Tohoku J. Exp. Med., 2007, **211**, 309-320

Invited Review

Recent Progress in the Treatment of Pulmonary Arterial Hypertension: Expectation for Rho-Kinase Inhibitors

YOSHIHIRO FUKUMOTO,[1] SHUNSUKE TAWARA[1] and HIROAKI SHIMOKAWA[1,2]

[1]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
[2]Technology Agency, CREST, Tokyo, Japan

FUKUMOTO, Y., TAWARA, S. and SHIMOKAWA, H. *Recent Progress in the Treatment of Pulmonary Arterial Hypertension: Expectation for Rho-Kinase Inhibitors*. Tohoku J. Exp. Med., 2007, **211** (4), 309-320 —— 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. However, the precise mechanism of PAH still remains to be elucidated. Although anticoagulant agents, vasodilators (e.g., prostaglandins, sildenafil, and bosentan), and lung transplantation are currently used for the treatment of PAH, more effective treatment needs to be developed. Rho-kinase causes vascular smooth muscle hyperconstriction and vascular remodeling through inhibition of myosin phosphatase and activation of its downstream effectors. In a series of experimental and clinical studies, we have demonstrated 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 expression, all of which may be involved in the pathogenesis of arteriosclerosis. We also have recently demonstrated that Rho-kinase is activated in animal models of PAH with different etiologies (monocrotaline and chronic hypoxia) associated with enhanced pulmonary vasoconstricting and proliferating responses, impaired endothelial vasodilator functions, and pulmonary remodeling. Indeed, we were able to demonstrate that intravenous fasudil, a selective Rho-kinase inhibitor, exerts acute pulmonary vasodilator effects in patients with severe PAH who were refractory to conventional therapies. Taken together, our findings indicate that Rho-kinase is a novel and important therapeutic target of PAH in humans and that Rho-kinase inhibitors are a promising new class of drugs for the fatal disorder. ———— pulmonary arterial hypertension; pulmonary arteriosclerosis; pulmonary arterial hyperconstriction; Rho-kinase

Pulmonary hypertension (PH) is defined as a mean pulmonary arterial pressure greater than 25 mmHg at rest or greater than 30 mmHg during exercise (Barst et al. 2004). Pulmonary arterial hypertension (PAH) is characterized by progressive elevation of pulmonary artery pressure and vascular resistance with poor prognosis (Barst et al. 2004). In 2003, World Health Organization

Received March 2, 2007; revision accepted for publication March 5, 2007.
Correspondence: Hiroaki Shimokawa, M.D., Ph.D., Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan.
e-mail: shimo@cardio.med.tohoku.ac.jp

(WHO) established the etiology-based new classification (Table 1) (Simonneau et al. 2004), which serves as a useful guide to physicians to evaluate a patient and develop a therapeutic plan. Although some progress has been made for the diagnosis and treatment of PAH (e.g., anticoagulant agents, vasodilators, and lung transplantation), more effective treatments need to be developed.

The Rho/Rho-kinase pathway has recently attracted much attention in various research fields, especially in the cardiovascular research field, for several reasons. First, the Rho/Rho-kinase pathway plays an important role in various cellular functions that are involved in the pathogenesis of cardiovascular diseases (Shimokawa 2000, 2002;

TABLE 1. World Health Organization classification of pulmonary hypertension.

1. Pulmonary arterial hypertension (PAH)
   - Idiopathic (IPAH)
   - Familial (FPAH)
   - Associated with (APAH):
     - Collagen vascular disease
     - Congenital systemic-to-pulmonary shunts
     - Portal hypertension
     - HIV infection
     - Drugs and toxins
     - Other (thyroid disorders, glycogen storage disease, Gaucher's disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy)
   - Associated with significant venous or capillary involvement
     - Pulmonary veno-occlusive disease (PVOD)
     - Pulmonary capillary hemangiomatosis (PCH)
   - Persistent pulmonary hypertension of the newborn
2. Pulmonary hypertension with left heart disease
   - Left-sided atrial or ventricular heart disease
   - Left-sided valvular heart disease
3. Pulmonary hypertension associated with lung diseases and/or hypoxemia
   - Chronic obstructive pulmonary disease
   - Interstitial lung disease
   - Sleep-disordered breathing
   - Alveolar hypoventilation disorders
   - Chronic exposure to high altitude
   - Developmental abnormalities
4. Pulmonary hypertension due to chronic thrombotic and/or embolic disease (CTEPH)
   - Thromboembolic obstruction of proximal pulmonary arteries
   - Thromboembolic obstruction of distal pulmonary arteries
   - Nonthrombotic pulmonary embolism (tumor, parasites, foreign material)
5. Miscellaneous
   - Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosing mediastinitis)

The table has been adapted from Simonneau et al. (2004)

Shimokawa and Takeshita 2005; Shimokawa and Rashid 2007). Second, this intracellular signaling pathway is substantially involved in the effects of many vasoactive substances that are implicated in the pathogenesis of cardiovascular diseases (Shimokawa 2000; Shimokawa and Takeshita 2005). Third, the so-called "pleiotropic" effects of statins may be mediated, at least in part, by their inhibitory effects on Rho with a resultant inhibition on Rho-kinase (Takemoto and Liao 2001; Shimokawa 2000; Shimokawa and Takeshita 2005). Indeed, we were recently able to demonstrate the roles of the Rho-kinase pathway in the pathogenesis of PAH (Shimokawa 2002; Abe et al. 2004; Fukumoto et al. 2005; Abe et al. 2006; Jiang et al. 2007; Tawara et al. 2007). In this article, we will briefly summarize the recent progress in the treatment of PAH, with a special reference to Rho-kinase inhibitors.

Pathophysiology of PAH

Normal pulmonary circulation

The lung is supplied by the pulmonary and bronchial arteries and drained by the pulmonary and azygos veins. The bronchial arteries arise from the aorta and supply the capillary plexus in the full length of the airway wall from the hilus to the respiratory bronchiole. The pulmonary artery branches run with the airways and the accompanying bronchial arteries in a single connective tissue sheath. The pulmonary artery goes into a capillary bed only when it reaches the alveoli of the respiratory bronchiole. It supplies all capillaries in the alveolar walls, which constitute the respiratory surface of the lung. The pulmonary veins drain the regions supplied by the pulmonary artery and also the airways within the lung that is supplied by the bronchial artery.

