohoku University is one of the top medical institutes with a long history of research and practice of pulmonary hypertension and indeed one of the two universities where both pulmonary and cardiac transplantation are approved in Japan.

In this International Workshop, three distinguished researchers will be invited from overseas, including Dr. Oka (University of Colorado), and Drs. Gebb and McMurtry (University of South Alabama) and five invited Japanese researchers will also present their most up-dated research findings on pulmonary hypertension. In addition, we will have poster sessions for graduate students with young investigator award competition.

I sincerely hope that this International Workshop helps young graduate students promote their research on vascular biology in general and pulmonary hypertension in particular.

Hiroaki Shimokawa, M.D., Ph.D.
Professor and Chairman
Department of Cardiovascular Medicine
Tohoku University Graduate School of Medicine

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(TUBERO), Sendai, Japan

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Chairperson

Hiroaki Shimokawa

Departments of Cardiovascular Medicine, Tohoku University

Graduate School of Medicine, Sendai, Japan

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Ivan F McMurtry

Department of Pharmacology and Center for Lung Biology
University of South Alabama, Mobile, Alabama, USA

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Hiroaki Shimokawa

Departments of Cardiovascular Medicine, Tohoku University
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Plenary Session 1

Rho kinase-mediated vasoconstriction in rat models of pulmonary hypertension

Masahiko Oka

Cardiovascular Pulmonary Research Laboratory, Department of Medicine, University of Colorado at Denver and Health Sciences Center, Denver, CO, USA

Although it is widely accepted that over time in the development of pulmonary hypertension (PH), the hypertensive component due to vasoconstriction decreases, while that due to vascular remodeling increases, the exact contribution of vasoconstriction in advanced stages of PH is unclear. Results of acute vasodilator testing using conventional agents in patients with severe PH suggest there is only a minor contribution of vasoconstriction. However, there is a possibility that these results may underestimate the contribution of vasoconstriction, because the most effective vasodilators have not yet been identified and tested.

Vascular smooth muscle tone is determined by the balance in activities of myosin light chain (MLC) kinase (contraction) and MLC phosphatase (relaxation). Inhibition of MLC phosphatase promotes MLC phosphorylation and contraction at a constant or decreasing cytosolic [Ca^{2+}], i.e., Ca^{2+} sensitization. Recent evidence indicates that while Ca^{2+}/calmodulin-dependent MLC kinase-mediated MLC phosphorylation is more important for triggering vascular smooth muscle cell contraction, Ca^{2+} sensitization is important for the sustained phase of contraction.

The small GTPase RhoA, a member of the Rho family of small GTP-binding proteins, and its downstream effector, Rho kinase (ROCK), play a major role in the regulation of MLC phosphatase activity. ROCK inhibits MLC phosphatase by phosphorylating its regulatory subunit MYPT1, and thereby induces Ca^{2+} sensitization. RhoA is activated by various G protein-coupled receptor (GPCR) agonists (vasoconstrictors), including endothelin-1 (ET-1) and serotonin (5-HT). Thus, RhoA/Rho kinase-mediated Ca^{2+} sensitization is thought to be a major component in the sustained vasoconstriction induced by GPCR agonists. In fact, selective ROCK inhibitors, such as Y-27632 and fasudil, effectively reverse the sustained vasoconstriction induced by many agonists, and are now regarded as a novel class of potent vasodilators with multiple other actions.

We have observed that ROCK inhibitors acutely and markedly reduce the high pulmonary artery pressure of rat PH models with occlusive neointimal lesions, suggesting that vasoconstriction can significantly be involved in PH with severely remodeled pulmonary vessels. While when administered systemically ROCK inhibitors are not pulmonary selective vasodilators, inhaled ROCK inhibitors reduce high pulmonary artery pressure of PH models with minimal systemic effects. We therefore propose that inhaled ROCK inhibitors could be useful for the chronic treatment as well as acute vasodilator testing of severe PH.

Memo

Plenary Session 2

Postnatal alveolarization is regulated through RhoA/ROCK suppression and deposition of the extracellular matrix protein tenascin-C (TN-C)

Sarah A. Gebb, Eiko Sako, Tetsu Nagaoka, Masahiko Oka, Jonathan Kaufman, Ivan F. McMurtry

Department of Cell Biology and Neuroscience and the Center for Lung Biology, University of South Alabama, Mobile, Alabama, USA

