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Review

RhoA/ROCK Signaling in Vascular Dysfunction: Emerging Insights and Therapeutic Perspectives

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Abstract: The Rho-associated coiled-coil containing protein kinase (ROCK) family is increasingly recognized as a key regulator of vascular biology and a contributor to the pathogenesis of cardiovascular diseases. Within the vascular system, the RhoA/ROCK pathway governs several essential functions, including endothelial homeostasis, vascular smooth muscle cell contraction, and maintenance of vascular tone. Given its central role in these processes, dysregulation of the RhoA/ROCK signaling pathway has been implicated in the development and progression of various vascular disorders, such as hypertension, atherosclerosis, restenosis, and abdominal aortic aneurysms (AAAs). In this review, we summarize the current understanding of the RhoA/ROCK signaling pathway under both physiological and pathological conditions and discuss the therapeutic potential and clinical applications of ROCK inhibitors in the treatment of vascular diseases.

Keywords: RhoA/ROCK signaling; vascular dysfunction; cardiovascular diseases; endothelial cells; vascular smooth muscle cells; therapeutic strategies

1. Introduction

Vascular diseases, together with their complications, encompassing hypertension, atherosclerosis, aortic aneurysm, aortic stiffness, and vascular calcification, represent a driver in most cardiovascular diseases and constitute a significant contributor to worldwide mortality rates. The RhoA/ROCK pathway is a crucial signaling pathway that maintains cardiovascular homeostasis. It serves as a master regulator of some vital cellular functions, including actin cytoskeletal reorganization, cellular contractility, adhesion, and motility, which can lead to a variety of cardiovascular diseases. Dysregulation of these processes contributes significantly to the pathogenesis of vascular disorders. Additionally, the RhoA/ROCK pathway mediates the effects of various vasoactive agents, including angiotensin II, 5-hydroxytryptamine (5-HT), and thrombin, all of which are highly implicated in the development of vascular disease. Thus, there is increasing interest in elucidating the role of RhoA/ROCK signaling in maintaining vascular homeostasis and etiopathogenesis of vascular pathology.

Studies have shown that the RhoA/ROCK signaling pathway has emerged as a regulator of endothelial barrier integrity, trans-endothelial leukocyte migration, oxidative stress, contraction, migration, and proliferation in vascular smooth muscle cells (VSMC) [1, 2], which are critically correlated with vascular tone, vascular remodeling, and inflammatory responses. An accumulating body of evidence suggests that aberrant activation of the RhoA/ROCK signaling pathway has been observed in coronary vasospasm [3], hypertension [4], aneurysms [5], and atherosclerotic plaques [6]. Hyperactivation of this signaling cascade has been implicated in the development of hypertension through sustained vasoconstriction mediated by

calcium sensitization. In atherosclerotic progression, the upregulation of leukocyte transmigration and subsequent foam cell formation are driven by the activation of the RhoA/ROCK pathway. Furthermore, pharmacological inhibition of the RhoA/ROCK pathway has proven efficacious in pulmonary hypertension [7]. Therefore, ROCK has recently been the subject of increasing investigation as a therapeutic target for various vascular diseases. This review focuses on the role of RhoA/ROCK signaling in endothelial dysfunction, vascular remodeling, and its potential clinical application as a therapeutic target for the prevention and treatment of vascular diseases.

2. RhoA/ROCK Signaling Pathway

Rho is a Ras-related small guanosine triphosphate (GTP) -binding protein, belonging to the Rho subfamily of GTPases. Rho proteins, such as RhoA, RhoB, and RhoC, exhibit high amino acid sequence homology (>85%), and function as molecular switches that cycle between an inactive GDP-bound state and an active GTP-bound state. Rho functions as a "molecular switch" during signal transduction under the three regulatory factors: guanine nucleotide exchange factors (GEFs), which activate Rho by accelerating exchange of GDP for GTP; GTPase-activating proteins (GAPs), which stimulate intrinsic GTP hydrolysis to revert Rho to its inactive state; and guanine nucleotide dissociation inhibitors (GDIs), which stabilize the GDP-bound form, and prevent Rho binding to the membrane. Activated Rho-GTP interacts with a variety of downstream signaling effectors, such as ROCK, mammalian diaphanous (mDia), Rhophilin-Rhotekin, Citron, and protein-kinase N, regulating various cellular functions [8].

