Advantages of Rho-associated kinases and their inhibitor fasudil for the treatment of neurodegenerative diseases

https://doi.org/10.4103/1673-5374.335827

Date of submission: June 21, 2021

Date of decision: September 1, 2021

Date of acceptance: November 6, 2021

Date of web publication: April 29, 2022

From the Contents

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Abstract

Ras homolog (Rho)-associated kinases (ROCKs) belong to the serine-threonine kinase family, which plays a pivotal role in regulating the damage, survival, axon guidance, and regeneration of neurons. ROCKs are also involved in the biological effects of immune cells and glial cells, as well as the development of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Previous studies by us and others confirmed that ROCKs inhibitors attenuated the symptoms and progression of experimental models of the abovementioned neurodegenerative diseases by inhibiting neuroinflammation, regulating immune imbalance, repairing the blood-brain barrier, and promoting nerve repair and myelin regeneration. Fasudil, the first ROCKs inhibitor to be used clinically, has a good therapeutic effect on neurodegenerative diseases. Fasudil increases the activity of neural stem cells and mesenchymal stem cells, thus optimizing cell therapy. This review will systematically describe, for the first time, the effects of abnormal activation of ROCKs on T cells, B cells, microglia, astrocytes, oligodendrocytes, and pericytes in neurodegenerative diseases of the central nervous system, summarize the therapeutic potential of fasudil in several experimental models of neurodegenerative diseases, and clarify the possible cellular and molecular mechanisms of ROCKs inhibition. This review also proposes that fasudil is a novel potential treatment, especially in combination with cell-based therapy. Findings from this review add support for further investigation of ROCKs and its inhibitor fasudil for the treatment of neurodegenerative diseases.

Key Words: Alzheimer's disease; cell-based therapy; central nervous system cells; fasudil; immunocytes; multiple sclerosis; Parkinson's disease; pericytes; Rho kinase inhibitor; Rho-associated kinases

Introduction

The Ras homolog (Rho) protein family is a member of the Ras protein superfamily, which is present in many tissue types and includes more than 20 intracellular signaling proteins, such as Ras homolog family member A (RhoA), Rac, and cell division control protein 42. The Rho family consists of small guanosine triphosphate (GTP)-binding proteins that are widely expressed in eukaryotes and have GTPase activity. Therefore, they are also known as Rho GTPases. The Rho GTPases play an important role in the construction of the cytoskeleton, as they can anchor to the cell membrane after lipid modification. Rho proteins act as a molecular switch as they can change between the active state of GTP binding and the inactive state of guanosine diphosphate (GDP) binding under the regulation of Rho guanine nucleotide exchange factors, Rho GTPase-activating proteins, and Rho guanine nucleotide dissociation inhibitors. The regulation of Rho proteins is complex. For instance, guanine nucleotide exchange factors promote the conversion of GDP to GTP and thus promote the activation of Rho GTPase, whereas guanine nucleotide dissociation inhibitors inhibit the catalytic effect of guanine nucleotide exchange factors, which prevents the dissociation of GDP from Rho GTPase and keeps Rho GTPase in an inactive state. GTPase-activating proteins, however, can activate endogenous GTP hydrolase, which induces the hydrolysis of GTP and causes Rho GTPase to lose its activity (Kobayashi et al., 2016; Shimokawa et al., 2016; Narumiya and Thumkeo, 2018).

Rho-associated kinases (ROCKs), important downstream effectors of Rho GTPase, play important roles in many cellular functions, including contraction, motility, proliferation, and apoptosis. Therefore, ROCKs regulate the damage, survival, axon guidance, and regeneration of neurons and are involved in the activation, migration, and proliferation of immune cells and glial cells. ROCKs exist as two isoforms, ROCK1 and ROCK2, which were initially reported to be ubiquitously expressed during embryogenesis and in adult tissues. Specifically, analysis of the distribution of ROCK1 and ROCK2 expressed sequence tags using the Tissue-Specific Gene Expression and Regulation database (Liu et al., 2008) revealed that their distribution patterns are similar and there are few specific organs and tissues with dramatically higher expression levels (Lu et al., 2020b). Both ROCK1 and ROCK2 activities are enhanced by binding to active GTP-bound RhoA. Many ROCK1 and ROCK2 substrates have been identified, including myosin light chain (MLC), myosin phosphatase target subunit 1, the ezrin/radixin/moesin family, adducin, phosphatase and tensin homolog (PTEN), endothelial nitric oxide synthase, tau, and LIM kinase. MLC is crucial for contraction of vascular smooth muscle cells and is phosphorylated by Ca^{2+}/calmodulin-activated dependent MLC kinase and is dephosphorylated by MLC phosphatase (**Figure 1**) (Kobayashi et al., 2016; Shimokawa et al., 2016; Narumiya and Thumkeo, 2018). We have reviewed the recent advances in the investigation of ROCKs for the treatment of central nervous system (CNS) diseases and the development of fasudil, a ROCKs inhibitor.

Search Strategy

We conducted an electronic search of the literature in the past 5 years in the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) using the following search terms (Tajouri et al., 2005; Yan et al., 2019; Ibrahim et al., 2020): RhoA, ROCK, central neurodegenerative diseases, T cells, B cells, microglia, macrophages, astrocytes, oligodendrocytes, and pericytes. Subsequently, we searched PubMed for research articles on the molecular mechanisms relating to the role of RhoA and ROCKs in the abnormal activation of various cell types in CNS neurodegenerative diseases.