Alveolarization of the post-natal lung is dependent upon precise temporal-spatial regulation of growth factors, cell signaling cascades, and cell specific environmental niches. The extracellular matrix (ECM) provides a dynamic cellular environment that can regulate inter- and intra-cellular signaling in the developing lung. The Fawn-hooded rat (FHR) develops severe pulmonary hypertension and this is associated with marked lung hypoplasia. ECM glycoprotein tenascin-C (TN-C) is decreased in FHR lungs bearing alveolarization defects. RhoA/ROCK activity regulates TN-C expression, accordingly we showed that higher RhoA activity coincided with reduced TN-C expression in alveolarization-defective FHR lungs. To test the hypothesis that suppression of RhoA/ROCK signaling is required for TN-C deposition and alveolarization, FH rats were treated with Fasudil, an orally available, specific ROCK inhibitor. ROCK inhibition prevented the development of severe pulmonary hypertension, restored TN-C and partially rescued the alveolarization defect. Collectively, this study demonstrates a novel role of TN-C in regulating lung development, and reveals a new function for RhoA/ROCK signaling in mediating this effect.

Memo

Oral Session 1

Smooth muscle Calponin, a crucial modulator of BMPR2 signaling that may have roles in the development of IPAH

Hiroyuki Kumagai

Tohoku University Biomedical Engineering Research Organization (TUBERO), Sendai, Japan

Idiopathic pulmonary arterial hypertension (IPAH) is a life-threatening disease characterized by pulmonary vasoconstriction and vascular remodeling, eventually leading to right heart failure due to pulmonary hypertension. Recent studies have provided important clues to the molecular events underlying this disorder. Loss-of-function mutations have been identified in the type II receptor for bone morphogenetic protein (BMPR2) in both familial and sporadic cases with IPAH. Reciprocally, elevated right ventricular pressures have been reported in mice genetically engineered to lose BMPR2 function in pulmonary vasculature. These observations point to a crucial and essential role for the BMP pathway in the pathogenesis of IPAH.

BMP signaling is mediated through multiple intracellular transducers, with Smad1/5/8 serving as specific nuclear transmitters. Upon ligand binding, the BMP receptor phosphorylates these Smads, allowing each of them to associate with a universal transducer, Smad4. The resultant complexes undergo nuclear translocation, thereby converting extracellular stimuli into alterations in gene expression.

Through a quest for the mechanism of arteriosclerotic calcification, we have identified a protein that serves as a cytoplasmic anchor for Smad1. Smooth muscle calponin, which is absent from arteriosclerotic lesions, bound exclusively to the unphosphorylated form of Smad1. This protein prevented nuclear localization of the Smad1-Smad4 complex that formed in the absence of extracellular stimuli. Upon BMP signaling, most of phosphorylated Smad1 was released from calponin, suggesting that calponin serves primarily to block premature activation of the BMP pathway.

Given the clear cause-effect relationship between attenuated BMP signaling and IPAH,

it is plausible to assume the presence of as yet unidentified etiologic factors that act by changing calponin expression.

Memo

Oral Session 2

Osteopontin could be a novel target for the treatment of pulmonary arterial hypertension

Yasushi Hoshikawa

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Rationale: Pulmonary arterial hypertension (PAH) is characterized by the formation of plexiform lesions composed of altered endothelial cells and by proliferation of smooth muscle cells in the mid- to small-sized pulmonary arteries. Pathogenesis of these lesions is still not fully understood. Chronic hypoxia causes pulmonary hypertension and pulmonary vascular remodeling in humans and most animals. However, the degree of the structural alterations of the lung vessels varies between species. For example, mice develop much less pulmonary vessel wall thickening after chronic hypoxia exposure than rats. Microarray gene analysis demonstrated that lung gene encoding osteopontin (OPN), a soluble secreted phosphoprotein, was significantly elevated in hypoxic rats, but was unchanged in hypoxic mice. Since OPN enhances proliferation of vascular smooth muscle cells and migration of vascular endothelial cells, we hypothesized that OPN was responsible for lung vascular remodeling and played a role in the pathogenesis of PAH. To test this hypothesis, we first measured OPN expression in blood and lung tissue from idiopathic PAH (IPAH) patients. We then examined the effects of OPN gene overexpression on pulmonary hypertension and pulmonary vascular remodeling in mice. We also tried to pharmacologically modulate hypoxia-induced OPN overexpression and pulmonary vascular remodeling in rats.