ROCKs are highly conserved serine/threonine protein kinases that are widely distributed throughout the body. As a critical downstream target effector molecule of the Rho proteins, it plays an important role in regulating a wide range of physiological processes, such as cytoskeleton rearrangement, vesicle transport, and cell adhesion. It consists of three domains: a *N*-terminal kinase domain, a C-terminal region comprising the pleckstrin homology (PH) domain, and a coiled-coil region followed by a Rho-binding domain (RBD)in its middle portion [9]. There are two isoforms of ROCK: ROCK1 (ROCKI or p160ROCK), located on chromosome 18, and ROCK2 (ROCKII or ROKα) on chromosome 12. The two isoforms share approximately 60% overall amino acid identity and 92% homology in the kinase domains. rROCK1 and ROCK2 exhibit differential expression patterns in various tissues. ROCK1 is predominantly expressed in the kidney, spleen, lung, and liver, whereas ROCK2 is primarily found in the brain and heart [10,11]. Both isoforms are widely expressed in vascular SMCs and endothelial cells. Under the inactive state, the C-terminal region of ROCK plays its auto-inhibitory role by interacting with the kinase domain in the *N*-terminal region [12]. ROCK proteins can be activated by binding to active Rho-GTP, thereby disrupting the negative regulatory interactions. It can also be activated by cleavage and removal of the inhibitory C-terminal region of ROCK1 mediated by caspase 3 [13,14] or ROCK2 mediated by granzyme B [10,15], respectively. Both isoforms play critical roles in the regulation of vascular function and biological processes [16–19]. Upon binding to the activated Rho GTPase, ROCK exposed the active sites at the *N*-terminal kinase domain. Subsequently, it phosphorylated a variety of downstream substrates, including myosin light chain (MLC) phosphatase target subunit 1 (MYPT-1), ezrin, radixin, and moesin (ERM), myocardin, and serum response factor (SRF), LIM (Lin-11, Isl-1, Mec-3)/cofilin.

The RhoA/ROCK signaling pathway can be activated by various membrane receptors, such as G protein-coupled receptors, tyrosine kinase receptors, and intracellular receptors. Activated ROCK can directly phosphorylate MYPT-1 and further phosphorylate myosin light chain phosphatase (MLCP), leading to persistent MLC phosphorylation. Other downstream targets of ROCK, as ezrin, radixin, and moesin (ERM), are involved in actin filament/plasma membrane interactions, which play a significant role in endothelial function and inflammation. LIM kinase, another downstream effector and substrate of ROCK, plays a key role in actin cytoskeleton dynamics. The actin-depolymerizing factor cofilin, when active, severs myofilaments and promotes actin filament depolymerization. Phosphorylation of LIM kinase by ROCK leads to the inactivation of cofilin through phosphorylation, thereby preventing filament disassembly and promoting actin filament elongation.

3. Physiological Role of RhoA/ROCK in Vascular Health

Emerging evidence suggests that RhoA, expressed in the vasculature, particularly in endothelial cells

and vascular smooth muscle cells, plays a crucial role in regulating vascular function.

