Rho-kinase inhibition: a novel therapeutic target for the treatment of cardiovascular diseases

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The Rho/rho-kinase (ROCK) pathway has an important role in the pathogenesis of several cardiovascular diseases. The activation of ROCK is involved in the regulation of vascular tone, endothelial dysfunction, inflammation and remodeling. The inhibition of ROCK has a beneficial effect in a variety of cardiovascular disorders. Evidence from animal models and from clinical use of ROCK inhibitors, such as Y-27632, fasudil and statins (i.e. pleiotropic effects), supports the hypothesis that ROCK is a potential therapeutic target. This review provides a current understanding of the role of ROCK pathway in the regulation of vascular function and the use of ROCK inhibitors in the treatment of cardiovascular disorders.

Introduction

The Rho kinases (ROCKs) were initially discovered as downstream targets of the small GTP-binding protein Rho. Given that ROCKs mediate various important cellular functions such as cell shape, motility, secretion, proliferation and gene expression, it is postulated that these pathways might interact with other signaling pathways known to contribute to cardiovascular disease. To date, ROCKs have been implicated in the regulation of vascular tone, proliferation, inflammation and oxidative stress. Evidence from animal studies suggests potential involvement of ROCK signaling in systemic and pulmonary hypertension, vascular inflammation, and atherosclerosis. Clinically, inhibition of ROCK pathway is believed to contribute to some of the cardiovascular benefits of statin therapy that are independent of lipid lowering (i.e. 'pleiotropic' effects). The extent to which ROCK activity is inhibited in patients on statin therapy is not known, although it might have important clinical implications. Various ROCK inhibitors are currently under development and in clinical trials as the next generation of therapeutic agents for cardiovascular diseases.

Rho/ROCK

Families of small G proteins such as Rho, Ras, Rab, Sarl/Arf and Ran are substantially involved in intracellular signaling [1]. The Rho family members, including Rho, Rac and Cdc42, regulate both cytoskeletal reorganization and gene expression. The effector domains of RhoA, RhoB and RhoC (collectively referred to here as 'Rho') have the same amino acid sequence, and these G proteins seem to have similar intracellular targets. As with other Rho GTPases, Rho acts as a molecular switch, cycling between an active GTP-bound state and an inactive GDP-bound state [2]. The exchange between the active and the inactive states is regulated by several regulatory proteins such as guanine dissociation inhibitor, guanine nucleotide exchange factor (GEF) and GTPase-activating protein. In unstimulated cells, Rho resides predominantly in the cytosol in its inactive GDP-bound form, and Rho guanine dissociation inhibitor binds to Rho-GDP and extracts it from the membrane to the cytosol. When cells are stimulated with certain agonists, Rho-GDP is converted to Rho-GTP through the action of Rho GEF. Rho-GTP is then targeted to the cell membrane, where it interacts with its specific targets (Fig. 1). Rho GTPase-activating protein inactivates Rho by dephosphorylating GTP to GDP. The best-characterized downstream effector of Rho is ROCK, which mediates various cellular functions [2]. ROCK was identified in the mid-1990s as one of the downstream effectors of Rho [1,2]. There are two isoforms of ROCK: ROCK1 and ROCK2 [1,2]. The genes expressing human ROCK1 and ROCK2 are located on chromosome 18 (18q11.1) and chromosome 2 (2p24), respectively [3,4]. ROCK1 and ROCK2 are highly homologous, sharing 65% homol-