Pulmonary gas exchange is performed in the pulmonary circulation with a high-flow and low-pressure system. Therefore, the right ventricle can operate at a low-energy cost, although the pulmonary circulation is very sensitive to mechanical influences because of the low pressures. The normal pulmonary vascular bed offers less than one-tenth the resistance to flow offered by the systemic bed. Pulmonary vascular resistance reflects a composite of variables that includes, but is not limited to, the cross-sectional area of small muscular pulmonary arteries and arterioles (Mandegar et al. 2004). Other determinants of pulmonary vascular resistance include blood viscosity, total mass of lung tissue, proximal vascular obstruction, and extramural compression of blood vessels (Rich and McLaughlin 2005).

Abnormal pulmonary circulation in PAH

Pulmonary vascular remodeling involves structural and functional changes in pulmonary arterial walls (Fig. 1). The process of pulmonary vascular remodeling may occur as endothelial dysfunction of pulmonary arteries, which can enhance pulmonary vasoconstriction and contribute to the development of PAH (Higenbottam and Laude 1998).

Pathohistological studies have demonstrated that idiopathic PAH (IPAH) is associated with abnormal vascular structures (Fig. 2), including medial and/or intimal hypertrophy, concentric and/or eccentric intimal fibrosis, obstruction in the arterial lumen, and aneurysmal dilatation (Palevsky et al. 1989). Pulmonary vascular remodeling characterized by these changes can easily elevate pulmonary vascular resistance and pulmonary arterial pressure in the high-flow and low-resistance vascular bed (Fig. 1). In the pathogenesis of PH, various mechanisms in all the three layers of pulmonary arteries appear to be involved, leading to vascular remodeling, hyperconstriction, and thrombosis, resulting in the increased pulmonary vascular resistance and vascular lesion formation (Fig. 1).

Genetic mutations

Several genetic mutations are associated with the occurrence of PAH, including bone morphogenetic protein receptor 2 (BMPR2) and activin-like kinase type-1 (ALK-1). BMPR2 is a member of the transforming growth factor-β (TGF-β) receptor superfamily, and has been shown to interrupt the bone morphogenetic protein signaling pathway, resulting in proliferation, rather than apoptosis of cells, within small arteri-

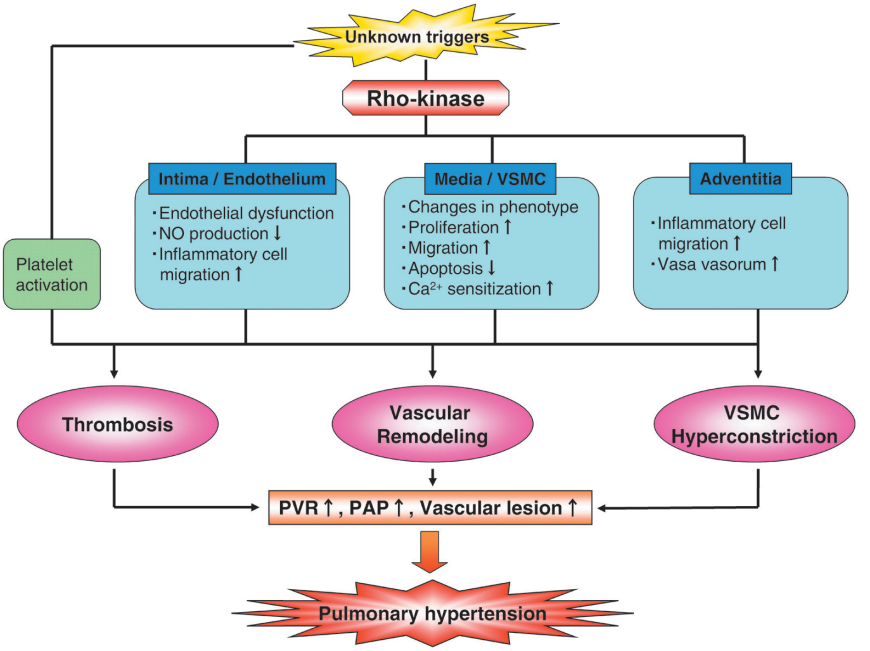

Fig. 1. Schematic illustration of pathophysiological components contributing to the development of pulmonary hypertension. Rho-kinase activation may be involved in the pathological changes in all the three layers of pulmonary arteries. NO, nitric oxide; VSMC, vascular smooth muscle cell; PVR, pulmonary vascular resistance; PAP, pulmonary arterial pressure.

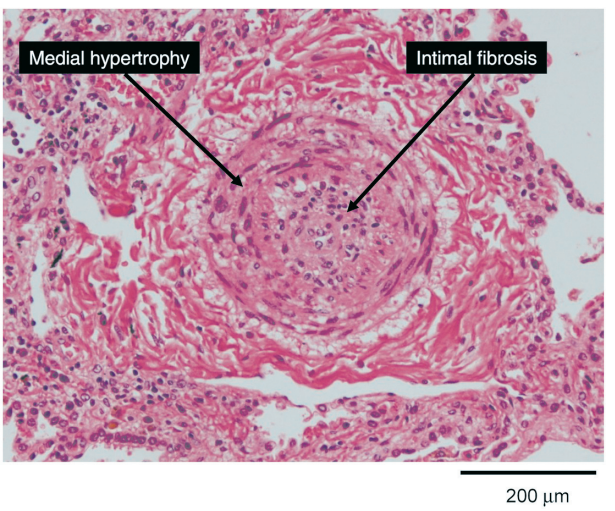

Fig. 2. Histology of pulmonary arteries from a patient with idiopathic pulmonary arterial hypertension. Medical hypertrophy and intimal fibrosis of small pulmonary arteries cause vascular obstruction. Bar indicates 200 μm.

oles (Morse et al. 2001). ALK-1 is another TGF-β family, and its mutations are found in a minority of patients with hereditary hemorrhagic telangiectasia and PAH as well (Newman et al. 2004).

Molecular and cellular mechanisms

prostacyclin

In patients with PAH, the release of vasodilator prostacyclin is reduced, whereas the release of the potent vasoconstrictor, thromboxane A2, is increased in patients with PAH, suggesting the involvement of platelet activation in the disorder (Christman et al. 1992).

Endothelin

Endothelin-1 (ET-1) is an endothelium-derived 21-residue peptide, which has been shown to be one of the most potent endogenous vasoconstrictors (Yanagisawa et al. 1988). The expression of ET-1 is increased in patients with PAH, which may contribute to the elevated pulmonary vascular resistance and the development of the disorder (Stewart et al. 1991; Giaid et al. 1993). Further-

more, circulating levels of ET-1 correlate with the severity of PAH and predict the prognosis of patients with the disorder (Rubens et al. 2001).

