Rho-Kinase Is an Important Therapeutic Target in Cardiovascular Medicine

Hiroaki Shimokawa, Akira Takeshita

Abstract—Rho-kinase has been identified as one of the effectors of the small GTP-binding protein Rho. Accumulating evidence has demonstrated that Rho/Rho-kinase pathway plays an important role in various cellular functions, not only in vascular smooth muscle cell (VSMC) contraction but also in actin cytoskeleton organization, cell adhesion and motility, cytokinesis, and gene expressions, all of which may be involved in the pathogenesis of cardiovascular disease. At molecular level, Rho-kinase upregulates various molecules that accelerate inflammation/oxidative stress, thrombus formation, and fibrosis, whereas it downregulates endothelial nitric oxide synthase. The expression of Rho-kinase itself is mediated by protein kinase C/NF-κB pathway with an inhibitory and stimulatory modulation by estrogen and nicotine, respectively. At cellular level, Rho-kinase mediates VSMC hypercontraction, stimulates VSMC proliferation and migration, and enhances inflammatory cell motility. In animal studies, Rho-kinase has been shown to be substantially involved in the pathogenesis of vasospasm, arteriosclerosis, ischemia/reperfusion injury, hypertension, pulmonary hypertension, stroke and heart failure, and to enhance central sympathetic nerve activity. Finally, in clinical studies, fasudil, a Rho-kinase inhibitor, is effective for the treatment of a wide range of cardiovascular disease, including cerebral and coronary vasospasm, angina, hypertension, pulmonary hypertension, and heart failure, with a reasonable safety. Thus, Rho-kinase is an important therapeutic target in cardiovascular medicine. (***Arterioscler Thromb Vasc Biol.* 2005;25:1767-1775.**)

Key Words: cardiovascular disease ■ Rho ■ small G proteins ■ signal transduction ■ Rho-kinase

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.[1,2] At least 10 members of the Rho family are present in mammals, including Rho (isoforms A to E, and G), Rac (isoforms 1 to 3), Cdc42, and TC10.[1,2] The effector domains of RhoA, RhoB, and RhoC (collectively referred to here as Rho) have the same amino acid sequence, and these G proteins appear to have similar intracellular targets.[1,2] Rho is known to modulate Ca^{2+}-sensitization of vascular smooth muscle cells (VSMCs) and is thought to act by inhibiting myosin phosphatase activity.[1,2]

In mid 1990s, 2 Japanese groups and 1 Singapore group independently identified one of the effectors of Rho and termed it as Rho-kinase α^3/ROKα^4/ROCK2.[5] Rho-kinase β^3/ROKβ^4/ROCK1[5] is an isoform of Rho-kinase. Hereafter, both Rho-kinase α/ROKα/ROCK2 and Rho-kinase β/ROKβ/ROCK1 are collectively referred to as Rho-kinase. Because systemic disruption of both Rho-kinase isoforms results in embryonic lethality in mouse (unpublished observations), studies with site-specific disruption of each isoform should be performed to elucidate the possible functional difference between the 2 isoforms.

In addition to Rho-kinase, several other proteins have been identified as effectors of Rho, including protein kinase N (PKN), rhophilin, rhotekin, citron, p140mDia, and citron kinase[6,7] (Figure 1). However, the roles of those effectors of Rho other than Rho-kinase remain to be examined. The substrates of Rho-kinase also have been identified, including the myosin-binding subunit (MBS) of myosin phosphatase (MLCPh), ERM (ezrin, radixin, moesin) family, adducin, intermediate filament (vimentin), Na^+-H^+ exchanger, and LIM-kinase[1] (Figure 1). It was subsequently demonstrated that Rho-kinase enhances myosin light chain (MLC) phosphorylation through inhibition of MBS of myosin phosphatase[8,9] (Figure 1).

The Rho/Rho-kinase pathway has recently attracted much attention in various research fields, especially in the cardiovascular research field, for several reasons (Figure 1). First, the Rho/Rho-kinase pathway plays an important role in various cellular functions that are involved in the pathogenesis of cardiovascular disease[10] (Figure 1). Second, this intracellular signaling pathway is substantially involved in the effects of many vasoactive substances that are implicated in the pathogenesis of cardiovascular disease.[10] Third, the so-called pleiotropic effects of statins are mediated, at least in

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From the Department of Cardiovascular Medicine (H.S., A.T.) and the Kyushu University COE Program on Lifestyle-Related Diseases (H.S.), Kyushu University Graduate School of Medical Sciences, Fukuoka; and Japan Science and Technology Agency (H.S.), CREST, Tokyo, Japan.

