Rho-Kinase Inhibitors

Yoshihiro Fukumoto and Hiroaki Shimokawa

Abstract Pulmonary arterial hypertension (PAH) is a fatal 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, pulmonary vasodilators, 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, it has been 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 expressions, all of which may be involved in the pathogenesis of arteriosclerosis. Rho-kinase is activated in animal models of PAH (monocrotaline and chronic hypoxia) associated with enhanced pulmonary vasoconstriction and proliferation, impaired endothelial vasodilator functions, and pulmonary remodeling. Therapeutic application of Rho-kinase inhibitors reverses established experimental pulmonary hypertension. Further, administration or inhalation of Rho-kinase inhibitors exerts acute pulmonary vasodilation in patients with PAH who were refractory to conventional therapies. Taken together, Rho-kinase is a novel and important therapeutic target of PAH, and Rho-kinase inhibitors are a promising new class of drugs for this fatal disorder.

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Contents

1 Introduction

In mid 1990s, two Japanese groups and one Singapore group independently identified Rho-kinase/ROK/ROCK as an effecter of the small GTP-binding protein Rho (Leung et al. 1995; Ishizaki et al. 1996; Amano et al. 1997), which plays an important role in various cellular functions, including smooth muscle contraction, actin cytoskeleton organization, cell adhesion and motility, cytokinesis, and gene expressions (Narumiya 1996; Shimokawa and Takeshita 2005; Loirand et al. 2006). The Rho/Rho-kinase pathway has recently attracted much attention in the cardiovascular research field for several reasons (Fig. 1). First, the Rho/Rho-kinase pathway plays an important role in various cellular functions that are involved in the pathogenesis of a variety of cardiovascular diseases (Shimokawa and Takeshita 2005; Shimokawa 2000). 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 and Takeshita 2005; Shimokawa 2000). Third, the so-called pleiotropic effects of statins, especially those of high-doses of statins, may be mediated, at least in part, by their inhibitory effects on Rho with a resultant inhibition on Rho-kinase (Shimokawa and Takeshita 2005; Shimokawa 2000). Fourth, the important roles of the Rho-kinase pathway have been recently demonstrated in the pathogenesis of pulmonary arterial hypertension (PAH) (Fig. 2) (Fukumoto et al. 2005, 2007; Shimokawa 2002; Abe et al. 2004, 2006; Do e et al. 2009; Fukumoto and Shimokawa 2011).

In this article, we will briefly review the role of Rho-kinase pathway on PAH, in terms of pathophysiology and treatment.

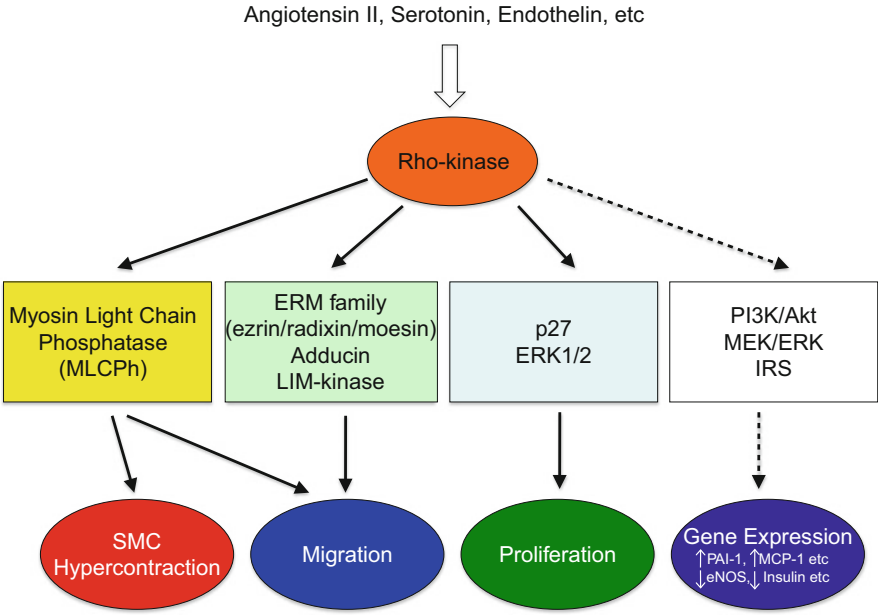

Fig. 1 Pathophysiology of Rho-kinase pathway. Rho-kinase pathway plays an important role in various cellular functions that are involved in the pathogenesis of a variety of cardiovascular diseases

2 Novel Pathophysiological Pathways of PAH

The pathological changes of the pulmonary arteries in PH include endothelial injury, proliferation and hyperconstriction of vascular smooth muscle cells (VSMC), and migration of inflammatory cells (Fig. 2) (Fukumoto and Shimokawa 2011; Humbert et al. 2004; McLaughlin and McGoon 2006).