3.1. Endothelial Cells

The vascular endothelium is a multifunctional organ essential to normal vascular physiology. Endothelial nitric oxide synthase (eNOS), secreted by endothelial cells, is a key mediator of vascular homeostasis by regulating the production of Nitric oxide (NO). A series of studies have demonstrated that activation of RhoA/ROCK inhibits eNOS mRNA stability or expression in endothelial cells [20–23]. For example, ROCK can directly inhibit the phosphorylation of eNOS by suppressing the protein kinase B/Akt signaling pathway, negatively regulating eNOS expression independent of Akt [20,22]. Excessive activation of RhoA/ROCK II was also found in mesenteric arteries of profilin1 mice, which show decreased eNOS protein levels together with reduced activation of eNOS [23]. In addition, ROCK inhibition directly affected the production and bioavailability of NO [24] or increased eNOS expression [25], subsequently improving vascular functions.

The endothelial barrier is regulated by a balance between the contractility of the actin cytoskeleton and adhesive tethering forces generated by endothelial intercellular junctions [26]. Accumulating data have demonstrated that RhoA is a well-known mediator of endothelial hyperpermeability via disrupting the endothelial intercellular junctions or reorganizing the actin cytoskeleton [27–30]. Increased myosin contractility caused by activation of RhoA/ROCK that pulls the endothelial cell and disrupts the barrier function, is considered the primary molecular mechanism of endothelial hyperpermeability [31]. Advanced glycation end products (AGEs) have been shown to activate the RhoA/ROCK signaling pathway by binding to the receptor for advanced glycation end products (RAGE), which is complexed with RhoA. This activation leads to actin cytoskeletal reorganization and intercellular gap formation, resulting in increased endothelial cell hyperpermeability [32]. Studies have shown that the ROCK inhibitor can block a dasatinib-induced increase in permeability [33]. Consistently, Wei et al. [28] reported that ulinastatin can inhibit the increased permeability of vascular endothelial cells induced by TNF-α via suppressing the expression of RhoA and ROCK2 in vascular endothelial cells. Taken together, the RhoA/ROCK pathway significantly affects endothelial homeostasis.

Clinically, dasatinib, a tyrosine kinase inhibitor primarily used as a targeted therapy for Philadelphia chromosome-positive leukemias, has been associated with adverse vascular effects, including increased endothelial permeability. This side effect is thought to result from dysregulation of the RhoA/ROCK signaling pathway. Notably, studies have shown that treatment with a ROCK inhibitor can attenuate dasatinib-induced endothelial hyperpermeability, underscoring the critical role of this pathway in maintaining vascular barrier integrity [33]. Consistently, Wei et al. reported that ulinastatin, a protease inhibitor with anti-inflammatory and cytoprotective properties, suppresses TNF-α-induced vascular endothelial hyperpermeability by downregulating the expression of RhoA and ROCK2 [28]. Taken together, these findings highlight the essential role of the RhoA/ROCK pathway in maintaining endothelial homeostasis.

3.2. Vascular Smooth Muscle Cells

Modulation of VSMC contraction is crucial for maintaining vascular tone and regulating blood pressure, which depends on the phosphorylation level and the activity of the myosin light chain (MLC) in VSMCs. RhoA is abundantly expressed in vascular smooth muscle cells (VSMCs), playing a crucial role in regulating VSMC contraction via the Ca^{2+} sensitization mechanism. Activated RhoA/ROCK phosphorylates MYPT-1, restraining the phosphatase activity of myosin light chain phosphatase (MLCP), leading to decreased dephosphorylation of MLC and subsequently increased vascular contraction [34,35]. RhoA/ROCK is also involved in the migration, proliferation, differentiation, and apoptosis of VSMC [1]. Migration of VSMCs involves multiple physiological processes and maintains vascular integrity by reorganizing the actin cytoskeleton. Extensive studies have shown that cytoskeleton remodeling in response to chemotactic factors, such as platelet-derived growth factor-BB (PDGF-BB), angiotensin II (Ang II), thrombin, and tumor necrosis factor-α (TNF-α), is a critical event in cell migration [1,36–38]. Previous studies have demonstrated that in response to PDGF-BB, the activated RhoA/ROCK pathway leads to cytoskeletal remodeling and ultimately precipitates VSMC migration [39–41]. Additionally, angiotensin II triggers the activation of RhoA/ROCK,