Correspondence to Hiroaki Shimokawa, MD, PhD, Chairman and Professor, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Seiryo-machi 1-1, Aoba-ku, Sendai 980-8574, Japan. E-mail shimo@cardio.med.tohoku.ac.jp

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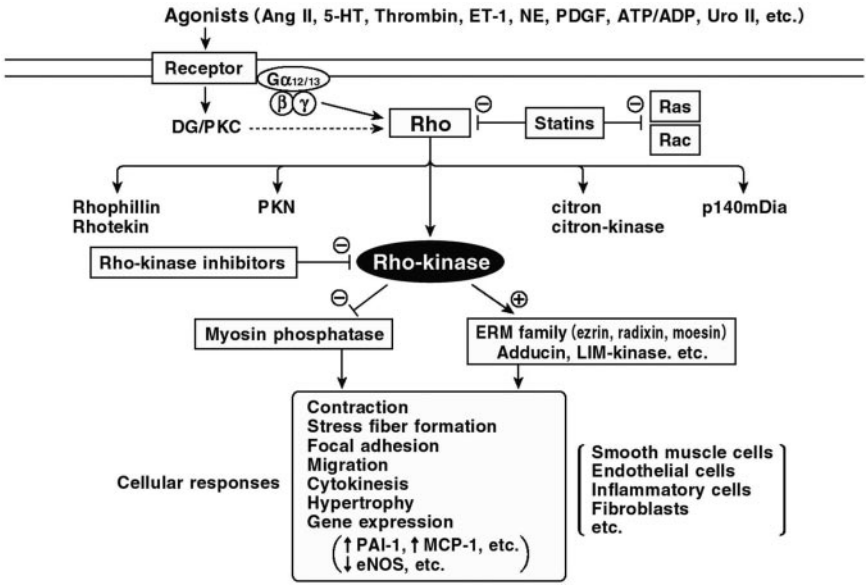

Figure 1. Role of Rho/Rho-kinase pathway in the pathogenesis of cardiovascular diseases. Rho/Rho-kinase–mediated pathway plays an important role in the signal transduction initiated by many agonists, including angiotensin II (Ang II), serotonin (5-HT), thrombin, endothelin-1 (ET-1), norepinephrine (NE), platelet-derived growth factor (PDGF), adenosine triphosphate (ATP)/adenosine diphosphate (ADP), and urotensin II (Uro II). Through the modulation of its target effectors, Rho-kinase is substantially involved in the vascular smooth muscle contraction (via inhibition of myosin phosphatase) and in the pathogenesis of arteriosclerosis (via activation of ERM, adducin, and other effectors). Whereas statins inhibit Rho at their relatively higher concentrations, they simultaneously inhibit pathways mediated by other G proteins, such as Ras and Rac. By contrast, Rho-kinase inhibitors selectively inhibit Rho-kinase pathway. DG indicates diacylglycerol; PKC, protein kinase C. Solid line indicates proven pathway; dashed line, proposed pathway.

part, by their inhibitory effects on Rho with a resultant inhibition on Rho-kinase[11] (Figure 1).

The initial works in the authors' laboratory on the therapeutic importance of Rho-kinase pathway were previously summarized.[10] Since then, a significant progress has been made in our knowledge of the therapeutic importance of Rho-kinase in cardiovascular medicine. This review briefly updates the recent progress in the translational research on the therapeutic importance of Rho-kinase in cardiovascular medicine, ranging from molecular and cellular levels to animal and clinical studies (Table).

Experimental Tools for Rho-Kinase

Rho-kinase consists of 3 major domains, including a catalytic (kinase) domain in its N-terminal domain, a coiled–coil domain in its middle portion, and a putative pleckstrin-homology (PH) domain in its C-terminal domain.[1,3] The Rho-binding (RB) domain of Rho-kinase is localized in the C-terminal portion of the coiled–coil domain and Rho-kinase activity is enhanced by binding GTP-Rho.[3] The kinase activity-deficient form or the C-terminal fragments that lack the kinase activity should theoretically serve as the dominant-negative form of Rho-kinase in cells.[1] Thus, the C-terminal fragment of Rho-kinase that contains the RB domain, deficient in Rho-binding activity after point mutations and the PH domain [RB/PH(TT)], serves as the dominant-negative form that specifically inhibits Rho-kinase.[1,10] As a pharmacological inhibitor of Rho-kinase, fasudil[12] and Y-27632[13] have been developed and they inhibit Rho-kinase activity in a competitive manner with ATP.[14] It has been recently demonstrated that hydroxyfasudil, a major active metabolite of fasudil after oral administration, has a more specific inhibitory effect on Rho-kinase.[15,16] The Ki value (μmol) of hydroxyfasudil and Y-27632 is 0.17 and 0.14 for Rho-kinase, 18 and 26 for protein kinase C (PKC), and 140 and >250 for MLC kinase (MLCK), respectively.[13,15]