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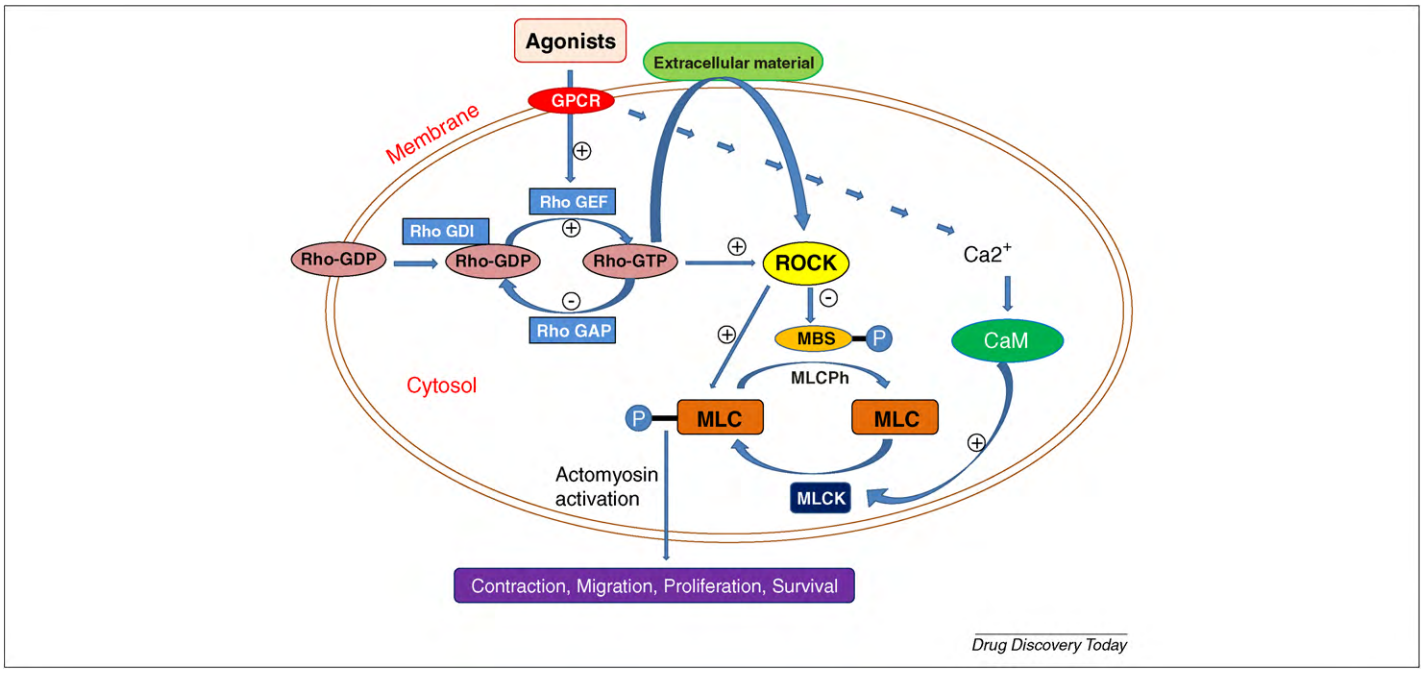

FIGURE 1

The Rho GDP–Rho GTP signaling pathway from membrane to the cytosol. With the binding of Rho GDI to Rho-GDP, inactivated Rho-GDP is extracted from the membrane to the cytosol. When cells are stimulated with certain agonists, Rho-GDP is converted to Rho-GTP through the action of Rho GEF. Rho-GTP is then targeted to the specific targets. Rho GAP inactivates Rho by dephosphorylating GTP to GDP. The downstream effector of Rho is ROCK. Stimulation of GPCR also leads to ROCK activation via Rho GEF. Activated ROCK, mediated through, phosphorylates various downstream targets including the MBS of MLCP. Phosphorylation of MBS inhibits MLCP activity leading to increased MLC phosphorylation and actomyosin activation. Abbreviations: CaM, calcium/calmodulin; GPCR, G-protein-coupled receptor; MBS, myosin-binding subunit; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; Rho GAP, Rho GTPase-activating protein; Rho GDI, Rho guanine dissociation inhibitor; Rho GEF, Rho guanine nucleotide exchange factor; ROCK, Rho kinase. Stimulation is denoted by +; inhibition is denoted by −.

ogy in amino acid sequence and 92% homology in their kinase domains. Both isoforms are ubiquitously expressed in human. ROCK2 is highly expressed in the brain and the heart, whereas ROCK1 is expressed preferentially in the lung, liver, spleen, kidney and testis [5].

The substrates of ROCK have been identified, including: the myosin-binding subunit of myosin light chain phosphatase (MLCP); the ezrin, radixin, moesin family; adducin; intermediate filaments (e.g. vimentin and desmin); the Na^+–H^+ exchanger; and LIM kinase [1]. In addition to ROCK, several other proteins have been identified as effectors of Rho, including protein kinase N, rhophilin, rhotekin, citron, p140mDia and citron kinase [1]. Although studies have shown the effectors' involvement of actin cytoskeleton organization [6] and neuronal differentiation [7], their roles remain to be examined.