Methods and Results: 1. Plasma OPN levels were significantly higher in six of fourteen IPAH patients than in nine non-PAH volunteers. The response to PGI2 infusion therapy, assessed by alterations in pulmonary vascular resistance, was significantly less in the six IPAH patients with higher plasma OPN levels than in the other eight IPAH patients with normal OPN levels. Two of three subjects with IPAH undergoing lung transplantation showed significantly higher lung OPN gene expression than did three age-matched controls. 2. OPN transgenic (Tg) and wild-type (WT) mice

were kept in normoxia (sea level) or exposed to hypobaric hypoxia (17,000 ft.) for 3 weeks. Then pulmonary hypertension and pulmonary vascular remodeling were assessed. At sea level, the OPN Tg and WT mice did not differ in right ventricular systolic pressure (RVSP) and wall thickness of the mid-sized pulmonary arteries (PAWT). Following chronic hypoxia, OPN Tg mice showed significantly higher RVSP and PAWT compared to WT animals. 3. Chronically hypoxia-exposed rats were treated with a peroxisome proliferator-activated receptor (PPAR) gamma ligand, pioglitazone, which has been reported to inhibit OPN gene expression in vitro, and whole lung OPN mRNA expression and pulmonary vascular remodeling were assessed. Lung OPN gene expression was increased in non-treated hypoxia-exposed rats compared to that of normoxic controls. Pioglitazone reduced both lung OPN gene expression and pulmonary vascular thickening in hypoxic rats.

Conclusions: These findings indicate that OPN may be responsible for pulmonary vascular remodeling and could be a novel target for the treatment of IPAH.

Memo

Oral Session 3

Rho-Kinase Is a Novel Therapeutic Target in Pulmonary Arterial Hypertension

Yoshihiro Fukumoto, Hiroaki Shimokawa

Departments of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Pulmonary arterial hypertension (PAH) is a disease with poor prognosis characterized by progressive elevation of pulmonary arterial pressure and vascular resistance due to pulmonary artery hyperconstriction and remodeling. Although anticoagulant agents, vasodilators (e.g. prostaglandins, sildenafil, and bosentan), and lung transplantation are currently used for the treatment of PAH, more effective treatment for the disorder needs to be developed.

Rho-kinase causes vascular smooth muscle hyperconstriction through inhibition of myosin phosphatase and vascular remodeling through activation of its downstream effectors. We have performed a series of experimental and clinical studies, demonstrating that Rho-kinase-mediated pathway plays an important role in various cellular functions, not only in vascular smooth muscle hyperconstriction but also in actin cytoskeleton organization, cell adhesion and motility, cytokinesis, and gene expressions, all of which could be involved in the pathogenesis of arteriosclerosis and vascular remodeling.

We also have recently demonstrated that Rho-kinase is activated in animal models of PH with different etiologies (monocrotaline and chronic hypoxia) associated with enhanced pulmonary vasoconstricting and proliferating responses, impaired endothelial vasodilator functions, and pulmonary remodeling. Importantly, long-term oral treatment with fasudil, an inhibitor of Rho-kinase, markedly ameliorated PH and pulmonary remodeling in those animal models of PAH. We have subsequently demonstrated that intravenous fasudil exerts acute and effective pulmonary vasodilator effects in patients with severe PAH, who were refractory to inhalation of NO or O_2 or to calcium channel blockers. Taken together, our findings indicate that Rho-kinase is an important therapeutic target in PAH in humans and that Rho-kinase inhibitors are a promising new class of drugs for the fatal disorder.

Memo

Meet the Expert 1

A Novel Long-Acting Prostacyclin Agonist with Thromboxane Inhibitory Activity for the Treatment of Pulmonary Hypertension

Atsuhiro Nakamura[1,2], Yoshiki Sakai[3], Noritoshi Nagaya[1,4], Hiroshi Kimura[2]

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The balance between prostacyclin and thromboxane plays an important role in the regulation of pulmonary vascular tone. Although prostacyclin is recognized as a therapeutic breakthrough for pulmonary hypertension, it needs continuous infusion because of its short action. Therefore we developed ONO-1301, a novel long-acting prostacyclin agonist with thromboxane synthase inhibitory activity.

As a model of pulmonary hypertension, rats were given a subcutaneous injection of either monocrotaline. There was significant development of pulmonary hypertension 3 wk after monocrotaline injection. Repeated subcutaneous injection of ONO-1301 twice per day for 3 wk significantly attenuated the increases in right ventricular systolic pressure, ratio of right ventricular weight to body weight and the increase in medial wall thickness of peripheral pulmonary arteries in monocrotaline rats. ONO-1301 improved survival rate in monocrotaline rats compared with vehicle administration. Next, we prepared a novel sustained-release prostacyclin analog polymerized with poly (D, L-lactic-co-glycolic acid) (PLGA) microspheres. Interestingly, a single injection of ONO-1301 MS attenuated monocrotaline-induced pulmonary hypertension and improved survival in rats. Finally, we assessed the effect of oral administration. Orally administered ONO-1301 improved survival in monocrotaline rats. Phosphorylation of extracellular signal-regulated protein kinase (ERK) in the lung was significantly increased in the control group, whereas this increase was markedly attenuated by the treatment.