Studies at Molecular Level

Rho-kinase mediates upregulation of pro-inflammatory molecules, including NAD(P)H,[16] IL-6,[17] monocyte chemoattractant protein (MCP)-1,[18] macrophage migration inhibitory factor (MIF),[19,20] and interferon (IFN)-γ.[19] It also upregulates thrombogenic molecules (eg, platelet-activating factor [plasminogen activator inhibitor (PAI)]-1[21] and tissue factor[22]) and fibrogenic molecules (eg, transforming growth factor [TGF]-β1[19] and Bcl-2[22]). By contrast, Rho-kinase downregulates endothelial nitric oxide synthase (eNOS)[23] and osteogenic molecules (leg, bone morphogenic protein [BMP]-2, and osteocalcin[24]). Thus, when Rho-kinase is activated, inflammatory processes, thrombosis, and tissue fibrosis are accelerated, whereas endothelial NO production and osteogenesis are inhibited.

The expression of Rho-kinase itself is accelerated by inflammatory stimuli, such as angiotensin II and IL-1β, through PKC/NF-κB pathway,[25] with a negative modulation by physiological concentration of estrogen and a positive modulation by clinical concentration of nicotine.[26] Remnant lipoproteins also upregulate Rho-kinase in human coronary VSMCs.[27] Indeed, mRNA expression of Rho-kinase is enhanced at the inflammatory and arteriosclerotic arterial lesions in animals[10,28,29] and humans,[30] causing hypercontraction of the artery. Interestingly, Rho-kinase is positively involved in its own expression.[31]

The promoter region of human Rho-kinase gene spanning $\approx$1200 base pairs was cloned and nucleotide sequences were determined. The promoter region was rich with guanine and cytosine. RNase protection assay revealed the presence of possible 2 transcription initiation sites. The database analysis suggests several possible cis DNA elements such as AP-1, Sp1, and Oct-1 in the Rho-kinase promoter region. However, the functions of these cis DNA elements have not been evaluated. The deletion analysis of the promoter region revealed that the DNA segment between -1 bp and -150 bp is responsible for the strong and constitutive promoter activity in

Translational Research on the Therapeutic Importance of Rho-Kinase in Cardiovascular Medicine

Study Level	References
Molecular level	
Gene expression	16–31
Promotor region analysis	—
Single nucleotide polymorphism	32
Role in signal transduction	16, 18, 21, 28, 33–41
Cellular level	
VSMC contraction	1, 10, 28, 29, 51
VSMC proliferation/migration	1, 29
Cell adhesion and motility	44, 53
Cytokinesis	45
Animal studies	
Coronary vasospasm	15, 27–29, 54–59
Cerebral vasospasm	60, 61
Arteriosclerosis/restenosis	19, 22, 53, 55, 62–64, 66, 67
Ischemia/reperfusion injury	68–70
Hypertension	13, 71–73
Pulmonary hypertension	74–77
Stroke	78, 79
Heart failure	80
Renal disease	81, 82
Glaucoma	85–88
Erectile dysfunction	89, 90
Clinical studies	
Angina	94–99
Hypertension	100
Pulmonary hypertension	101
Stroke	102
Heart failure	103

— indicates unpublished observations.

VSMCs, in which 4 possible Sp1 sites are present (unpublished observations). A novel missense mutation, G930T, has been identified in the catalytic domain of Rho-kinase (ROCK2) that is associated with enhanced Rho-kinase activity.[32] The prevalence of the mutation is higher in patients with vasospastic angina than in controls and is also higher in Japanese patients with ischemic heart disease than in white patients.[32]

Importantly, Rho-kinase is substantially involved in the vascular effects of various vasoactive factors, including angiotensin II,[16,18,21,33] serotonin,[28] thrombin,[34,35] endothelin-1,[36,37] norepinephrine,[38] platelet-derived growth factor,[39] extracellular nucleotides,[40] and urotensin II[41] (Figure 1).