ROCK at cellular level

ROCK and vascular smooth muscle cells

The Rho/ROCK pathway is a major regulator of vascular smooth muscle cell contraction and is important in controlling migration, proliferation, differentiation, apoptosis, survival and gene transcription [8]. The major mediator of smooth muscle contraction is phosphorylation and dephosphorylation of myosin light chain (MLC) [9]. MLC is phosphorylated by the Ca^{2+}-calmodulin-activated MLC kinase and dephosphorylated by the Ca^{2+}-independent MLCP. However, stimulation of tyrosine kinase and G-protein-coupled receptors leads to activation of Rho via recruitment and activation of Rho GEF [10]. Phosphorylation and inhibition of MLCP by ROCK increases MLC phosphorylation and cellular contraction by facilitating interaction of myosin with F-actin (Fig. 1). This ROCK-mediated contraction can occur independently of intracellular Ca^{2+} changes and is known as Ca^{2+} sensitization [11]. It has recently been shown that in normotensive arteries, ROCK-mediated calcium sensitization is involved in the maintenance of basal myogenic tone and, to a lesser extent, in pressure-dependent development [12]. Pressure-dependent activation of this enzyme is enhanced in hypertension with greater contribution to the maintenance of myogenic tone [12].

ROCK and endothelial cells

Endothelium-derived nitric oxide (NO) plays an important part in the regulation of vascular tone, inhibition of platelet aggregation, suppression of smooth muscle cell proliferation and prevention of leukocyte recruitment to the vessel wall. Increased bioavailability of NO is partially dependent on increased expression and activity of endothelial nitric oxide synthase (eNOS) and decreased inactivation of NO by reactive oxygen species. Rho/ROCK activation plays a part in oxidized LDL-induced endothelial cell contractility [13] and in the modulation of endothelial fibrinolytic activity [14]. The Rho/ROCK signaling pathway is involved in the regulation of endothelial barrier function, inflammation and transendothelial leukocyte migration, platelet activation, thrombosis, gene expression and oxidative stress [15]. Studies have found that ROCK activation decreases the expression of eNOS by reducing eNOS

mRNA stability [16]. Direct inhibition of Rho by C3 transferase, inhibition by ROCK inhibitors or overexpression of dominant-negative ROCK prevent downregulation of eNOS expression and eNOS mRNA stability [16]. ROCKs also negatively regulate eNOS function via a tonic inhibitory effect on the PI3-kinase/Akt pathway [17] and possibly by stimulation of arginase activity, leading to the damage of vessel function [18].

In addition to lipid-lowering effects, statins exert pleiotropic effects, such as inhibition of vascular inflammation and atherosclerosis through Rho GTPases (Rho, Rac1 and Ras) on the vascular wall [19]. Statins upregulate and activate eNOS expression through inhibition of Rho geranylgeranylation [19]. Clinically, statins inhibit Rho geranylgeranylation at lipid-lowering doses [20], and statin-induced improvement in endothelial-dependent vasomotion is mediated, in part, by inhibition of ROCK activity [21].

ROCK and adventitia

In a porcine model of coronary arteriosclerosis, treatment with fasudil (a selective ROCK inhibitor) markedly reduces macrophage accumulation in the adventitia and migration into the media [22]. The importance of adventitial accumulation of inflammatory cells has been suggested for the pathogenesis of arteriosclerosis [23] and acute coronary syndrome and coronary artery restenosis after percutaneous coronary intervention [24]. Furthermore, ROCK inhibition suppresses in-stent neointimal formation in porcine coronary arteries by reducing vascular inflammation, enhancing apoptosis and decreasing collagen deposition [25]. Long-term treatment with fasudil or adenovirus-mediated transfer of dominant-negative ROCK induces regression of both constrictive remodeling and coronary vasospastic activity in a porcine model with adventitial inflammation [26].

ROCK and cardiomyocytes

Mitochondrial RNA of both ROCK1 and ROCK2 is expressed in the developing heart [27]. ROCK inhibition can block migration of pre-cardiac mesoderm and cardiac tube fusion in cultured chick and mouse embryos [27]. In cultured mouse embryos, inhibition of ROCK decreases cell proliferation but does not lead to programmed cell death, suggesting that ROCK regulates cardiomyocyte division but not apoptosis during heart development [28]. This effect is mediated through the regulation of cell-cycle protein expression, cyclin D3, CDK6 and p27Kip1 in cardiomyocytes [28].