Nitric oxide

Nitric oxide (NO) is a potent endothelium-derived vasodilator and an inhibitor of smooth muscle proliferation, which is synthesized in endothelial cells from L-arginine by endothelial NO synthase (eNOS) in pulmonary arteries (Abe et al. 2006). NO pathway involves the production of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells (VSMCs) (Moncada and Higgs 2006), and then activates cGMP kinase with resultant opening of potassium channels, membrane hyperpolarization, and calcium channel inhibition (Moncada and Higgs 2006). Decrease in calcium entry and calcium release from sarcoplasmic stores diminishes activation of the contractile apparatus and leads to vasodilatation (Moncada and Higgs 2006). Since the expression of eNOS is diminished in patients with PAH (Giaid and Saleh 1995), the impaired NO release may contribute to pulmonary vasoconstriction and pulmonary vascular remodeling seen in patients with PAH.

Serotonin (5-hydroxytryptamine)

Serotonin (5-HT) is a potent vasoconstrictor (Shimokawa et al. 1983; Fukumoto et al. 1996; Fukumoto et al. 1997) and has long been implicated in PAH. Indeed, elevated plasma levels of serotonin and reduced serotonin content in platelets have been observed in patients with PAH (Herve et al. 1995). Serotonin transporter facilitates the proliferation of pulmonary arteries by transporting the indolamine into pulmonary VSMCs (Eddahibi et al. 2002). Furthermore, the expression of 5-HT_{1B} receptor, which mediates serotonin-induced vasoconstriction, is increased in small pulmonary arteries of patients with PAH (Morecroft et al. 1999).

Inflammation

A number of studies have suggested that inflammation may be involved as a mechanism of some forms of PAH (Tuder et al. 1994; Dorfmuller et al. 2003). Some patients with IPAH have immunological disturbances (e.g., circulating auto-antibodies, such as antinuclear antibodies) and elevated circulating levels of pro-inflammatory cytokines (e.g., interleukin-1 and -6) (Dorfmuller et al. 2003).

Platelet activation and hypercoagulable state

It has been reported that increased platelet activation and thrombin-antithrombin III levels are, at least in part, associated with PAH (Singer et al. 2006). Hypercoagulable state can be associated with endothelial dysfunction and PAH as well. It has been reported that in patients with PAH, circulating levels of pro-coagulants (e.g., von Willebrand factor, plasma fibrinopeptide A, plasminogen activator inhibitor [PAI]-1, serotonin, and thromboxane) are increased while those of anti-coagulants (e.g., tissue plasminogen activator, thrombomodulin, NO, and prostacyclin) are reduced (McLaughlin and McGoon 2006).

Adrenomedullin

Adrenomedullin is synthesized by several cell populations in the normal lung and dilates pulmonary vessels and thus increases pulmonary blood flow (de Vroomen et al. 1997). The plasma levels of adrenomedullin are elevated in patients with PAH (Kakishita et al. 1999). There noted a significant correlation between plasma levels of adrenomedullin and mean right atrial pressure, stroke volume, total pulmonary resistance, mean pulmonary arterial pressure, the natural logarithm of plasma atrial natriuretic peptide, and total pulmonary resistance (Kakishita et al. 1999).

Current Treatment of PAH

In the treatment of PAH, as well as PH due to chronic thrombotic and/or embolic disease (CTEPH), it is important to first examine the acute pulmonary vasodilator response to conventional medical therapy, including O_2 and NO inhalation and calcium channel blockers (Fig. 3). The positive response to NO inhalation is defined as more than 30% reduction in pulmonary vascular resistance. After PAH/CTEPH is diagnosed, disease severity should be assessed in order to

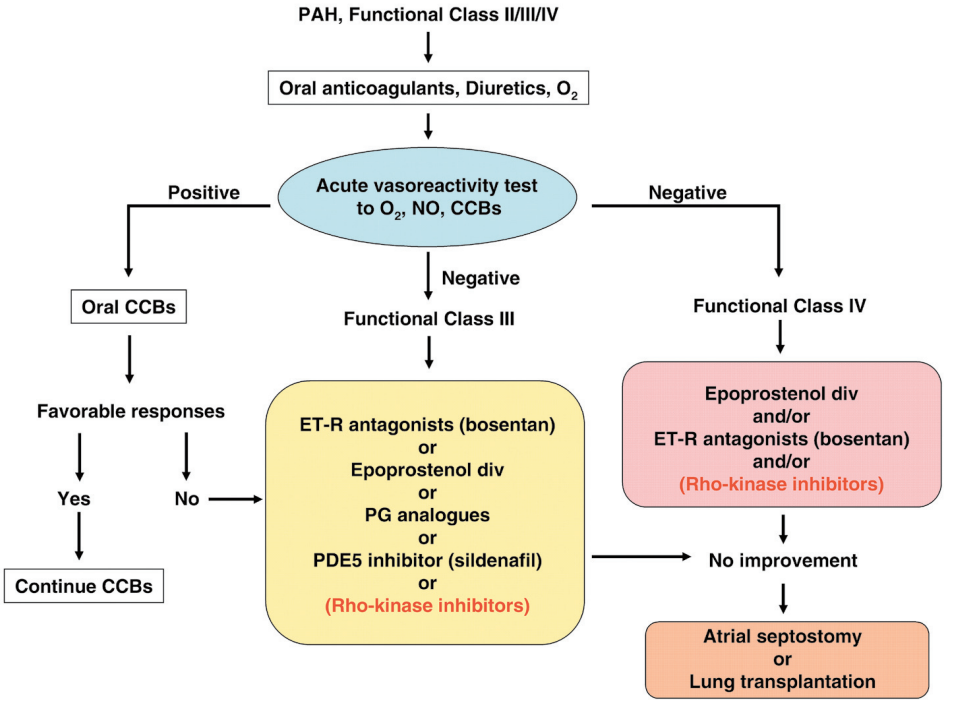

Fig. 3. Diagnosis and treatment algorithm for pulmonary arterial hypertension (PAH). NO, nitric oxide; CCBs, calcium channel blockers; ET-R, endothelin receptor; PG, prostaglandin; PDE5, phosphodiesterase-5.

accurately determine the risk-benefit profiles for various therapeutic options. Predictors of PAH include functional class, exercise capacity (e.g., 6 minute-walking test), pulmonary hemodynamics, acute vasoreactivity, right ventricular function, as well as plasma levels of brain natriuretic peptide, ET-1, uric acid, and troponin (Nagaya et al. 1999, 2000; Rubens et al. 2001; Barst et al. 2004). These examinations need to be repeated at an interval of 0.5-1 years in order to closely follow-up the patients with PAH/CTEPH. Patients with PAH/CTEPH are currently treated with anticoagulants plus vasodilators (e.g., prostacyclin, sildenafil, and bosentan) as a monotherapy or in combination, and in advanced stage, with lung transplantation (Fig. 3) (Fuster et al. 1984; Rich et al. 1992; Chapelier et al. 1993; Barst et al. 1996; Michelakis et al. 2002). However, since the effects of the current therapy are not satisfactory, more effective and non-invasive treatments need to be developed.