It has previously been shown that 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitors (statins) enhance mRNA expression of eNOS by cholesterol-independent mechanisms involving inhibition of Rho geranylgeranylation.[11] Statins could inhibit intracellular signal transduction mediated by Rho, Ras, and Rac[11] (Figure 1). Qualitative and quantitative differences in the inhibitory effects of statins and Rho-kinase inhibitors on the Rho/Rho-kinase pathway remain to be fully elucidated.

Studies at Cellular Level

Rho-kinase plays an important role in mediating various cellular functions, not only VSMC contraction[1,10,28] but also actin cytoskeleton organization,[6,42,43] cell adhesion and motility,[44] cytokinesis,[45] and, as discussed, gene expression, all of which may be involved in the pathogenesis of arteriosclerosis/atherosclerosis (Figure 1).

Arteriosclerosis is a slowly progressing inflammatory process of arterial wall that involves all 3 layers (Figure 2).[46,47] In the intima, endothelial function is impaired, inflammatory cell adhesion to the endothelium with subsequent migration into the subintimal area is enhanced, and tissue factor and matrix metalloproteinases are upregulated. In the media, proliferation and migration of VSMCs are enhanced with increased vasoconstrictor responses and phenotypic changes. At the adventitia, inflammatory cell accumulation also is enhanced, fibroblasts are transformed into myofibroblasts, and the density of vasa vasorum is increased (Figure 2). Accumulating evidence has indicated that Rho-kinase–mediated pathway is substantially involved in all these processes (Figure 2). For instance, activated Rho-kinase downregulates eNOS,[23] whereas hydroxyfasudil rapidly increases endothelial eNOS activity and exerts cardiovascular protection.[48]

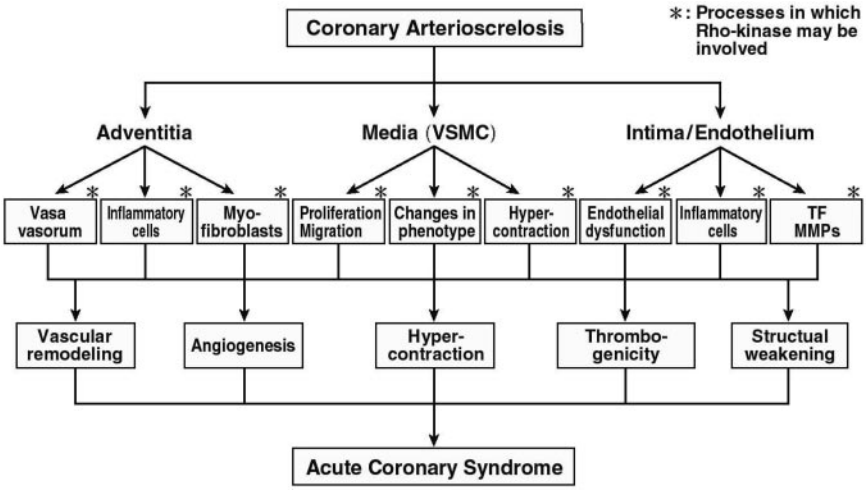

Figure 2. Involvement of Rho-kinase pathway in the pathogenesis of arteriosclerosis. Arteriosclerosis is a slowly progressing inflammatory process of arterial wall, in which all 3 layers of blood vessels, intima, media, and adventitia, are involved. In each layer, several cellular processes are induced, resulting in the development of structural weakening, angiogenesis, thrombus formation, hypercontraction, and vascular remodeling with a resultant occurrence of acute vascular events. Rho-kinase pathway may be involved in all of the cellular processes in all the 3 vascular layers. VSMC indicates vascular smooth muscle cells.

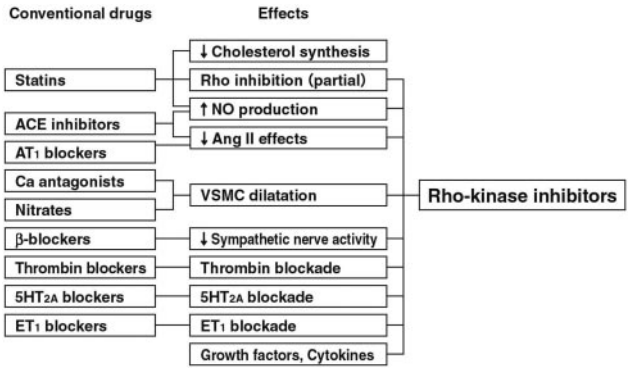

Figure 3. Broad pharmacological properties of Rho-kinase inhibitors. ACE indicates angiotensin-converting enzyme inhibitor; AT1, angiotensin II type 1 receptor; $5HT_{2A}$, $5HT_{2A}$ serotonergic receptor; VSMC, vascular smooth muscle cells.