ROCK signaling is also involved in cell proliferation and migration during endocardial cushion development, myocardial hypertrophy and cardiac fibrosis [29]. Recent studies in ROCK1-deficient mice indicate that ROCK1 is required for the development of cardiac fibrosis [30]. Hattori *et al.* [31] reported that ROCK is substantially involved in the pathogenesis of left ventricular remodeling after myocardial infarction associated with upregulation of pro-inflammatory cytokines, indicating a potential therapeutic target for preventing post-infarct heart failure.

ROCK and the central nervous system

In the central nervous system (CNS), Rho GTPases are essential regulators of neuronal growth, axonal migration and dendritic spine morphogenesis. In addition, recent studies have identified Rho GTPases as central players in the molecular pathways that determine neuronal survival and death. Interestingly, individual Rho family members have either a pro-death or a pro-survival role in the nervous system, depending on both the type of neuron and the particular neurodegenerative insult involved [32].

Rho/ROCK pathway is involved in neurite outgrowth, and upregulation of eNOS with ROCK inhibition has a potential neuroprotective effect [19]. Thus, ROCK regulators might be potential important targets for axonal repair strategies in CNS injury, ischemic stroke and Alzheimer's disease [33]. In patients with stroke, fasudil increases cerebral blood flow in both ischemic and non-ischemic areas, reduces cerebral infarct size and improves neurological deficits [19]. A study by Yamashita *et al.* [34] demonstrated an increase in neural (axonal) ROCK expression and activity in ischemic brain tissue, which was reduced by fasudil that inhibited oxygen-glucose deprivation-induced PC12 cell death and glutamate-induced neurotoxicity. In another study, Yamaguchi *et al.* [35] demonstrated that ROCK regulated the tumor necrosis factor alpha (TNF-α)-induced interleukin (IL)-6 release, suggesting that ROCK inhibition might alleviate cerebral vasospasms. TNF-α is widely recognized as a prototypic proinflammatory cytokine. In the central nervous system, TNF-α is released from neurons, astrocytes and microglia [36]. Therefore, ROCK inhibitor can be considered to be a new clinical candidate for the treatment of CNS disorders, in addition to cerebral vasospasms.

ROCK and cardiovascular diseases

Inflammation and atherosclerosis

Atherosclerosis is characterized by progressive inflammation, accumulation of lipids and fibrosis in the arterial wall [37], and mRNA expression of ROCK has been enhanced in atherosclerotic lesions in both animal [38] and human studies [39]. Within fibroblasts and inflammatory cells, ROCK upregulates pro-inflammatory molecules including activator protein-1, NF-κB [40], NAD(P)H [29], IL-6 [41], monocyte chemoattractant protein-1 [42], macrophage migration inhibitory factor [31] and interferon-γ [31], all of which are involved in the pathogenesis of atherosclerosis. The expression of ROCK itself is accelerated by inflammatory stimuli such as nicotine [43] and angiotensin II and IL-1β, which are mediated through the PKC/NF-κB pathway [44], and is negatively modulated by estrogen. Interestingly, ROCK is positively involved in its own expression [44]. Because ROCK itself mediates the intracellular signaling initiated by those inflammatory agonists [45], it is also conceivable that ROCK and inflammatory agonists activate each other and promote the process of arteriosclerosis.

Cumulating evidence indicates that ROCK-mediated pathway is involved in all stages of the inflammatory process. Activated ROCK downregulates eNOS [16], whereas ROCK inhibition by hydroxyfasudil rapidly increases endothelial eNOS activity [46]. Nitric oxide itself antagonizes the vasoconstrictor effect of ROCK through activation of myosin phosphatase [47]. ROCK activation leads to endothelial hyperpermeability and hence enhances atherosclerosis [48]. Furthermore, ROCK1 has a key role in macrophage chemotaxis, cholesterol uptake and foam cell formation, all of which are hallmark events in the pathogenesis of atherosclerosis [49]. ROCK1 also mediates neointimal proliferation via recruit-

ment of circulating leukocytes and infiltration of inflammatory cells into the vessel wall [50]. In a low-density lipoprotein receptor knockout mice model, activation of the transcription factor NF-κB via the Rho/ROCK pathway was enhanced after a high-fat cholate-free diet, and inhibition of ROCK was associated with suppression of early atherosclerotic plaque development [51].