Lifestyle modification

Heavy physical activity or isotonic exercise often causes right ventricular failure in patients with PAH/CTEPH (Mereles et al. 2006). Thus, low-level of exercise is recommended for those with PAH/CTEPH in their daily life, since low-level of physical training rather improves endothelial function, exercise capacity and quality of life, not only in those with coronary artery disease but also in those with PAH (Hambrecht et al. 2000; Belardinelli et al. 2001; Mereles et al. 2006). High-altitude and infections should also be avoided; since high-altitude may produce hypoxic pulmonary vasoconstriction, and infections are fatal in some patients. Special attention should also be paid for the drug interactions with warfarin, bosentan and/or sildenafil. Pregnancy is not recommended in young women with PAH in general.

Anticoagulation

Anticoagulation with warfarin is important for the treatment of PAH/CTEPH and should be

maintained in the range of an international normalized ratio between 1.5 and 3.0 (McLaughlin and McGoon 2006). However, the effect of warfarin on PH is controversial because only a few studies have examined them in small, uncontrolled trials (Fuster et al. 1984; Rich et al. 1992).

Calcium channel blockers

Some patients with PAH have an acute pulmonary vasodilator response to calcium channel blockers, for whom those drugs may be indicated (Fig. 3). However, it was reported that only 6.8% of patients with PAH had a favorable response to long-term treatment with calcium channel blockers (Sitbon et al. 2005).

Prostacyclins

Intravenous epoprostenol improves symptoms, 6 minute-walk distance, hemodynamics, and survival in patients with IPAH (Barst et al. 1996). It was also reported that intravenous epoprostenol improved survival rate in comparison with historical controls, with 1-, 2-, 3-, and 5-year survival rates of 85%, 70%, 63%, and 55%, respectively (Sitbon et al. 2002), although this drug needs to be delivered by continuous intravenous infusion. Thus, it is important for the patients with PAH/CTEPH to learn the techniques of sterile preparation of the drug, operation of the ambulatory infusion pump, and sterile handling of a central venous catheter. The epoprostenol therapy should be started during hospitalization and the starting dose of epoprostenol ranges 0.5~1 ng/kg/min (Sitbon et al. 2002). Its dose should be carefully increased in a step-wise manner on the basis of symptoms and side effects of the drug, whereas chronic overdose can lead to high cardiac output failure. The side effects include headache, jaw pain, flushing, nausea, diarrhea, skin rash, and musculoskeletal pain (McLaughlin and McGoon 2006). In addition, infections and infusion interruptions can be life threatening. Due to its considerable complexity, the epoprostenol therapy should be performed in experienced centers.

Phosphodiesterase (PDE) inhibitors

Sildenafil, a potent and highly specific PDE-5 inhibitor, has been demonstrated to improve exercise capacity, symptoms, and hemodynamics in patients with PAH in the Sildenafil Use in Pulmonary Hypertension (SUPER) trial (Galie et al. 2005). Side effects of sildenafil include headache, flushing, dyspepsia, and epistaxis.

Endothelin receptor antagonists

Bosentan is the first drug in this class of ET antagonists that block both ET_A and ET_B receptors. Clinical trials have shown that the treatment with bosentan increases exercise capacity, and improves symptoms and pulmonary hemodynamics, not only in patients with PAH but also in those with Eisenmenger syndrome (Channick et al. 2001; Galie et al. 2003; Roman et al. 2006). The adverse effects of bosentan include headache, hypotension, and liver dysfunction (McLaughlin et al. 2005). Therefore, careful dosing of bosentan is required, starting at a low dose (15.5~62.5 mg/day) with a close follow-up, in order to avoid the adverse effects, especially in those with heart failure and liver dysfunction.

Rho-Kinase as a Novel Therapeutic Target of PAH

Important role of Rho-kinase in the pathogenesis of PAH

Recent advances in molecular biology have elucidated the substantial involvement of intracellular signaling pathways mediated by small GTP-binding proteins (G proteins), such as Rho, Ras, Rab, Sarl/Arf, and Ran families (Fukata et al. 2001; Takai et al. 2001). In mid 1990s, 2 Japanese groups and 1 Singapore group independently identified one of the effectors of Rho and termed it as Rho-kinase (Ishizaki et al. 1996). The Rho/Rho-kinase pathway has recently attracted much attention in various research fields, especially in the cardiovascular research field (Shimokawa 2000, 2002; Shimokawa and Takeshita 2005; Shimokawa and Rashid 2007).

We have previously demonstrated that Rho-kinase is a novel therapeutic target in ischemic heart disease (Shimokawa 2002; Shimokawa and Takeshita 2005). Rho-kinase suppresses myosin