Importantly, NO antagonizes the vasoconstrictor effect of Rho-kinase through activation of myosin phosphatase.[49] Rho-kinase also upregulates tissue factor in the intima[22] and is involved in endothelial contraction that increases endothelial permeability and hence enhances atherosclerosis.[50] Activated Rho-kinase causes VSMC hypercontraction through inhibition of myosin phosphatase[28,29,51] and accelerates VSMC proliferation and migration and inhibits VSMC apoptosis in the media,[1,33,52] and enhances accumulation of inflammatory cells at the adventitia.[53] Those Rho-kinase–mediated cellular responses lead to the development of structural weakening, increased thrombogenicity, hypercontraction, pathological angiogenesis, and vascular remodeling, resulting in vascular crisis such as acute coronary syndrome (Figure 2). Thus, Rho-kinase is an important therapeutic target for the treatment of arteriosclerotic cardiovascular disease.

Animal Studies

Accumulating evidence indicates that Rho-kinase inhibitors have broad pharmacological properties that could cover those of many cardiovascular drugs currently used in cardiovascular medicine, except for lipid-lowering effect of statins (Figure 3). Because of their unique pharmacological properties, Rho-kinase inhibitors could cover the pharmacological effects of many conventional cardiovascular drugs, including statins, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II type 1 receptor blockers, calcium channel blockers, nitrates, β-blockers, and blockers of thrombin, serotonin, and endothelin. One of the exceptions is the cholesterol lowering effect of statins (Figure 3).

The beneficial effects of long-term inhibition of Rho-kinase for the treatment of cardiovascular disease have been demonstrated in animal models for various cardiovascular diseases, including coronary and cerebral vasospasm, arteriosclerosis/restenosis, ischemia/reperfusion injury, hypertension, pulmonary hypertension, stroke, heart failure, and others (Table, Figure 4).

Coronary Vasospasm

Coronary vasospasm plays an important role in a wide variety of ischemic heart diseases, not only in variant angina but also in other forms of angina pectoris, myocardial infarction, and sudden death.[28] Accumulating evidence indicates that Rho-kinase is substantially involved in the pathogenesis of coronary vasospasm. Intracoronary administration of fasudil[54] and of hydroxyfasudil[15] markedly inhibits coronary spasm in a porcine model with long-term treatment with IL-1β.[55] This also is the case in other porcine models of coronary spasm with long-term treatment with MCP-1[53] and remnant lipoproteins (from patients with sudden cardiac death).[27] Importantly, the inhibition of Rho-kinase with fasudil/hydroxyfasudil is associated with the suppression of enhanced myosin light chain (MLC) phosphorylations (both MLC monophosphorylations and diphosphorylations) at the spastic coronary segments in those models.[15,54] The activity and the expression of Rho-kinase are enhanced at the inflammatory/arteriosclerotic coronary lesions, thereby suppressing myosin phosphatase through phosphorylation of its MBS with resultant increase in MLC phosphorylations and coronary spasm.[28,29] Anti-ischemic effect of fasudil has also been demonstrated in a rabbit model of myocardial ischemia induced by intrave-