resulting in the polymerization of G (globular)-actin into F (fiber)-actin. This polymerization initiated VSMC migration by downregulating contractile proteins [38]. As another upstream signal, asymmetric dimethylarginine (ADMA) also facilitates VSMC migration by activating RhoA/ROCK, which in turn stimulates ERK to downregulate contractile protein expression. Additionally, ADMA can relieve the repressive effect of eNOS on RhoA/ROCK, which in turn, suppresses Ang II-induced vascular migration [37].

Active RhoA/ROCK stimulates the MEK/ERK signaling axis, a key driver of VSMC proliferation [36, 42]. RhoA/ROCK-induced ERK activation upregulates the expression of cell cycle regulators, such as cyclin D1 and proliferating cell nuclear antigen (PCNA). Inhibition of ROCK1 and ROCK2 activity using small interfering RNA significantly reduces the expression levels of cyclin D1 and PCNA, along with a marked decrease in VSMC proliferation [36]. Furthermore, RhoA/ROCK signaling promotes VSMC proliferation during neointima formation following balloon injury, while its inhibition has been shown to suppress neointima formation [43]. Consistent with these findings, $ROCK1^{+/-}$ mice exhibit significantly reduced VSMC proliferation and neointima formation [44].

In healthy vasculature, VSMCs remain in a contractile, stationary, and quiescent state. In response to various stimuli, VSMCs can undergo phenotypic switching from contractile to a synthetic, migratory, and proliferative phenotype, characterized by downregulation of contractile proteins, increased proliferation, and extracellular matrix (ECM) remodeling to facilitate migration [45, 46]. The RhoA/ROCK pathway plays a critical role in regulating VSMC phenotype [1]. On one hand, RhoA/ROCK promotes the contractile phenotype by stabilizing actin stress fibers, which help maintain cell shape and adhesion [47]. Activated RhoA or ROCK enhances stress fiber formation and can directly activate myosin light chain kinase (MLCK) to stabilize the cytoskeleton further. Additionally, RhoA/ROCK signaling regulates the expression of VSMC-specific contractile and cytoskeletal genes. Inhibition of this pathway by Y27632 significantly reduces the expression of these contractile genes [48]. On the other hand, in the presence of pro-migratory and pro-proliferative stimuli, RhoA/ROCK signaling contributes to the synthetic phenotype by promoting cytoskeletal reorganization and cell migration [38,49].

3.3. Fibroblasts

Actin cytoskeleton remodeling in fibroblasts is a fundamental process that facilitates their activation, migration, and contractile function during tissue repair or the wound healing process. In the context of cardiovascular disease, increased ROCK activity in fibroblasts promotes their transdifferentiation into myofibroblasts through signaling pathways involving TGF-β, MRTF/SRF, and YAP/TAZ [50–52]. Emerging evidence suggests that TGF-β-mediated ROCK activation leads to the upregulation of profibrotic genes and enhances F-actin polymerization, thereby driving myofibroblast activation and differentiation [53]. In contrast, inhibition of ROCK has been shown to attenuate cardiac fibrosis and remodeling, particularly in models treated with angiotensin-II or Nω-nitro-L-arginine methyl ester (L-NAME).