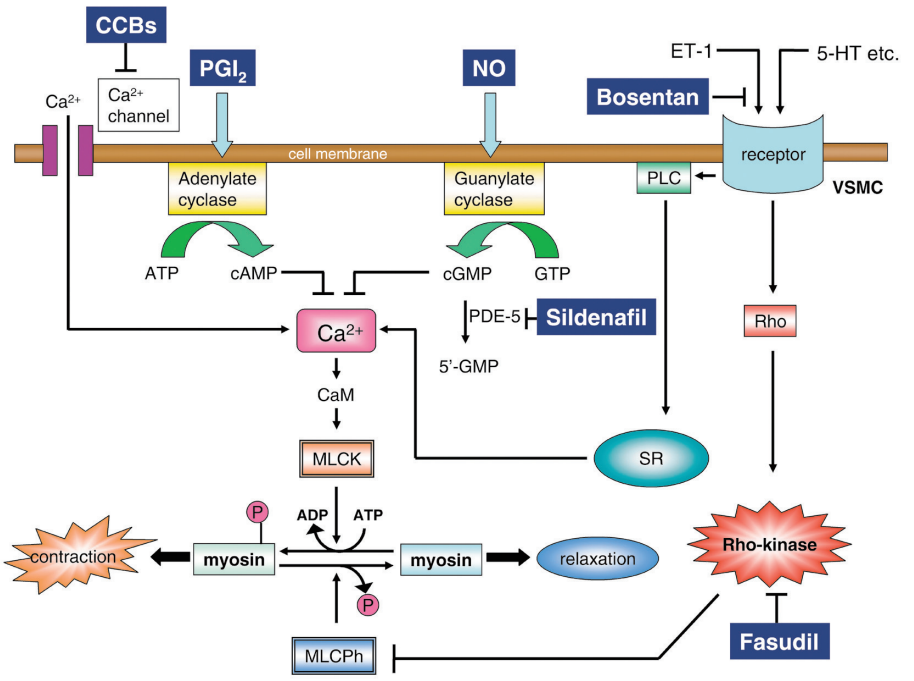

Fig. 4. Mechanism of pulmonary dilatation in response to conventional drugs and Rho-kinase inhibitors. 5-HT, serotonin; CaM, calmodulin; CCBs, calcium channel blockers; ET-1, endothelin-1; MLCK, myosin light chain kinase; MLCPh, myosin light chain phosphatase; NO, nitric oxide; PDE-5, phosphodiesterase-5; VSMC, vascular smooth muscle cell; PGI_2, prostacyclin; PLC, phospholipase C; SR, sarcoplasmic reticulum.

phosphatase activity by phosphorylating the myosin-binding subunit of the enzyme and thus augments VSMC contraction at a given intracellular calcium concentration (Fig. 4) (Uehata et al. 1997; Somlyo and Somlyo 2000). It also has been demonstrated that the Rho-kinase pathway is associated with enhanced myosin light chain (MLC) phosphorylations at the hyperconstrictive artery segments in animals (Shimokawa 2000; Shimokawa and Takeshita 2005). The activity and the expression of Rho-kinase are enhanced at the hyperconstrictive coronary segments, thereby suppressing myosin phosphatase through phosphorylation of its myosin-binding subunit with a resultant increase in MLC phosphorylations and hyperconstriction (Shimokawa 2000; Shimokawa and Takeshita 2005; Hizume et al. 2006). Thus, VSMC hyperconstriction mediated by activated Rho-kinase plays a key role in patients with coronary artery spasm (Masumoto et al. 2001, 2002; Shimokawa 2002; Shimokawa and Takeshita 2005), suggesting that Rho-kinase inhibition is an important therapeutic strategy for vasospastic angina (Masumoto et al. 2002; Shimokawa 2002). Moreover, we have recently demonstrated that Rho-kinase inhibition increases eNOS expression and decreases inflammatory cell migration and anigiotensin II-induced upregulation of atherogenic molecules (e.g., monocyte chemoattractant protein [MCP]-1, PAI-1, and NADPH oxidase) and cardiovascular hypertrophy both in vitro and in vivo (Shimokawa 2002; Higashi et al. 2003; Shimokawa and Takeshita 2005).

Increased pulmonary vascular resistance in PAH is caused by both pulmonary vascular remodeling and sustained pulmonary vasoconstriction (Giaid and Saleh 1995; Higenbottam and Laude 1998; Yuan et al. 1998), in which endothelial dysfunction (Higenbottam and Laude 1998) and VSMC hyperconstriction (Yuan et al. 1998) may be involved (Fig. 1). Indeed, in patients with PAH, eNOS expression is reduced (Giaid and Saleh 1995) and pulmonary VSMC are hyperreactive (Yuan et al. 1998). It is thus conceivable that

the Rho-kinase pathway plays an important role in the pathogenesis of PAH (Figs. 1 and 4).

Potential importance of Rho-kinase inhibitors for the treatment of PAH

We have recently demonstrated that Rho-kinase is a novel therapeutic target not only in ischemic heart disease and essential hypertension but also in PAH (Shimokawa 2002; Abe et al. 2004; Fukumoto et al. 2005; Abe et al. 2006; Jiang et al. 2006; Tawara et al. 2007). Fasudil is a potent and selective inhibitor of Rho-kinase (Uehata et al. 1997; Shimokawa et al. 1999) with its inhibitory effect on Rho-kinase being 100 times and 1,000 times more potent than on protein kinase C and myosin light chain kinase, respectively (Shimokawa et al. 1999; Shimokawa 2002; Shimokawa and Rashid 2007). Rho-kinase inhibition ameliorates VSMC hyperconstriction, increases eNOS expression, and decreases inflammatory cell migration and anigiotensin II-induced up-regulation of MCP-1 and PAI-1 (Morishige et al. 2001; Shimokawa 2002).

In the rat model of monocrotaline-induced PAH, fasudil markedly suppressed the development of PAH when started simultaneously with monocrotaline, and even induced a regression of the disorder when started after the establishment of PAH (Abe et al. 2004). Oral treatment with fasudil is also effective to inhibit the development of PAH induced by chronic hypoxia in mice, in which both eNOS-dependent and -independent mechanisms were involved (Abe et al. 2006). Inhalation of fasudil is effective to reduce pulmonary vascular resistance in animal models of PAH with various etiologies (Nagaoka et al. 2005). Rho-kinase inhibitors appear to have pulmonary vasodilator effects through mechanism different from conventional drugs (Fig. 4). Therefore, it is possible that the combination of a Rho-kinase inhibitor and conventional drugs exerts additive or synergistic effects. Indeed, prostacyclin lacks direct inhibitory effects on Rho-kinase (Abe et al. 2005) and the combination of oral prostacyclin analogue, beraprost, and a Rho-kinase inhibitor, fasudil, is more effective than each monotherapy for ameliorating monocrotaline-induced PH in rats (Tawara et al. 2007). Finally, we were recently able to demonstrate the acute beneficial effects of intravenous fasudil in patients with severe PAH without adverse effects (Fukumoto et al. 2005).

We plan to perform clinical trials with a long-term oral treatment with fasudil in patients with PAH/CTEPH in Japan. Through those trials, we expect to learn whether the long-term inhibition of Rho-kinase is a novel therapeutic strategy for the treatment of PAH/CTEPH in humans.

Future Directions and Conclusions

PAH is characterized by a progressive elevation of pulmonary artery pressure and pulmonary vascular resistance, leading to right ventricular failure and premature death. Although several treatments are currently used, the outcome still remains unsatisfactory. Animal studies have demonstrated that Rho-kinase pathway plays an important role in the pathogenesis of PAH and that long-term treatment with a Rho-kinase inhibitor ameliorates the disorder. Recent clinical studies have suggested that this is indeed the case in humans. It is expected that clinical trials with long-term oral treatment with Rho-kinase inhibitors will elucidate their effectiveness and safety for the treatment of PAH in humans.