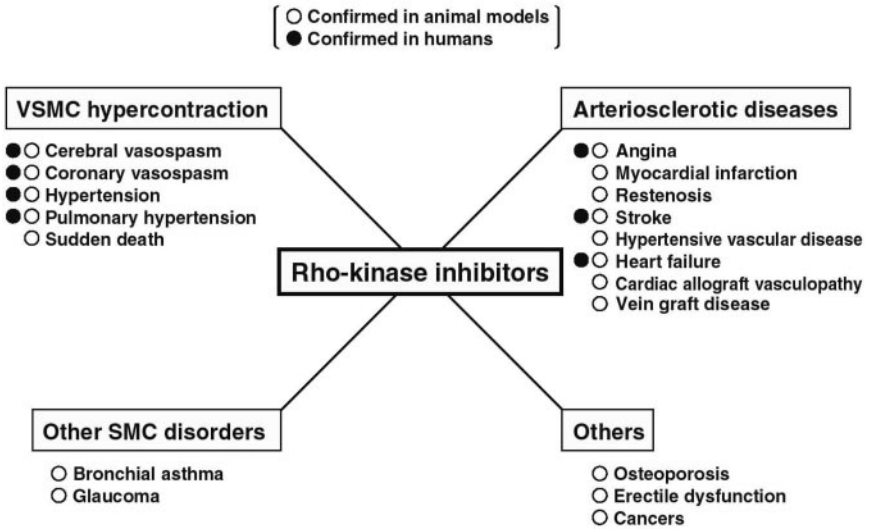

Figure 4. Therapeutic targets of Rho-kinase inhibitors. Rho-kinase inhibitors appear to be useful for the treatment of disorders caused by vascular smooth muscle hyperconstriction, including cerebral vasospasm, coronary vasospasm, hypertension, pulmonary hypertension, and possibly vasospasm-related sudden death. They also may be useful for the treatment of various arteriosclerotic cardiovascular diseases, including angina, myocardial infarction, restenosis, stroke, hypertensive vascular disease, heart failure, cardiac allograft vasculopathy, and vein graft disease. They also may be useful for the treatment of disorders associated with smooth muscle hyperreactivity, such as bronchial asthma and glaucoma. Recent studies suggested that they also might be useful for the treatment of osteoporosis, erectile dysfunction, and cancers. The clinical usefulness of Rho-kinase inhibitors remain to be fully elucidated in future studies.

nous administration of endothelin-1,[56] a dog model of pacing-induced myocardial ischemia in the presence of coronary stenosis,[57] and a rat model of vasopressin-induced chronic myocardial ischemia.[58] Recently, it has been demonstrated that sustained elevation of serum level of cortisol, one of the important stress hormones, causes coronary hyperreactivity through activation of Rho-kinase in pigs in vivo.[59]

Cerebral Vasospasm

Rho-kinase pathway is involved in the pathogenesis of cerebral vasospasm after subarachnoid hemorrhage as myosin phosphatase in cerebral VSMCs is suppressed by activated Rho-kinase in dogs in vivo.[60] Rho-kinase and PKC play an important role in oxyhemoglobin-induced cerebral contractions.[61]

Arteriosclerosis/Restenosis

Both in vivo gene transfer of dominant-negative Rho-kinase[62] and long-term treatment with a Rho-kinase inhibitor[63,64] suppress balloon injury-induced neointimal formation in animals in vivo. Long-term treatment with MCP-1 and oxidized low-density lipoproteins (ox-LDL) causes vascular lesions characterized by neointimal formation and constrictive remodeling in porcine coronary arteries in vivo.[53] Long-term oral treatment with fasudil significantly suppressed this vascular lesion formation caused, at least in part, by the inhibition of macrophage migration in vivo.[53] The porcine model of coronary vasospasm/arteriosclerosis with IL-1β is characterized by constrictive remodeling,[55] which is also an important mechanism for restenosis after coronary intervention.[65] Importantly, the long-term inhibition of Rho-kinase by either hydroxyfasudil[66] or in vivo gene transfer of dominant-negative Rho-kinase[67] induces a marked regression of the constrictive remodeling in this porcine model in vivo. The regression of constrictive remodeling is associated with functional inhibition of ERM family (ezrin, radixin, and moesin) and adducin, suggesting that these effectors of Rho-kinase may be involved in the development and maintenance of the vascular remodeling.[66,67] The long-term treatment with fasudil also effectively suppresses in-stent restenosis in porcine coronary arteries, for which multiple mechanisms (eg, reduced vascular inflammation, enhanced apoptosis, and decreased collagen deposition) are involved.[22]

Rho-kinase also is involved in the pathogenesis of cardiac allograft vasculopathy and of vein graft disease as the long-term treatment with fasudil significantly inhibits the coronary vascular lesion formation in a mouse model of cardiac allograft vasculopathy[19] and in a rabbit model of vein graft disease (unpublished observations), respectively.

Ischemia/Reperfusion Injury

Rho-kinase activation is involved in the pathogenesis of myocardial ischemia/reperfusion injury and pretreatment with fasudil before reperfusion prevents endothelial dysfunction and suppresses the development of myocardial infarction in dogs in vivo.[68] Rho-kinase inhibition seems to be a novel strategy independent of PKC for ischemic preconditioning in dogs in vivo.[69] Inhibition of Rho-kinase with fasudil also is effective in inhibiting cold ischemia/reperfusion injury after liver transplantation in rats.[70]