4. Relationship between RhoA/ROCK and Vascular Diseases

4.1. Arterial Hypertension

Arterial hypertension (HTN) is a major cardiovascular risk factor characterized by increased peripheral vascular resistance, mainly due to enhanced contractility of VSMCs and structural changes in the arterial wall [54]. The RhoA/ROCK signaling pathway is a well-established regulator of basal vascular tone and vasoconstriction during hypertension development [4]. Extensive studies have shown that elevated levels of active RhoA, induced by circulating vasoconstrictors, increase calcium sensitivity of the VSMC contractile machinery in various animal models of experimental hypertension [55–58]. For example, a microarray-based global gene expression analysis revealed increased RhoA gene expression in arteries from rats with deoxycorticosterone acetate (DOCA)-salt-induced hypertension, and administration of ROCK inhibitors in these models significantly lowered blood pressure [59–61]. Similarly, L-NAME-induced hypertension, which involves nitric oxide synthase inhibition, is associated with increased RhoA and ROCK activity [55]. ROCK inhibitors such as Y27632 and SAR407899 have been shown to exert antihypertensive effects in this model [55,60]. Additionally, mutations in Cullin3, a scaffolding protein involved in ubiquitin ligase complexes, have been reported to enhance RhoA/ROCK signaling, leading to augmented vascular contraction and

hypertension [62,63]. Likewise, tacrolimus, an immunosuppressive agent, increases Ang II-induced vasoconstriction and blood pressure through activation of the RhoA/ROCK/MYPT-1 pathway, implicating RhoA signaling in tacrolimus-related vascular dysfunction [64]. In contrast, pharmacological inhibition of the RhoA/ROCK pathway with Y27632 or fasudil attenuates Ang II-induced vasoconstriction and lowers blood pressure [64–66].

4.2. Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by a progressive increase in pulmonary artery pressure (mPAP > 20 mmHg at rest), ultimately leading to right heart failure. The disease involves multiple pathological mechanisms, including increased vascular resistance, exacerbated vascular inflammation, and vascular remodeling in pulmonary arterioles [67]. A key contributor to pulmonary vascular remodeling is the excessive proliferation and migration of VSMCs, which reduces the internal diameter of blood vessels and consequently increases vascular resistance [68]. In addition, phenotypic switching of pulmonary artery smooth muscle cells from a contractile to a synthetic state further contributes to disease progression [69].

Substantial evidence supports the involvement of the RhoA/ROCK signaling pathway in the pathogenesis of PAH [70–72]. Both the expression and activity of ROCK are significantly elevated in the lung tissues of patients with PAH compared to healthy controls, and ROCK activity has been shown to correlate positively with disease severity [73].

Experimental studies have demonstrated that long-term inhibition of Rho kinase ameliorates PAH in monocrotaline or hypoxia-induced animal models [74,75]. Notably, VSMC-specific knockout of ROCK2 significantly attenuated hypoxia-induced increase in pulmonary artery pressure in mice [76]. In a clinical study, intraventricular administration of fasudil (30 mg/kg) in PAH patients significantly reduced pulmonary artery vascular resistance and lowered pulmonary artery pressure [77]. Similarly, the ROCK inhibitor Y-27632 has shown therapeutic potential by up-regulating eNOS expression in pulmonary arteries [78]. Interestingly, sildenafil, a widely used treatment for PAH, may also exert part of its therapeutic effects by inhibiting the RhoA/ROCK pathway [79]. Together, these findings suggest that aberrant RhoA/ROCK activation plays a critical role in the development and progression of pulmonary hypertension. Although current treatments, such as vasodilators, anticoagulants, and other symptomatic therapies, can provide temporary relief, the prognosis for patients with advanced PAH remains poor due to progressive right ventricular failure. The identification of novel and effective therapeutic strategies targeting the RhoA/ROCK pathway is a promising area that addresses this urgent clinical need.