2.1 Important Role of Rho-Kinase in the Cardiovascular Fields

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). The Rho/Rho-kinase pathway has recently attracted much attention in various research fields, especially in the cardiovascular research field (Shimokawa and Takeshita 2005; Shimokawa 2000, 2002; Shimokawa and Rashid 2007a).

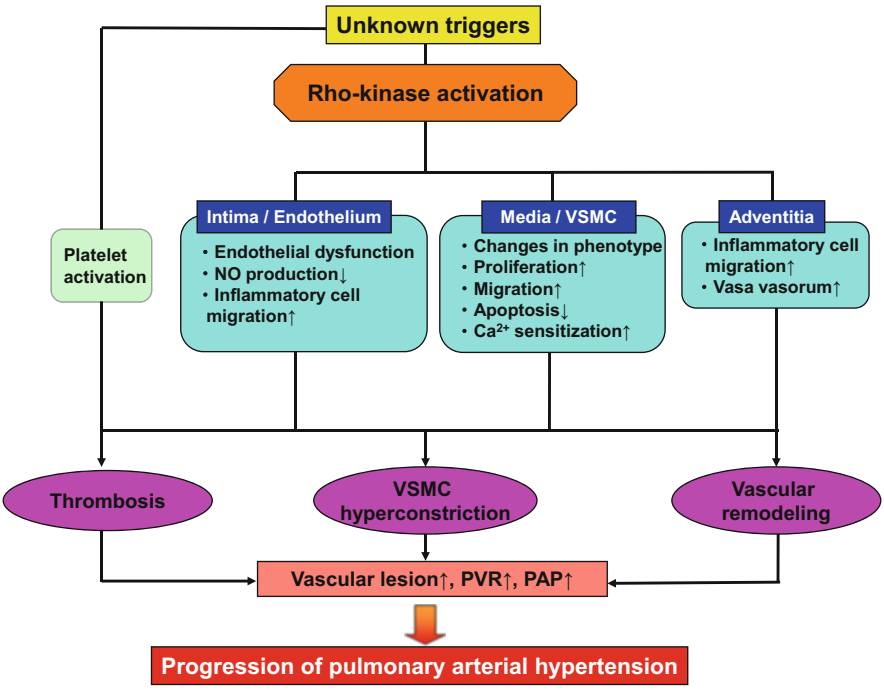

Fig. 2 Roles of Rho-kinase pathway in the pathogenesis of pulmonary hypertension. Rho-kinase activation has been confirmed to be substantially involved in the pathological changes of the pulmonary arteries. *NO* nitric oxide, *PAP* pulmonary arterial pressure, *PVR* pulmonary vascular resistance, *VSMC* vascular smooth muscle cell [from Fukumoto and Shimokawa 2011 with permission)

It has been previously demonstrated that Rho-kinase is a novel therapeutic target in ischemic heart disease (Shimokawa and Takeshita 2005; Shimokawa 2002). Rho-kinase suppresses myosin phosphatase activity by phosphorylating the myosin-binding subunit of the enzyme and thus augments VSMC contraction at a given intracellular calcium concentration (Fig. 3) (Uehata et al. 1997; Somlyo and Somlyo 2000). It has been also demonstrated that the Rho-kinase pathway is associated with enhanced myosin light chain (MLC) phosphorylations at the hyperconstrictive artery segments in animals (Shimokawa and Takeshita 2005; Shimokawa 2002). 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 and Takeshita 2005; Shimokawa 2002). Thus, VSMC hyperconstriction mediated by activated Rho-kinase plays a key role in patients with coronary artery spasm (Shimokawa and Takeshita 2005; Shimokawa 2002; Masumoto et al. 2001, 2002), suggesting that Rho-kinase inhibition is an important therapeutic strategy for vasospastic angina (Shimokawa 2002; Masumoto et al. 2002). Moreover, it has recently been