Hypertension

Short-term administration of Y-27632 preferentially reduces systemic blood pressure in various rat models of systemic hypertension irrespective of the mechanisms of hypertension, suggesting an involvement of Rho-kinase in the pathogenesis of hypertension in general.[13] In spontaneously hypertensive rats (SHR), the expression and the activity of Rho-kinase are significantly enhanced even before the development of hypertension, suggesting that Rho-kinase pathway is substantially involved in the pathogenesis of hypertension and hypertensive vascular disease.[71] Importantly, long-term treatment with a nonhypotensive dose of fasudil significantly suppresses coronary vascular lesion formation (medial thickening and perivascular fibrosis) in SHR, indicating that Rho-kinase pathway is involved in the pathogenesis of hypertensive vascular disease that is distinct from that of systemic hypertension.[71] To further address the inhibitory effect of a Rho-kinase inhibitor on hypertensive vascular disease, the effect of fasudil was examined in a rat model with long-term infusion of angiotensin II that is also characterized by hypertension and coronary vascular lesions (medial thickening and perivascular fibrosis). The treatment with nonhypotensive doses of fasudil again significantly suppressed the coronary vascular lesion formation in this rat model along with normalization of endothelial NAD(P)H oxidase activity and endothelial production of superoxide anions and resultant improvement of endothelial vasodilator function.[16] Furthermore, the treatment with fasudil also inhibited the angiotensin II-induced cardiac hypertrophy.[16] These results indicate that Rho-kinase is substantially involved in both hypertensive vascular disease and hypertensive cardiac hypertrophy. The blood pressure-lowering effect of fasudil is strictly dependent on the dose of the Rho-kinase inhibitor administered.

Local administration of a small amount of hydroxyfasudil or of adenovirus solution containing dominant-negative Rho-kinase into nucleus tractus solitarii causes sustained decrease in heart rate and blood pressure in SHR but not in normotensive WKY, suggesting that Rho-kinase may also be involved in the central mechanisms of sympathetic nerve activity.[72] Inhibition of Rho-kinase in the brain stem also augments baroreflex control of heart rate in rats.[73]

Pulmonary Hypertension

Primary pulmonary arterial hypertension is a fatal disease characterized by endothelial dysfunction, VSMC hypercontraction and proliferation and inflammatory cell migration, for which Rho-kinase may also be substantially involved. Long-term treatment with fasudil suppresses the development of monocrotaline-induced pulmonary hypertension in rats when started simultaneously and even induces a marked regression when started after establishment of pulmonary hypertension.[74] Fasudil also is effective to inhibit the development of pulmonary hypertension induced by chronic hypoxia in mice through eNOS-dependent and eNOS-independent mechanisms.[75] Inhalation of fasudil may also be effective to reduce pulmonary vascular resistance in animal

model of pulmonary hypertension with various etiologies.[76] Because prostacyclin lacks the inhibitory effects on Rho-kinase,[77] the combination therapy with prostacyclin and a Rho-kinase inhibitor may provide a useful therapeutic strategy for this fatal disorder.

Stroke

In a rat model of stroke (lacunar infarction) caused by pharmacological damage of endothelial cells and subsequent thrombotic occlusion, intraperitoneal administration of fasudil shortly after the endothelial damage reduces cerebral infarct size and resultant neurological deficit.[78] In a rat model of microembolization stroke, intravenous administration of hydroxyfasudil prevents neutrophil accumulation, reduces cerebral infarct size, and improves neurological functions.[79] These results suggest the efficacy of fasudil/hydroxyfasudil for the treatment of ischemic brain damage.

Heart Failure

In a dog model of tachypacing-induced heart failure, the Ca^{2+}-sensitizing mechanism of conduit artery (femoral artery) is augmented, resulting in the enhanced vasoconstrictor response to norepinephrine.[80] Y-27632 attenuates this response without a significant change in intracellular Ca^{2+} concentrations in VSMC, suggesting an involvement of Rho/Rho-kinase pathway in the increased vasoconstrictor response in heart failure.[80]

Other Forms of Vascular Diseases

Inhibition of Rho-kinase has been shown to be effective to attenuate interstitial fibrosis in rats with unilateral ureteral obstruction[81] and glomerulosclerosis in Dahl salt-sensitive rats.[82] Long-term treatment with a Rho-kinase inhibitor may also be useful for the treatment of arteriosclerosis obliterans and Raynaud disease although these points remain to be examined.

Disorders Other Than Cardiovascular Diseases

The strategy to inhibit Rho-kinase may also be useful for the treatment of other disorders associated with smooth muscle hyperreactivity, such as bronchial asthma and glaucoma (Figure 4). It has been recently demonstrated that Rho-kinase is involved in bronchial smooth muscle contraction[83,84] and the regulation of aqueous humor outflow and other related mechanisms.[85–88] Because Rho-kinase negatively regulates osteogenesis,[24] inhibition of Rho-kinase may be a new strategy for the treatment of osteoporosis. Rho-kinase inhibitors may also be useful for the treatment of erectile dysfunction as they improve cavernosal smooth muscle relaxation.[89,90] Because of its inhibitory effects on cell replication and migration (metastasis) and neovascular formation, Rho-kinase inhibitors also are implicated in the treatment of cancers,[91] although this point remains to be examined in future studies (Figure 4).