Acknowledgments

The authors' work presented in this article was supported in part by the grants-in-aid (10177223, 10357006, 12032215, 12470158, 12877114, 13307024, 13557068, 15256003, 16209027, 18652918) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan, the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan, and Technology Agency, CREST, Tokyo, Japan. The authors thank the coworkers at the Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences (especially Drs K. Abe, and B.H. Jiang) and those at the Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan (especially Drs. J. Nawata and J. Demachi) for their collaboration in the present studies. The authors also thank Professor K. Kaibuchi and Dr. M. Amano at the Department of Cell Pharmacology, Nagoya Univer-

sity Graduate School of Medicine, T. Asano, M. Seto, K. Tsurui, A. Takashima, and other staff at the Asahi Kasei Pharma, for their cooperation in the present studies and M. Sonoda, E. Gunshima, and N. Shintani for excellent technical assistance.

H.S. is the recipient of the 2006 Jeffrey M. Hoeg Arteriosclerosis, Thrombosis, and Vascular Biology Award of the American Heart Association.

References

Abe, K., Shimokawa, H., Morikawa, K., Uwatoku, T., Oi, K., Matsumoto, Y., Hattori, T., Nakashima, Y., Kaibuchi, K., Sueishi, K. & Takeshit, A. (2004) Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. *Circ. Res.,* **94**, 385-393.

Abe, K., Morikawa, K., Hizume, T., Uwatoku, T., Oi, K., Seto, M., Ikegaki, I., Asano, T., Kaibuchi, K. & Shimokawa, H. (2005) Prostacyclin does not inhibit rho-kinase: an implication for the treatment of pulmonary hypertension. *J. Cardiovasc. Pharmacol.,* **45**, 120-124.

Abe, K., Tawara, S., Oi, K., Hizume, T., Uwatoku, T., Fukumoto, Y., Kaibuchi, K. & Shimokawa, H. (2006) Long-term inhibition of Rho-kinase ameliorates hypoxia-induced pulmonary hypertension in mice. *J. Cardiovasc. Pharmacol.,* **48**, 280-285.

Barst, R.J., Rubin, L.J., Long, W.A., McGoon, M.D., Rich, S., Badesch, D.B., Groves, B.M., Tapson, V.F., Bourge, R.C., Brundage, B.H., Koerner, S.K., Langleben, D., Keller, C.A., Murali, S., Uretsky, B.F., Clayton, L.M., Jobsis, M.M., Blackburn, S.D., Shortino, D. & Crow, J.W. (1996) A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. *N. Engl. J. Med.,* **334**, 296-302.

Barst, R.J., McGoon, M., Torbicki, A., Sitbon, O., Krowka, M.J., Olschewski, H. & Gaine, S. (2004) Diagnosis and differential assessment of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.,* **43**, 40S-47S.

Belardinelli, R., Paolini, I., Cianci, G., Piva, R., Georgiou, D. & Purcaro, A. (2001) Exercise training intervention after coronary angioplasty: the ETICA trial. *J. Am. Coll. Cardiol.,* **37**, 1891-1900.

Channick, R.N., Simonneau, G., Sitbon, O., Robbins, I.M., Frost, A., Tapson, V.F., Badesch, D.B., Roux, S., Rainisio, M., Bodin, F. & Rubin, L.J. (2001) Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet,* **358**, 1119-1123.

Chapelier, A., Vouhe, P., Macchiarini, P., Lenot, B., Cerrina, J., Le Roy Ladurie, F., Parquin, F., Herve, P., Brenot, F. & Lafont, D. (1993) Comparative outcome of heart-lung and lung transplantation for pulmonary hypertension. *J. Thorac. Cardiovasc. Surg.,* **106**, 299-307.

Christman, B.W., McPherson, C.D., Newman, J.H., King, G.A., Bernard, G.R., Groves, B.M. & Loyd, J.E. (1992) An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N. Engl. J. Med.,* **327**, 70-75.

de Vroomen, M., Takahashi, Y., Gournay, V., Roman, C., Rudolph, A.M. & Heymann, M.A. (1997) Adrenomedullin increases pulmonary blood flow in fetal sheep. *Pediatr. Res.,* **41**, 493-497.

Dorfmuller, P., Perros, F., Balabanian, K. & Humbert, M. (2003) Inflammation in pulmonary arterial hypertension. *Eur. Respir. J.,* **22**, 358-363.

Eddahibi, S., Raffestin, B., Hamon, M. & Adnot, S. (2002) Is the serotonin transporter involved in the pathogenesis of pulmonary hypertension? *J. Lab. Clin. Med.,* **139**, 194-201.

Fukata, Y., Amano, M. & Kaibuchi, K. (2001) Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol. Sci.,* **22**, 32-39.

Fukumoto, Y., Shimokawa, H., Kozai, T., Kadokami, T., Kuwata, K., Owada, M.K., Shiraishi, T., Kuga, T., Egashira, K. & Takeshita, A. (1996) Tyrosine kinase inhibitor suppresses the (re) stenotic changes of the coronary artery after balloon injury in pigs. *Cardiovasc. Res.,* **32**, 1131-1140.

Fukumoto, Y., Shimokawa, H., Kozai, T., Kadokami, T., Kuwata, K., Yonemitsu, Y., Kuga, T., Egashira, K., Sueishi, K. & Takeshita, A. (1997) Vasculoprotective role of inducible nitric oxide synthase at inflammatory coronary lesions induced by chronic treatment with interleukin-1beta in pigs in vivo. *Circulation,* **96**, 3104-3111.

Fukumoto, Y., Matoba, T., Ito, A., Tanaka, H., Kishi, T., Hayashidani, S., Abe, K., Takeshita, A. & Shimokawa, H. (2005) Acute vasodilator effects of a Rho-kinase inhibitor, fasudil, in patients with severe pulmonary hypertension. *Heart,* **91**, 391-392.

Fuster, V., Steele, P.M., Edwards, W.D., Gersh, B.J., McGoon, M.D. & Frye, R.L. (1984) Primary pulmonary hypertension: natural history and the importance of thrombosis. *Circulation,* **70**, 580-587.

Galie, N., Hinderliter, A.L., Torbicki, A., Fourme, T., Simonneau, G., Pulido, T., Espinola-Zavaleta, N., Rocchi, G., Manes, A., Frantz, R., Kurzyna, M., Nagueh, S.F., Barst, R., Channick, R., Dujardin, K., Kronenberg, A., Leconte, I., Rainisio, M. & Rubin, L. (2003) Effects of the oral endothelin-receptor antagonist bosentan on echocardiographic and doppler measures in patients with pulmonary arterial hypertension. *J. Am. Coll. Cardiol.,* **41**, 1380-1386.