A novel prostacyclin agonist (ONO-1301) markedly attenuated monocrotaline-induced

pulmonary hypertension and improved survival in rats. A novel long-acting prostacyclin agonist with thromboxane synthase inhibitory activity, ONO-1301 may be a promising compound for the treatment of the pulmonary hypertension.

Memo

Meet the Expert 2

Anti-inflammatory Therapy for Pulmonary Arterial Hypertension in Rats

Masafumi Takahashi[1], Takayuki Ito[2], Keiya Ozawa[2], and Uichi Ikeda[1]

[1]Department of Cardiovascular Medicine, Shinshu University Graduate School of Medicine, Matsumoto, [2]Division of Genetic Therapeutics, Jichi Medical University, Tochigi, Japan

Pulmonary arterial hypertension (PAH) is an intractable disease that leads to increased pulmonary arterial (PA) pressure, progressive right ventricular (RV) hypertrophy, and premature death; however, no satisfactory treatment for PAH has been established. Since accumulating evidence suggests important roles of vascular inflammation in the pathogenesis of PAH, we examined 2 types of anti-inflammatory therapies in a rat model of monocrotaline (MCT) induced-PAH. (1) Immunosupressive treatment with mycophenolate mofetil (MMF): MMF treatment decreased RV systolic pressure and RV hypertrophy, and reduced the medial hypertrophy of PA. MMF also prevented macrophage infiltration, the number of proliferating cell nuclear antigen (PCNA)-positive cells, and expression of P-selectin and interleukin (IL)-6 on the endothelium of PA. *In vitro* experiments revealed that mycophenolic acid (MPA), an active metabolite of MMF, inhibited proliferation of human pulmonary arterial vascular smooth muscle cells. (2) Gene therapy with anti-inflammatory cytokine IL-10: Rats were intramuscularly transduced with an adeno-associated virus (AAV) vector expressing IL-10, followed by MCT injection. The IL-10-transduced rats showed increased serum IL-10 levels, compared to the control rats. Systemic IL-10 expression prevented increased PA pressure, RV hypertrophy, the medial hypertrophy of PA, macrophage infiltration, and the pulmonary tissue levels of TGFbeta1 and IL-6. In particular, the serum IL-10 concentration negatively correlated with PA pressure and RV hypertrophy. These findings suggest that anti-inflammatory therapy is effective for

the development of PAH and provide a new insight into the potential role of inflammation in this disease.

Memo

Special Lecture

Animal Models of Pulmonary Arterial Hypertension (PAH)

Ivan F McMurtry

Department of Pharmacology and Center for Lung Biology
University of South Alabama, Mobile, Alabama, USA

A recent meta-analysis of clinical trials of prostacyclin analogs, endothelin receptor antagonists, and phosphodiesterase type 5 inhibitors in severe PAH shows that although the pharmacological agents moderately improve symptoms and hemodynamic parameters, none significantly reduces mortality. These disappointing results are not predicted by animal model studies, which show these classes of drugs largely prevent, and in some cases reverse, chronic hypoxia- and monocrotaline-induced pulmonary hypertension in rats. The limitations of using chronically hypoxic or monocrotaline-injected rats as models of human PAH have been previously noted. It is apparent that preventing or reversing sustained constriction and increased muscularization of pulmonary arteries in these rodent models is not equivalent to "dissolving" obstructive neointimal and other complex vascular lesions that seemingly account for the high pulmonary vascular resistance in human severe PAH. Investigators evaluating new therapies for PAH should consider using more recent rodent models of neointimal lesion-associated pulmonary hypertension, rather than the classic chronically hypoxic and monocrotaline-injected models. At least six different rodent models of distal pulmonary artery neointimal lesion formation have been described. These include: left pneumonectomized plus monocrotaline-injected rats, VEGF receptor blocker (Sugen 5416)-injected plus chronic hypoxia-exposed rats, Sugen 5416-injected athymic nude rats, chronically hypoxic athymic nude rats, monocrotaline-injected endothelin B receptor-deficient rats, and S100A4/Mts1 protein overexpressing mice infected with M1-gammaherpesvirus 68. These models develop pulmonary hypertension accompanied by formation of obstructive cellular lesions in the lumen of small pulmonary arteries and arterioles, in addition to increased muscularization of pulmonary arteries. The proliferative neointimal lesions are variously reported to comprise phenotypically abnormal smooth muscle cells, endothelial cells, or cells expressing both endothelial and smooth muscle cell markers. In some of the models, the lesions are

considered to resemble the plexogenic arteriopathy of human PAH. Although it is not yet clear how closely any of these neointimal models mimic the cellular and molecular pathobiology of human PAH, it is likely they will provide insights into pathological molecular signaling pathways and potentially efficacious therapies that would not be revealed or rigorously tested in the classic chronically hypoxic and monocrotaline-injected models.

Memo

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