The anti-inflammatory properties of statins (hydroxy-3-methyl-glutaryl coenzyme-A reductase inhibitors) might be mediated, at least in part, by the inhibition of Rho protein isoprenylation, which prevents membrane attachment of Rho proteins and the subsequent activation of downstream effectors such as ROCK [52]. Nohira *et al.* were the first to demonstrate statins inhibited ROCK's activity and improved endothelial function in patients with stable atherosclerosis [53]. Intriguingly, high-dose statin (40 mg/d) monotherapy seems to have greater inhibitory effects on ROCK activity and potentially more improvement in endothelial function than low-dose statin plus ezetimibe (10/10 mg/d) [21]. It is postulated that ezetimibe, either alone or in combination with statin, is less effective in improving endothelial function than high-dose statin monotherapy [54].

Thrombosis

ROCK upregulates thrombogenic molecules (e.g. platelet-activating factor [plasminogen activator inhibitor]-1 [55] and tissue factor [25]) and fibrogenic molecules (e.g. transforming growth factor-β1 [31] and Bcl-2 [25]). Thrombin-induced tissue factor expression is regulated positively by Rho/ROCK and p38 MAP kinase. Inhibition of Rho/ROCK can prevent endothelial tissue factor induction through and activation of Akt [56]. Thrombin is also an independent potent stimulator of endothelial permeability via the ROCK pathway [57].

Coronary and cerebral vasospasm

There is increasing evidence that ROCK is substantially involved in the pathogenesis of coronary and cerebral vasospasm. Coronary spasm is caused by hypercontraction of coronary smooth muscle triggered by an increase of intracellular Ca^{2+} in the presence of an increased Ca^{2+} sensitivity [58]. It has been shown that enhanced Rho/ROCK activity reduces endothelial NO activity, resulting in increased Ca^{2+} sensitivity. ROCKs modulate sensitivity of contractile apparatus to intracellular Ca^{2+} by increasing MLC phosphorylation, either directly via phosphorylation or via inhibition of the myosin-binding subunit of MLCP [45]. Studies have shown that Rho/ROCK activity is enhanced in rat arteries with hypertension and vasospasm [1,59]. Shimokawa and colleagues [1] developed the swine model of coronary spasm and have shown that ROCK activity is enhanced in smooth muscles of the coronary artery involved in spasm, as well as in human arteries [39].

Intracoronary administration of fasudil [60] and hydroxyfasudil [61] markedly inhibit coronary spasm induced by IL-1β in a porcine model via suppression of enhanced MLC phosphorylations at the spastic coronary segments [61]. The effect of fasudil has also been demonstrated in other animal coronary spasm models, including a rabbit myocardial ischemic model induced by intravenous administration of endothelin-1 [62], a dog model of pacing-induced myocardial ischemia in the presence of coronary stenosis [63] and a rat model of vasopressin-induced chronic myocardial ischemia [64]. Similar results were found in a cerebral vasospasm dog model using Y27632 [65].

ROCKs are negative upstream regulators of eNOS expression and activation eNOS in endothelial cells [16]. In the coronary artery, decrease in endothelial NO activity causes increase in Rho/ROCK activity [1,11]. Rho/ROCK inhibition leads to stabilization of eNOS mRNA and increases expression of eNOS [66]. In cerebral vasospasm, increased contractility of vascular muscle owing to increased rhoA/Rho-kinase activity is now thought to cause severe arterial narrowing observed in cerebral vasospasm after subarachnoid hemorrhage [67].

Recently, inhibition of ROCK's activity by fasudil relieves coronary spasm induced by acetylcholine in humans [68]. Fasudil inhibits coronary vasospasm in patients with unstable angina pectoris [69] and for the treatment of cerebral vasospasm after subarachnoid hemorrhage [70]. The vasodilatory effect of fasudil is more potent than that of nitroglycerin [71] and has been shown to further dilate segments of vasospastic coronary artery that have already been pre-treated with nitroglycerin [72]. These findings support the potential of fasudil as a novel therapeutic agent for coronary and cerebral vasospasm.