Galie, N., Ghofrani, H.A., Torbicki, A., Barst, R.J., Rubin, L.J., Badesch, D., Fleming, T., Parpia, T., Burgess, G., Branzi, A., Grimminger, F., Kurzyna, M. & Simonneau, G. (2005) Sildenafil citrate therapy for pulmonary arterial hypertension. *N. Engl. J. Med.,* **353**, 2148-2157.

Giaid, A., Yanagisawa, M., Langleben, D., Michel, R.P., Levy, R., Shennib, H., Kimura, S., Masaki, T., Duguid, W.P. & Stewart, D.J. (1993) Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.,* **328**, 1732-1739.

Giaid, A. & Saleh, D. (1995) Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.,* **333**, 214-221.

Hambrecht, R., Wolf, A., Gielen, S., Linke, A., Hofer, J., Erbs, S., Schoene, N. & Schuler, G. (2000) Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N. Engl. J. Med.,* **342**, 454-460.

Herve, P., Launay, J.M., Scrobohaci, M.L., Brenot, F., Simonneau, G., Petitpretz, P., Poubeau, P., Cerrina, J., Duroux, P. & Drouet, L. (1995) Increased plasma serotonin in primary pulmonary hypertension. *Am. J. Med.,* **99**, 249-254.

Higashi, M., Shimokawa, H., Hattori, T., Hiroki, J., Mukai, Y.,

Morikawa, K., Ichiki, T., Takahashi, S. & Takeshita, A. (2003) Long-term inhibition of Rho-kinase suppresses angiotensin II-induced cardiovascular hypertrophy in rats in vivo: effect on endothelial NAD(P)H oxidase system. *Circ. Res.,* **93**, 767-775.

Higenbottam, T.W. & Laude, E.A. (1998) Endothelial dysfunction providing the basis for the treatment of pulmonary hypertension: Giles F. Filley lecture. *Chest,* **114**, 72S-79S.

Hizume, T., Morikawa, K., Takaki, A., Abe, K., Sunagawa, K., Amano, M., Kaibuchi, K., Kubo, C. & Shimokawa, H. (2006) Sustained elevation of serum cortisol level causes sensitization of coronary vasoconstricting responses in pigs in vivo: a possible link between stress and coronary vasospasm. *Circ. Res.,* **99**, 767-775.

Ishizaki, T., Maekawa, M., Fujisawa, K., Okawa, K., Iwamatsu, A., Fujita, A., Watanabe, N., Saito, Y., Kakizuka, A., Morii, N. & Narumiya, S. (1996) The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. *EMBO J.,* **15**, 1885-1893.

Jiang, B.H., Tawara, S., Abe, K., Takaki, A., Fukumoto, Y. & Shimokawa, H. (2007) Acute vasodilator effect of fasudil, a Rho-kinase inhibitor, in monocrotaline-induced pulmonary hypertension in rats. *J. Cardiovasc. Pharmacol.,* **49**, 85-89.

Kakishita, M., Nishikimi, T., Okano, Y., Satoh, T., Kyotani, S., Nagaya, N., Fukushima, K., Nakanishi, N., Takishita, S., Miyata, A., Kangawa, K., Matsuo, H. & Kunieda, T. (1999) Increased plasma levels of adrenomedullin in patients with pulmonary hypertension. *Clin. Sci. (Lond.),* **96**, 33-39.

Mandegar, M., Fung, Y.C., Huang, W., Remillard, C.V., Rubin, L.J. & Yuan, J.X. (2004) Cellular and molecular mechanisms of pulmonary vascular remodeling: role in the development of pulmonary hypertension. *Microvasc. Res.,* **68**, 75-103.

Masumoto, A., Hirooka, Y., Shimokawa, H., Hironaga, K., Setoguchi, S. & Takeshita, A. (2001) Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans. *Hypertension,* **38**, 1307-1310.

Masumoto, A., Mohri, M., Shimokawa, H., Urakami, L., Usui, M. & Takeshita, A. (2002) Suppression of coronary artery spasm by the Rho-kinase inhibitor fasudil in patients with vasospastic angina. *Circulation,* **105**, 1545-1547.

McLaughlin, V.V., Sitbon, O., Badesch, D.B., Barst, R.J., Black, C., Galie, N., Rainisio, M., Simonneau, G. & Rubin, L.J. (2005) Survival with first-line bosentan in patients with primary pulmonary hypertension. *Eur. Respir. J.,* **25**, 244-249.

McLaughlin, V.V. & McGoon, M.D. (2006) Pulmonary Arterial Hypertension. *Circulation,* **114**, 1417-1431.

Mereles, D., Ehlken, N., Kreuscher, S., Ghofrani, S., Hoeper, M.M., Halank, M., Meyer, F.J., Karger, G., Buss, J., Juenger, J., Holzapfel, N., Opitz, C., Winkler, J., Herth, F.F., Wilkens, H., Katus, H.A., Olschewski, H. & Grunig, E. (2006) Exercise and respiratory training improve exercise capacity and quality of life in patients with severe chronic pulmonary hypertension. *Circulation,* **114**, 1482-1489.

Michelakis, E., Tymchak, W., Lien, D., Webster, L., Hashimoto, K. & Archer, S. (2002) Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: comparison with inhaled nitric oxide. *Circulation,* **105**, 2398-2403.

Moncada, S. & Higgs, E.A. (2006) Nitric oxide and the vascular endothelium. *Handb. Exp. Pharmacol.*, 213-254.

Morecroft, I., Heeley, R.P., Prentice, H.M., Kirk, A. & MacLean, M.R. (1999) 5-hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: importance of the 5-HT1B receptor. *Br. J. Pharmacol.,* **128**, 730-734.

Morishige, K., Shimokawa, H., Eto, Y., Kandabashi, T., Miyata, K., Matsumoto, Y., Hoshijima, M., Kaibuchi, K. & Takeshita, A. (2001) Adenovirus-mediated transfer of dominant-negative rho-kinase induces a regression of coronary arteriosclerosis in pigs in vivo. *Arterioscler. Thromb. Vasc. Biol.,* **21**, 548-554.

Morse, J.H., Deng, Z. & Knowles, J.A. (2001) Genetic aspects of pulmonary arterial hypertension. *Ann. Med.,* **33**, 596-603.

Nagaoka, T., Fagan, K.A., Gebb, S.A., Morris, K.G., Suzuki, T., Shimokawa, H., McMurtry, I.F. & Oka, M. (2005) Inhaled Rho kinase inhibitors are potent and selective vasodilators in rat pulmonary hypertension. *Am. J. Respir. Crit. Care Med.,* **171**, 494-499.