Clinical Studies

Although fasudil is the only clinically available Rho-kinase inhibitor at present, several other Rho-kinase inhibitors are currently undergoing investigation. Whereas the intravenous form of fasudil is used for the treatment of cerebral vasospasm only in Japan,[92,93] its oral form is undergoing clinical trials for angina pectoris in Japan[94] and Northern America.[95] The adverse effects of fasudil are minimal so far;[94,95] however, careful development of Rho-kinase inhibitors is needed. Clinical studies with fasudil have suggested that the Rho-kinase inhibitor may be useful for the treatment of a wide range of cardiovascular diseases in addition to cerebral vasospasm, including angina pectoris, hypertension, pulmonary hypertension, stroke, and heart failure (Figure 4).

Angina

In patients with vasospastic angina, intracoronary fasudil markedly inhibits acetylcholine-induced coronary spasm and related myocardial ischemia, demonstrating that Rho-kinase pathway is substantially involved in the pathogenesis of coronary spasm in humans.[96] Fasudil is also effective in treating patients with microvascular angina, indicating an involvement of Rho-kinase-mediated hyperreactivity of coronary microvessels.[97] The clinical trials for the antianginal effects of fasudil in Japanese patients with stable effort angina have demonstrated that the long-term oral treatment with the Rho-kinase inhibitor is effective in ameliorating exercise tolerance in those patients with adequate safety profiles.[94] Intracoronary administration of fasudil is effective in reducing tachypacing-induced myocardial ischemia in patients with stable effort angina without changing heart rate or blood pressure.[98] These results suggest that inappropriate coronary vasoconstriction may be involved even in the pathogenesis of effort angina that is effectively suppressed by Rho-kinase inhibitors. Intracoronary fasudil also is effective for the treatment of intractable coronary spasm resistant to maximal vasodilator therapy with calcium channel blockers and nitrates after coronary artery bypass surgery.[99] The potential usefulness of Rho-kinase inhibitors for the treatment of unstable angina and myocardial infarction remain to be examined in future studies.

Hypertension

The vasodilator responses of forearm circulation in response to intra-arterial infusion of fasudil are markedly enhanced in hypertensive patients as compared with normotensive controls, whereas those to nitroprusside were comparable between the 2 groups.[100] This suggests that Rho-kinase is involved in the increased peripheral vascular resistance in hypertension in humans (Figure 4). It remains to be examined whether long-term inhibition of Rho-kinase also ameliorates hypertensive vascular disease and/or cardiac hypertrophy in humans.

Pulmonary Hypertension

Intravenous infusion of fasudil significantly reduces pulmonary vascular resistance in patients with pulmonary hypertension, indicating an involvement of Rho-kinase pathway in the pathogenesis of pulmonary hypertension in humans.[101] The long-term effects of oral administration of fasudil in patients with pulmonary hypertension remain to be examined.

Stroke

A clinical trial with intravenous form of fasudil in the acute phase of stroke in Japan demonstrates that the Rho-kinase inhibitor exerts beneficial effects on ischemic neuronal damage without any serious adverse effects.[102]

Heart Failure

In patients with heart failure, intra-arterial infusion of fasudil causes preferential increase in forearm blood flow as compared with control subjects, suggesting an involvement of Rho/Rho-kinase pathway in the increased peripheral vascular resistance in heart failure in humans.[103] The long-term effects of fasudil as a vasodilator therapy in the treatment of heart failure remain to be examined.

Concluding Remarks

Translational research from gene levels to clinical studies on the therapeutic importance of Rho-kinase in cardiovascular medicine is briefly summarized in this article. Accumulating evidence suggests that Rho-kinase is substantially involved in the pathogenesis of a wide spectrum of cardiovascular diseases and that Rho-kinase inhibitors are useful for the treatment of those cardiovascular diseases with a broad spectrum of pharmacological properties (Figures 3 and 4). Importantly, the clinical trials with fasudil demonstrate its efficacy and safety profile in humans. However, further careful studies are needed to confirm the potential therapeutic importance of Rho-kinase and clinical usefulness of Rho-kinase inhibitors in cardiovascular medicine in humans.

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