Ischemia and reperfusion injury

ROCK activation is involved in the pathogenesis of myocardial ischemia and reperfusion injury. Pre-treatment with fasudil before reperfusion prevents endothelial dysfunction and suppresses the development of myocardial infarction in dogs *in vivo* [73]. Upregulated expression of Rho in ischemic myocardium and subsequent activation of ROCKs occurs specifically during early reperfusion [74]. The mechanisms by which fasudil acts against cerebral infarction are thought to involve an increase in regional cerebral blood flow and a decrease in the inflammatory response [19]. Increasing regional cerebral blood flow via inhibition of ROCK is believed to be achieved through vascular dilatation [19], hemodilution and decrease in blood viscosity [75]. The latest findings indicate that the vascular dilatation induced by fasudil is achieved not only via an inhibition of smooth muscle contraction but also through an upregulation of eNOS [19]. The decrease in inflammatory responses induced by ROCK blockade is achieved by an inhibition of neutrophil migration [76]. These observations suggest that ROCK might play an important part in mediating the inflammatory response to ischemic and reperfusion injury [17]. Y-27632 reduces myocardial infarct size in rats via the same mechanism, confirming that ROCK activation might be deleterious through suppression of the reperfusion injury salvage kinase pathway [74]. Furthermore, Y-27632 enhances post-ischemia cardiac function, reduces myocardial apoptosis and decreases accumulation of neutrophils in the heart after ischemic and reperfusion injury in mice [77]. These findings suggest that ROCK activation occurs during early reperfusion and inhibition of ROCK at this crucial period might limit infarct size.

ROCK inhibitors

Given the role of ROCKs in vascular function and inflammation, the development of selective and nonselective ROCK inhibitors has gained considerable interest. Current evidence supports ROCK inhibition as a potential treatment of various cardiovascular disorders caused by vascular smooth muscle cell hyperconstriction,