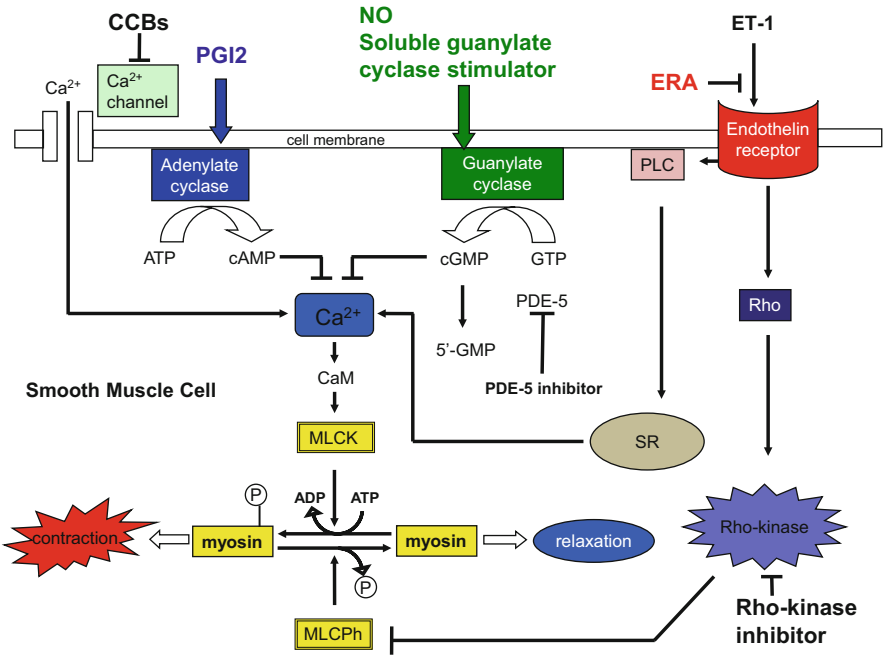

Fig. 3 Mechanism of pulmonary vasodilatation in response to conventional drugs and Rho-kinase inhibitors. *5-HT* serotonin, *CaM* calmodulin, *CCBs* calcium channel blockers, *ERA* endothelin receptor antagonist, *ET-1* endothelin-1, *MLCK* myosin light chain kinase, *MLCPh* myosin light chain phosphatase, *NO* nitric oxide, *PDE-5* phosphodiesterase-5, *PLC* phospholipase C, *VSMC* vascular smooth muscle cell, PGI_2 prostacyclin, *PLC* phospholipase C, *SR* sarcoplasmic reticulum

demonstrated that Rho-kinase inhibition increases eNOS expression and decreases inflammatory cell migration and angiotensin II-induced upregulation of atherogenic molecules [e.g., monocyte chemoattractant protein (MCP)-1, plasminogen activator inhibitor (PAI)-1, and NADPH oxidase] and cardiovascular hypertrophy both in vitro and in vivo (Shimokawa and Takeshita 2005; Shimokawa 2002; Higashi et al. 2003).

2.2 *PAH and Rho-Kinase Pathway*

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 and VSMC hyperconstriction may be involved through Rho-kinase activation (Fig. 2) (Higenbottam and Laude 1998; Yuan et al. 1998). Indeed, in patients with PAH, eNOS expression is reduced and pulmonary VSMC are

hyperreactive (Giaid and Saleh 1995; Yuan et al. 1998). It is thus conceivable that the Rho-kinase pathway plays an important role in the pathogenesis of PAH (Figs. 2 and 3).

Rho-kinase suppresses myosin phosphatase activity by phosphorylating the myosin-binding subunit of the enzyme, thus augmenting VSMC contraction at a given intracellular calcium concentration (Uehata et al. 1997; Somlyo and Somlyo 2000). VSMC hypercontraction mediated by activated Rho-kinase plays a key role not only in coronary artery spasm but also in PAH (Fukumoto et al. 2005, 2007; Shimokawa 2002; Fukumoto and Shimokawa 2011; Masumoto et al. 2002; Mohri et al. 2003; Fujita et al. 2010). Rho-kinase inhibition may be preferable to calcium channel blockers because of its selective spasmolytic effect on vascular hyperconstrictive segments (Fukumoto et al. 2007; Shimokawa 2002; Masumoto et al. 2002).