Nagaya, N., Uematsu, M., Satoh, T., Kyotani, S., Sakamaki, F., Nakanishi, N., Yamagishi, M., Kunieda, T. & Miyatake, K. (1999) Serum uric acid levels correlate with the severity and the mortality of primary pulmonary hypertension. *Am. J. Respir. Crit. Care Med.,* **160**, 487-492.

Nagaya, N., Nishikimi, T., Uematsu, M., Satoh, T., Kyotani, S., Sakamaki, F., Kakishita, M., Fukushima, K., Okano, Y., Nakanishi, N., Miyatake, K. & Kangawa, K. (2000) Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation,* **102**, 865-870.

Newman, J.H., Trembath, R.C., Morse, J.A., Grunig, E., Loyd, J.E., Adnot, S., Coccolo, F., Ventura, C., Phillips, J.A., 3rd, Knowles, J.A., Janssen, B., Eickelberg, O., Eddahibi, S., Herve, P., Nichols, W.C. & Elliott, G. (2004) Genetic basis of pulmonary arterial hypertension: current understanding and future directions. *J. Am. Coll. Cardiol.,* **43**, 33S-39S.

Palevsky, H.I., Schloo, B.L., Pietra, G.G., Weber, K.T., Janicki, J.S., Rubin, E. & Fishman, A.P. (1989) Primary pulmonary hypertension. Vascular structure, morphometry, and responsiveness to vasodilator agents. *Circulation,* **80**, 1207-1221.

Rich, S., Kaufmann, E. & Levy, P.S. (1992) The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N. Engl. J. Med.,* **327**, 76-81.

Rich, S. & McLaughlin, V.V. (2005) *Braunwald's Heart Disease*, 7th ed., Chap.67, Elsevier Inc., Pennsylvania, pp. 1807-1842.

Roman, A., Gispert, P., Monforte, V., Bravo, C., Domingo, E. & Morell, F. (2006) Long-term Outcomes of Treatment With Bosentan in Pulmonary Hypertension. *Arch. Bronconeumol.,* **42**, 616-620.

Rubens, C., Ewert, R., Halank, M., Wensel, R., Orzechowski, H.D., Schultheiss, H.P. & Hoeffken, G. (2001) Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension. *Chest,* **120**, 1562-1569.

Shimokawa, H., Tomoike, H., Nabeyama, S., Yamamoto, H., Araki, H., Nakamura, M., Ishii, Y. & Tanaka, K. (1983) Coronary artery spasm induced in atherosclerotic miniature swine. *Science,* **221**, 560-562.

Shimokawa, H., Seto, M., Katsumata, N., Amano, M., Kozai, T., Yamawaki, T., Kuwata, K., Kandabashi, T., Egashira, K., Ikegaki, I., Asano, T., Kaibuchi, K. & Takeshita, A. (1999) Rho-kinase-mediated pathway induces enhanced myosin

light chain phosphorylations in a swine model of coronary artery spasm. *Cardiovasc. Res.,* **43**, 1029-1039.

Shimokawa, H. (2000) Cellular and molecular mechanisms of coronary artery spasm: lessons from animal models. *Jpn. Circ. J.,* **64**, 1-12.

Shimokawa, H. (2002) Rho-kinase as a novel therapeutic target in treatment of cardiovascular diseases. *J. Cardiovasc. Pharmacol.,* **39**, 319-327.

Shimokawa, H. & Takeshita, A. (2005) Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler. Thromb. Vasc. Biol.,* **25**, 1767-1775.

Shimokawa, H. & Rashid, M. (2007) Rho-kinase inhibitors for cardiovascular medicine. Its rationale and current status. *Trends Pharmacol. Sci.,* (in press)

Simonneau, G., Galie, N., Rubin, L.J., Langleben, D., Seeger, W., Domenighetti, G., Gibbs, S., Lebrec, D., Speich, R., Beghetti, M., Rich, S. & Fishman, A. (2004) Clinical classification of pulmonary hypertension. *J. Am. Coll. Cardiol.*, **43**, Suppl. 12, 5S-12S.

Singer, S.T., Kuypers, F.A., Styles, L., Vichinsky, E.P., Foote, D. & Rosenfeld, H. (2006) Pulmonary hypertension in thalassemia: association with platelet activation and hypercoagulable state. *Am. J. Hematol.,* **81**, 670-675.

Sitbon, O., Humbert, M., Nunes, H., Parent, F., Garcia, G., Herve, P., Rainisio, M. & Simonneau, G. (2002) Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. *J. Am. Coll. Cardiol.,* **40**, 780-788.

Sitbon, O., Humbert, M., Jais, X., Ioos, V., Hamid, A.M., Provencher, S., Garcia, G., Parent, F., Herve, P. & Simonneau, G. (2005) Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. *Circulation,* **111**, 3105-3111.

Somlyo, A.P. & Somlyo, A.V. (2000) Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *J. Physiol.,* **522**, 177-185.

Stewart, D.J., Levy, R.D., Cernacek, P. & Langleben, D. (1991) Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann. Intern. Med.,* **114**, 464-469.

Takai, Y., Sasaki, T. & Matozaki, T. (2001) Small GTP-binding proteins. *Physiol. Rev.,* **81**, 153-208.

Takemoto, M. & Liao, J.K. (2001) Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. *Arterioscler. Thromb. Vasc. Biol.,* **21**, 1712-1719.

Tawara, S., Abe, K., Morikawa, K., Fukumoto, Y. & Shimokawa, H. (2007) Prostacyclin lacks inhibitory effects on Rho-kinase -Possible benefits of combined therapy with prostacyclin and a Rho-kinase inhibitor in pulmonary hypertension. *Circ. J.*, **71**, Suppl. I, 232.

Tuder, R.M., Groves, B., Badesch, D.B. & Voelkel, N.F. (1994) Exuberant endothelial cell growth and elements of inflammation are present in plexiform lesions of pulmonary hypertension. *Am. J. Pathol.,* **144**, 275-285.

Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M. & Narumiya, S. (1997) Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature,* **389**, 990-994.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. & Masaki, T. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature,* **332**, 411-415.

Yuan, J.X., Aldinger, A.M., Juhaszova, M., Wang, J., Conte, J.V., Jr., Gaine, S.P., Orens, J.B. & Rubin, L.J. (1998) Dysfunctional voltage-gated K+ channels in pulmonary artery smooth muscle cells of patients with primary pulmonary hypertension. *Circulation,* **98**, 1400-1406.