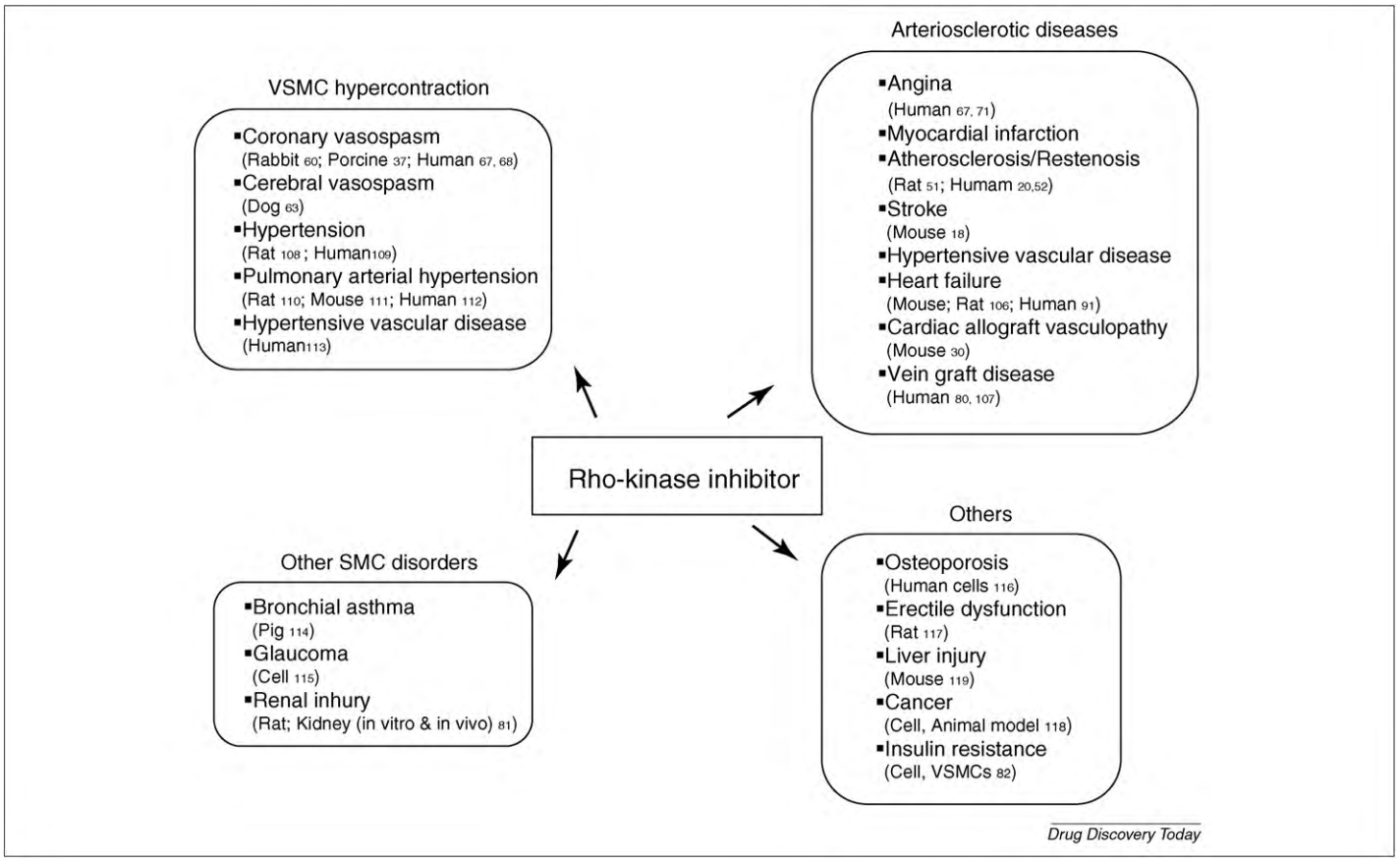

FIGURE 2

Current studies of ROCK inhibitors. ROCK inhibitors seem to be useful for treating disorders caused by arteriosclerotic diseases, vascular smooth muscle cell (VSMC) hypercontraction, other smooth muscle cell (SMC) disorders (e.g. bronchial asthma and glaucoma) and other diseases. The clinical usefulness of ROCK inhibitors remains to be fully elucidated. See Refs. [1–119] in the reference list for the reference citations in the figure.

including coronary and cerebral vasospasm [65,61], hypertension, and pulmonary hypertension [78].

The benefit of ROCK inhibition might extend to the treatment of atherosclerosis [25,26], ischemia–reperfusion injury [73], stroke [79], myocardial hypertrophy [29], heart failure [80], cardiac allograft vasculopathy [31] and vein graft disease [81]. Furthermore, they could potentially be used for the treatment of disorders associated with smooth muscle hyper-reactivity, such as asthma and glaucoma [1], and renal injury [82]. Finally, recent studies indicate that ROCK inhibitors could potentially be used to treat osteoporosis, renal disease, erectile dysfunction, cancers [1] and insulin resistance [83] (Fig. 2).

Fasudil

Fasudil (HA-1077) [84] was the first ROCK inhibitor approved for clinical use in Japan in 1995. Fasudil selectively inhibits ROCK by competing with ATP for binding to the kinase [85]. It was initially approved for the treatment of cerebral vasospasm complicating intracranial hemorrhage [70]. Fasudil has major vasodilatory activity [86] and is currently undergoing clinical trials for the treatment of ischemic heart disease [87]. Hydroxyfasudil (HA-1100), a major active metabolite of fasudil after oral administration, has a more selective inhibitory effect on ROCK than does the parent drug [29,61]. Another dimethylated analog of fasudil, H-1152P, is the most potent inhibitor of the three (HA-1077, H-1152P and HA-1100) ligands against ROCK [88]. H-1152P has potent intraocular pressure-lowering effect on an ocular hypertension model. These results suggested that H-1152P could be a candidate for the next generation of glaucoma therapy [89].

Intracoronary administration of fasudil is effective at reducing coronary spasm and myocardial ischemia in patients with vasospastic angina and microvascular angina [68]. Long-term oral treatment with fasudil is also effective at ameliorating exercise tolerance in patients with stable-effort angina and adequate safety profiles [45]. Intra-arterial infusion of fasudil markedly enhances the vasodilator responses of forearm circulation in hypertensive patients [90]. Intravenous infusion of fasudil reduces pulmonary vascular resistance in patients with pulmonary hypertension [91]. Intra-arterial infusion of fasudil causes a preferential increase in forearm blood flow in patients with heart failure when compared with control subjects [92]. However, the potential usefulness of the oral administration of ROCK inhibitors for the treatment of unstable angina, myocardial infarction, pulmonary hypertension, hypertensive vascular disease and/or cardiac hypertrophy remains to be examined in humans. Another clinical trial of the intravenous administration of fasudil in the acute phase of stroke demonstrates that ROCK inhibitors exert beneficial effects on ischemic neuronal damage without causing serious adverse effects [93].