2.2.1 Rho-Kinase and Inflammation

A number of studies have suggested that inflammation may be involved in the pathogenesis of PAH (Fukumoto et al. 2007; Dorfmuller et al. 2003). Some patients with idiopathic PAH have immunological disturbances (e.g., circulating autoantibodies, such as antinuclear antibodies) and elevated circulating levels of pro-inflammatory cytokines (e.g., interleukin-1 and -6) (Dorfmuller et al. 2003). It has been demonstrated that Rho-kinase is upregulated by inflammatory stimuli (Shimokawa and Takeshita 2005; Hiroki et al. 2004; Liao et al. 2007) and that Rho-kinase inhibition increases endothelial nitric oxide synthase (eNOS) expression and inhibits inflammatory cell migration and angiotensin II-induced upregulation of monocyte chemoattractant protein-1 and plasminogen activator inhibitor-1 in vivo or in vitro (Shimokawa 2002), in which the Rho-kinase pathway may play an important role in the development of PAH.

2.2.2 Rho-Kinase and PAH

Indeed, it has been demonstrated that the long-term inhibition of Rho-kinase ameliorates monocrotaline (MCT)-induced PAH and hypoxia-induced PAH in animal models (Fukumoto et al. 2007; Abe et al. 2004, 2006; Fukumoto and Shimokawa 2011; Oka et al. 2008; Dahal et al. 2010). In those studies, Rho-kinase activity of pulmonary arteries was enhanced irrespective of the different etiologies and long-term treatment with Rho-kinase inhibitors ameliorated endothelial dysfunction and suppressed hypercontraction and proliferation of VSMC and migration of inflammatory cells (Abe et al. 2004, 2006; Oka et al. 2008). In clinical studies, it also has been demonstrated that a Rho-kinase inhibitor, fasudil, acutely improves pulmonary hemodynamics in patients with PAH (Fukumoto et al. 2005; Fujita et al. 2010; Ishikura et al. 2006).

3 Novel Therapeutic Target as Rho-Kinase Pathway in PAH

3.1 Potential Importance of Rho-Kinase Inhibitors for the Treatment of PAH

We have It has recently been 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, 2006; Fukumoto et al. 2005; Jiang et al. 2007; Tawara et al. 2007; Yasuda et al. 2011). Potent and selective inhibitors of Rho-kinase like fasudil and azaindole-1 are available possessing an inhibitory effect on Rho-kinase being 100 times and 1,000 times more potent than on other protein kinases C and myosin light chain kinase, respectively (Shimokawa 2002; Shimokawa et al. 1999; Shimokawa and Rashid 2007b; Kast et al. 2007). Rho-kinase inhibition ameliorates VSMC hyperconstriction, increases eNOS expression, and decreases inflammatory cell migration and angiotensin II-induced upregulation of MCP-1 and PAI-1 (Shimokawa 2002; Morishige et al. 2001).

In the rat model of monocrotaline (MCT)-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). Further, the highly selective Rho-kinase inhibitor azaindole-1 has shown to reverse MCT-induced pulmonary hypertension (Dahal et al. 2010; Lohn et al. 2009). Oral treatment with fasudil and azaindole-1 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; Dahal et al. 2010; Lohn et al. 2009; Pankey et al. 2012; Peng et al. 2012). 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. 3). 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 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; Abe et al. 2005). Finally, the acute beneficial effects of intravenous fasudil have been shown in patients with PAH (Fukumoto et al. 2005; Fujita et al. 2010).

We have performed a clinical trial to examin the mid-term effects of Rho-kinase inhibitor, addressing the efficacy and safety in patients with PAH. (Fukumoto et al. 2013). Through those trials, we expect to learn whether the long-term inhibition of Rho-kinase is a novel therapeutic strategy for the treatment in patients with PAH.