4.3. Atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by vascular inflammation, lipid accumulation and deposition, and arterial wall fibrosis. The pathological process is initiated by endothelial dysfunction, triggering the recruitment and infiltration of inflammatory cells, extracellular lipid deposition, and ultimately resulting in plaque progression and vascular stenosis [80,81]. Considerable evidence indicates that the RhoA/ROCK signaling pathway is involved in the inflammatory atherosclerotic processes. ROCK activity is significantly elevated in patients with acute coronary syndromes and is positively correlated with an increased risk of cardiovascular events [82]. Pharmacological inhibition of ROCK limits early atherosclerotic plaque formation but also promotes regression of arteriosclerotic coronary lesions in various animal models, including porcine [83], LDL-receptor-deficient ($LDLR^{-/-}$) mice [84], and apolipoprotein E-deficient mice ($ApoE^{-/-}$) [85, 86]. In particular, ROCK1 deficiency in bone-marrow-derived cells result in reduced chemotaxis, cholesterol uptake, and foam-cell formation, ultimately leading to decreased atherosclerosis in $LDLR^{-/-}$ mice [87]. Similarly, bone marrow-specific ROCK2 deficiency in $LDLR^{-/-}$ mice also resulted in a significant decrease of foam cell formation by inhibiting peroxisome proliferator-activated receptor (PPAR)-γ–mediated reverse cholesterol transport in macrophages [87]. These findings highlight the crucial roles of both ROCK1 and ROCK2 in macrophage-mediated atherogenesis. Furthermore, fasudil treatment significantly reduced atherosclerotic plaque burden in ApoE knockout mice, an effect associated with inhibition of ERM protein phosphorylation [84]. These preclinical findings provide strong evidence for

targeting ROCK as a potential therapeutic strategy for endothelial dysfunction and atherosclerosis.

Consistently, clinical studies have demonstrated a strong association between ROCK activity and endothelial dysfunction, particularly in individuals with metabolic syndrome or coronary artery disease. For example, Nohria A et al. reported a correlation between elevated ROCK activity and impaired endothelial function in patients with coronary artery disease [88]. Furthermore, fasudil treatment reduced ROCK hyperactivation and improved endothelium-dependent vasodilation in these atherosclerosis patients, supporting the translational relevance of ROCK inhibition in atherosclerosis management.

4.4. Aortic Aneurysm

Aortic aneurysm is a life-threatening vascular disorder defined as an enlarged aorta exceeding 50% of its normal diameter, resulting from structural weakening of the vessel wall [89]. The main pathological features include chronic vascular inflammation, apoptosis, and senescence of VSMCs, elevated oxidative stress, and degradation of extracellular matrix components [90]. A series of studies has demonstrated that RhoA expression is markedly reduced in the aneurysm area compared to adjacent segments in patients with abdominal aortic aneurysm (AAA). Chronic inhibition of ROCK by fasudil effectively reduces Ang II-induced aortic aneurysm formation in mice [5]. Furthermore, genetic deletion of RhoA in VSMCs increases susceptibility to AAA following pharmacological stimulation. This RhoA depletion leads to aberrant activation of mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) and its downstream MAPK signaling, which promotes vascular inflammation and extracellular matrix breakdown, making the aortic wall more prone to damage from hemodynamic stress [91]. In another study, zoledronate, an inhibitor of farnesyl pyrophosphate synthase, crucial for prenylation of small GTPases, exerts protective effects on AAA formation by suppressing Ang-II-induced RhoA/ROCK activation [92]. Moreover, Ang II – induced AAA models exhibit increased VSMC apoptosis, which is significantly reduced by fasudil treatment, indicating that ROCK inhibition can limit both cell death and aneurysm progression [5]. Collectively, these findings highlight the complex and context-dependent role of the RhoA/ROCK pathway in AAA pathogenesis, where both overactivation and deficiency can contribute to disease development. Further investigation is warranted to fully elucidate the therapeutic potential of targeting this pathway in aortic aneurysms.