Clinical trials of the anti-anginal effects of fasudil in patients with stable-effort angina in Japan [45] and the USA [94] have

demonstrated that long-term oral treatment with this ROCK inhibitor is effective at ameliorating exercise tolerance in patients with adequate safety profiles. This Phase II dose-finding trial in patients with stable angina showed that ST segment depression was improved with fasudil at both peak and trough compared with placebo. In addition, fasudil improved Seattle Angina Questionnaire scores, was well tolerated and did not affect heart rate or blood pressure. No major adverse events were noted with fasudil treatment; most of the adverse events were mild and not considered to be related to study medication [94]. An effort has been made to develop more specific and more potent ROCK inhibitors [95]. Moreover, fasudil analogs have been designed on the basis of the complex structure of PKA and HA1077, and it was found that glycine derivatives of HA1077 are highly specific inhibitors of ROCK [95]. These inhibitors were applied to rabbit ocular hypertensive models, in which they reduced intraocular pressure—indicating that they might be useful for glaucoma, in addition to other ROCK-related diseases [95].

Other ROCK inhibitors

Several pyridine derivative compounds such as Y27632 have been developed with potent ROCK inhibitory effect [59]. Y27632 inhibits smooth muscle contractility and normalizes blood pressure in rat hypertensive models [59]. It was originally reported that Y27632 is a non-specific inhibitor of both ROCK1 and ROCK2 by competing with ATP for binding to their catalytic sites [96]. Y27632 is also a potent inhibitor of Rho-dependent protein kinase C-related kinase 2 [85]. This results in decreased phosphorylation of myosin, arterial smooth muscle relaxation and vasodilation of blood vessels. Y27632 has other inhibitory properties against RhoA-mediated cell transformation [97], tumor cell invasion [98] and neutrophil chemotaxis [99]. These findings raised the possibility that inhibitors of ROCK might have additional potential therapeutic use in the treatment of cardiovascular and other diseases. More recently, a closely related molecule, Y32885, was found to inhibit protein kinase C-related kinase 1, or PRK1 (a distinct Rho-dependent protein kinase), at a concentration similar to that with which it inhibits ROCK isoforms [100]. PRK1 is present in various tumor cell development and progression stages and is a potential drug target for pharmacological intervention of RhoA-mediated signaling pathway [101].

Recently, two aminofurazan-based inhibitors, GSK269962A and SB772077B, were characterized as members of a novel class of compounds [102]. These compounds highly inhibit both ROCK1 and ROCK2, and their potency is higher than that of Y27632 or fasudil, especially on inhibiting ROCK1 [102]. These compounds are potent vasodilators, and they lower blood pressure in spontaneously hypertensive rats and deoxycorticosterone acetate-salt-treated hypertensive rats [102]. Other highly ROCK2-selective inhibitors such as SR-715 and SR-899 have been developed [103]. Another ROCK2 inhibitor, SLx-2119, reduced connective tissue growth factor mRNA and remodeled the actin cytoskeleton in fibrosis-derived smooth muscle cells [104]. A weak ROCK inhibitor, Rockout (3-(4-pyridyl)indole), was discovered using automated microscopic screening for compounds that affected cell migration and mitotic progression [105]. Recently, the 3D crystal structure of ROCK and its binding site for fasudil have been determined, which should facilitate the development of more selective ROCK inhibitors [106].

Concluding remarks

The Rho/ROCKs pathway has been demonstrated to have an important role in the pathogenesis of various cardiovascular diseases. Currently, fasudil is the only ROCK inhibitor available for clinical use. Inhibition of Rho/ROCKs might be an attractive therapeutic target in the treatment of coronary and cerebral artery vasospasm and systemic and pulmonary hypertension. Further studies are warranted to determine the role of ROCK inhibitors in clinical practice.

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