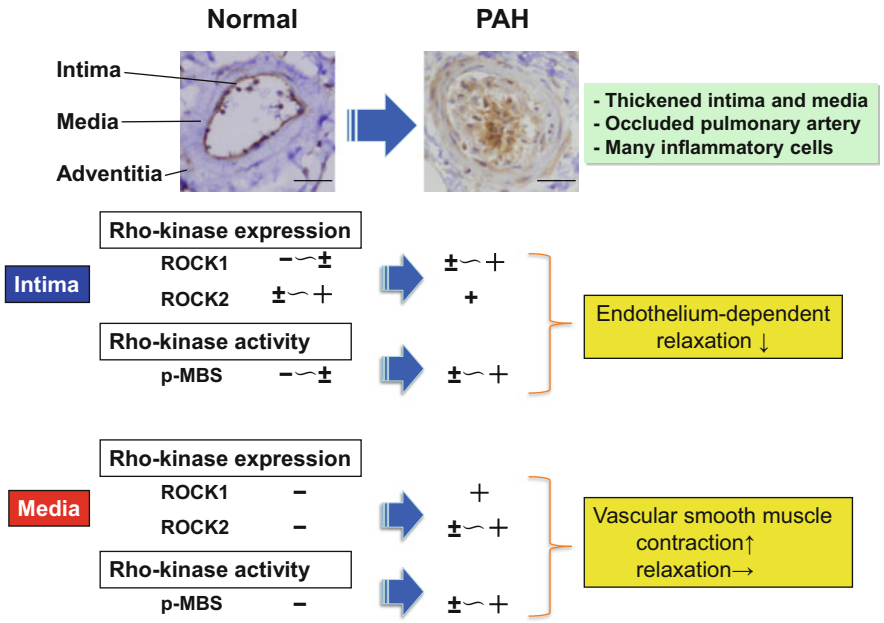

Fig. 4 Enhanced Rho-kinase expression and activity of pulmonary arteries from patients with pulmonary arterial hypertension. Immunohistological findings of Rho-kinase expression (both isoforms, ROCK 1 and 2) and activity in pulmonary arteries from patients with pulmonary arterial hypertension. Activated Rho-kinase expression and activity causes impairment of endothelium-dependent relaxation and enhances vascular smooth muscle contraction. *Scale bar*, 50 μm (from Fukumoto and Shimokawa 2011 with permission)

3.2 *Enhanced Rho-Kinase Expression and Activity in Patients with PAH*

Recently, the direct evidence for Rho-kinase activation has been demonstrated in patients with PAH (Do e et al. 2009), where Rho-kinase activity is enhanced in circulating neutrophils and the pulmonary arteries from patients with PAH, resulting in hypercontractions of the artery (Fig. 4). These findings support the previous findings in animal models of PAH and during right heart cardiac catheterization in patients with PAH (Shimokawa and Takeshita 2005; Abe et al. 2004, 2006; Fukumoto et al. 2005; Fujita et al. 2010; Oka et al. 2008; Ishikura et al. 2006). Thus, increased pulmonary vascular resistance may be caused, at least in part, by activated Rho-kinase pathway (Do e et al. 2009). In addition, in patients with PAH, eNOS expression is reduced and pulmonary VSMC are hyperreactive (Do e et al. 2009; Giaid and Saleh 1995; Xu et al. 2004). Indeed, activated Rho-kinase causes several important abnormalities, including eNOS downregulation in endothelial cells, VSMC hypercontraction through inhibition of myosin phosphatase, VSMC proliferation and migration, and inhibition of VSMC apoptosis (Figs. 1 and 2)