4.5. Vascular Stiffness

Vascular stiffness is strongly associated with the risk and progression of cardiovascular diseases. There is a bidirectional relationship between vascular stiffness and blood pressure: elevated blood pressure can damage the vascular wall and accelerate stiffening, while increased vascular stiffness contributes to greater pulse pressure and elevated systolic blood pressure. Although the precise causal relationship between vascular stiffness and hypertension remains unresolved, accumulating evidence indicates that RhoA/ROCK is closely associated with aortic stiffness and hypertension. Early studies identified ROCK activity as an independent predictor of increased pulse wave velocity (PWV), a widely used marker of arterial stiffness, suggesting its involvement in the pathogenesis of aortic stiffening [93]. Consistently, aging and smoking have been shown to elevate ROCK activity, leading to excessive oxidative stress and subsequent arterial stiffening. More recently, Yuxin et al. found that aortic ROCK activity increases with age, and that haploinsufficiency of ROC1 or ROCK2 confers protective effects against age-associated aortic stiffness [94]. In another study, treatment with angiotensin-converting enzyme (ACE) inhibitor perindopril in spontaneously hypertensive rats (SHR) significantly upregulated the expression of GDIs in the arterial wall, alleviating aortic stiffness, which is partially through inhibiting the RhoA/ROCK signaling pathway, suggesting that targeting this pathway may offer therapeutic benefit in the management of arterial stiffness [95].

5. ROCK Inhibitors

Given the critical role of RhoA/ROCK signaling pathway in vascular function, the potential therapeutic applications of ROCK inhibitors in vascular diseases have gained considerable attention. Several classic ROCK inhibitors, such as fasudil, hydroxyfasudil, and Y27632, have been investigated in various diseases [96]. These inhibitors exhibit moderate kinase selectivity and function by targeting the ATP-dependent kinase domain of ROCK to block its kinase activity [97]. In recent years, more selective and potent ROCK inhibitors

have been developed, enhancing the precision of pharmacological targeting [98]. To date, four ROCK inhibitors have received clinical approval for use in specific vascular and neurological conditions, including fasudil [99], Ripasudil [100], Netarsudil [101] and Belumosudil [98]. Among which, Belumosudil has been demonstrated that can effectively treat patients with chronic graft-versus-host disease as a selective inhibitor of ROCK2 [98,102].

Fasudil (HA-1077) is a selective isoquinoline sulfonamide ROCK inhibitor, initially approved for the treatment of cerebral vasospasm following subarachnoid hemorrhage. More recently, inhaled formulations of fasudil have been employed for targeted delivery to the lungs in the treatment PAH [7]. Clinical trials have shown that fasudil can significantly reduce pulmonary vascular resistance in PAH patients [71]. Combination therapies of fasudil with prostacyclin [103] or sildenafil [104] or simvastatin [105] have demonstrated superior efficacy compared to monotherapy.

In other cardiovascular disease models, fasudil markedly reduced macrophage accumulation in the adventitia, inhibited migration into the media, and suppressed coronary lesion formation in a porcine model of coronary arteriosclerosis [106]. Additionally, IL-1β-induced coronary spasm was significantly attenuated by intracoronary administration of fasudil, likely through inhibition of enhanced MLC phosphorylation [107]. Similar benefits were observed in acetylcholine-induced coronary spasm in humans [108]. Consistently, intra-arterial infusion of fasudil markedly enhanced vasodilator responses in forearm circulation in hypertensive patients, further supporting its vascular benefits [109].

6. Conclusions and Future Directions

While systemic pharmacological inhibition of ROCK shows promising therapeutic benefits, it also affects physiological functions of the pathway, potentially causing adverse effects, such as hypotension, reflex tachycardia, lymphopenia and even cardiovascular collapse. Therefore, future studies should focus on developing isoform-selective ROCK inhibitors, such as ROCK1- or ROCK2-specific inhibitors, to minimize off-target effects and enhance safety profiles. Additionally, ongoing challenges include mitigating off-target kinase interactions, particularly with long-term RCK inhibitor therapy. Although promising effects have been observed in unstable angina, myocardial infarction, pulmonary hypertension, and systemic hypertension, the efficacy and safety of oral ROCK inhibitors require validation in large-scale clinical trials. Finally, further investigation into tissue-specific delivery, chronic administration, and combination therapies is necessary to optimize the clinical utility of ROCK-target treatments.

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