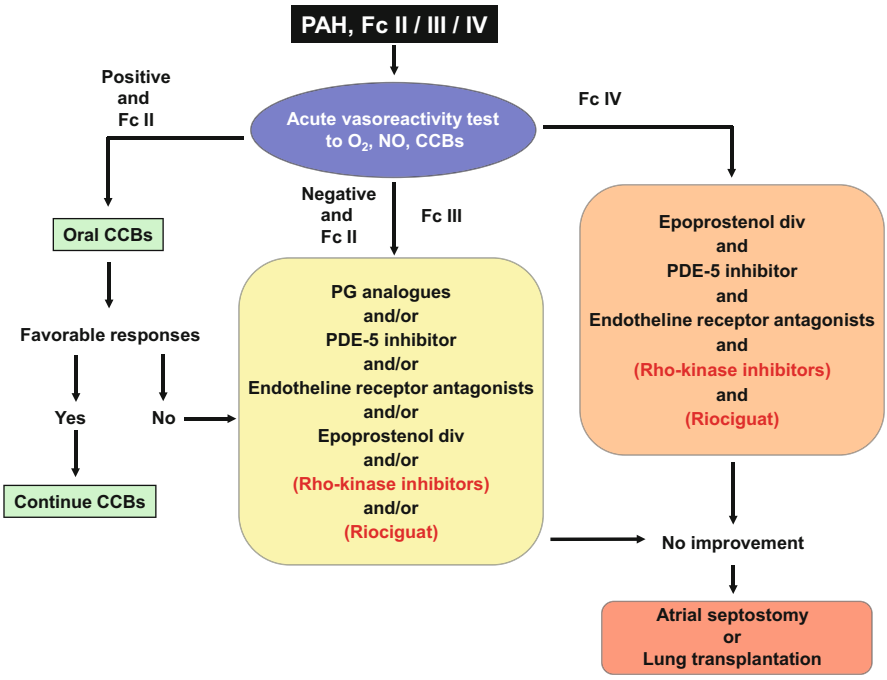

Fig. 5 Potential treatment algorithm for pulmonary arterial hypertension. In Fc II/III, the combination therapy can be beneficial compared with the mono-therapy. In Fc IV, the maximum use of medicines can be required. *CCBs* calcium channel blockers, *Fc* functional class, *NO* nitric oxide, *PAH* pulmonary arterial hypertension, *PG* prostaglandin, *PDE-5* phosphodiesterase-5 (from Fukumoto and Shimokawa 2011 with permission)

(Shimokawa and Takeshita 2005; Shimokawa 2002; Liao et al. 2007; Shimokawa and Rashid 2007b; Takemoto et al. 2002). Also, the direct evidence has been recently demonstrated that endothelial vasodilator function is impaired and VSMC contraction is enhanced in pulmonary arteries from patients with PAH (Fig. 4) (Do e et al. 2009). These findings are consistent with the previous studies with MCT-induced PH in rats and hypoxia-induced PH in mice and previous clinical studies with PAH patients (Abe et al. 2004, 2006; Fukumoto et al. 2005; Fujita et al. 2010; Oka et al. 2008; Ishikura et al. 2006). Furthermore, the inhibition of Rho-kinase abolishes VSMC hypercontraction of pulmonary arteries from idiopathic PAH patients (Do e et al. 2009), which is also consistent with the previous clinical study that acute inhibition of Rho-kinase improves pulmonary hemodynamics in PAH patients (Fukumoto et al. 2005; Fujita et al. 2010; Ishikura et al. 2006). However, it still remains to be examined whether those functional abnormalities of pulmonary arteries could be ameliorated by the long-term treatment with a Rho-kinase inhibitor in patients with PAH. For this purpose, the effects of long-acting oral form of fasudil in PAH patients are examined in a clinical trial.

4 New Developments of Novel Rho Kinase Inhibitors

Although a clinical trial with long-acting oral form of fasudil was done for the mid-term treatment, further clinical trials are required. Ahead of cardiovascular fields including PAH (Fukumoto et al. 2013), clinical use of Rho-kinase inhibitors has begun for glaucoma and ocular hypertension in ophthalmology (K-115 from D Western Therapeutics Institute/Kowa, AR-12286 from Aerie Pharmaceuticals Inc, AMA-0076 from Amakem NV) (Williams et al. 2011). As Rho-kinase pathway can be a new therapeutic target in various fields, new developments are required to establish safe and effective Rho-kinase inhibitors.

5 Future Perspectives and Conclusions

PAH still remains a fatal disease, leading to right ventricular failure and premature death. Although significant research progress has been made on the pathogenesis of PAH, especially with regard to Rho-kinase, the detailed mechanisms of the disorder still remain to be elucidated. In the clinical practice, significant progress has also been made for new treatment (e.g., endothelin receptor antagonists, Rho-kinase inhibitors, and riociguat, Fig. 5). The usefulness of those new drugs remains to be fully examined in future studies.

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.

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