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Funding: *This work was supported by the National Natural Science Foundation of China, Nos. 81473577 (to CGM), 81903596 (to QW), 82004028 (to LJS); China Postdoctoral Science Foundation, No. 2020M680912 (to LJS); Open Project of The Key Laboratory of Medicinal Resources and Natural Pharmaceutical Chemistry, The Ministry of Education of China,No. 2019004 (to CGM); Science and Technology Innovation Project of Shanxi Colleges of China, Nos. 2019L0728 (to QW); and Cultivation Project of Shanxi Universtity of Chinese Medicine of China, No. 2019PY130 (to QW).*

How to cite this article: *Wang Q, Song LJ, Ding ZB, Chai Z, Yu JZ, Xiao BG, Ma CG (2022) Advantages of Rho-associated kinases and their inhibitor fasudil for the treatment of neurodegenerative diseases. Neural Regen Res 17(12):2623-2631.*

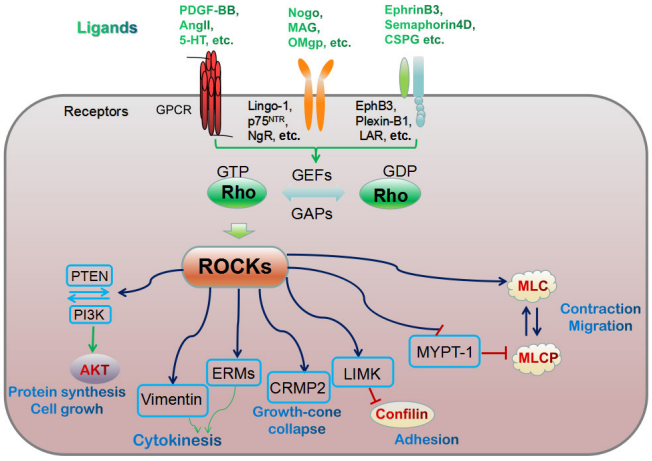

Figure 1 | **Rho/ROCK signaling.**
Activated ROCK acts directly or indirectly on several target proteins such as LIM kinase (LIMK), myosin light chain (MLC), myosin light chain phosphatase (MLCP), dihydropyrimidinase-related protein 2 (CRMP2), phosphatase and tensin homolog (PTEN), and AKT, and leads to contraction, adhesion, growth-cone collapse, protein synthesis, and cell growth. 5-HT: 5-Hydroxytryptamine; AngII: angiotensin II; CSPG: chondroitin sulfate proteoglycans; EphB3: Ephrin receptors B3 ; GAP: GTPase-activating protein; GDP: guanosine diphosphate; GEF: guanine nucleotide exchange factor; GPCR: G-protein-coupled receptor; GTP: guanosine triphosphate; LAR: leukocyte common antigen-related phosphatase; MAG: myelin-associated glycoprotein; MYPT-1: myosin phosphatase target subunit-1; NgR: Nogo receptor; OMgp: oligodendrocyte-myelin glycoprotein; p75NTR: p75 neurotrophin receptor; PDGF-BB: platelet-derivedgrowth factor-BB; PI3K: phosphatidylinositol 3-kinase; Rho: Ras homologus; ROCK: Rho-associated kinase.

In addition, we performed an electronic search of the PubMed database for the therapeutic effects of Rho kinase inhibitors, particularly fasudil, on CNS degenerative diseases. The search conditions were fasudil, multiple sclerosis, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. We excluded articles that did not correspond to human models of CNS degenerative diseases.

The Role of Ras Homolog-Associated Kinases in Neurodegenerative Disease

ROCKs activity is present in many types of nerve cells in the CNS. Excess ROCKs activity in the CNS leads to oxidative stress, uncontrolled inflammation, immune abnormality, energy metabolism disorders, neuronal cell loss, reactive gliosis, and impaired synaptic transmission, thus promoting the development of neurodegenerative diseases (Chong et al., 2017). ROCKs overexpression has been detected in the lesions of Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS), revealing that ROCKs are involved in the pathology of these diseases and might be important initiators of pathogenesis (**Figure 2**) (Tajouri et al., 2005; Yan et al., 2019; Ibrahim et al., 2020). AD is a common age-related neurodegenerative disease, and its pathogenesis has not been fully elucidated. However, we know that the typical pathological features of AD, including hyperphosphorylated tau protein in neurons and extracellular amyloid-β (Aβ) deposits, are the main causes of synaptic damage and dendritic spine loss. ROCKs overactivation not only induces the formation of toxic $A\beta_{1-42}$ and the promotion of Aβ deposition but also induces synaptic damage and loss in combination with Aβ deposition (Henderson et al., 2016; Ferrera et al., 2017). Abnormally high expression of α-synuclein (α-syn) in PD induces progressive degeneration of dopaminergic neurons; activation of the ROCKs signaling pathway is also involved in processes including impaired transmission of α-syn-affected neurons, oxidative stress, mitochondrial degeneration, lipid metabolism defects, and endoplasmic reticulum stress (Hou et al., 2018). MS is a chronic inflammatory demyelinating disease. ROCKs are highly expressed in the central and peripheral regions of MS patients, and the ROCKs upstream effector RhoA is upregulated in MS plaques (Tajouri et al., 2005; Ibrahim et al., 2020). RhoA regulates the recruitment and activation of inflammatory cells in injured and stressed sites and aggravates the inflammatory response and subsequent demyelination (Fang et al., 2017; Yan et al., 2019).

Thus, ROCKs have become new targets for the treatment of neurodegenerative diseases. Inhibition of ROCKs causes several biological events, such as increased neurite outgrowth, axonal regeneration, and activation of prosurvival protein kinase B (AKT), and exhibits broad potential for clinical application in the treatment of primary or secondary nerve injury (Koch et al., 2018). In previous studies, we also found that ROCKs inhibitors (RKIs) such as fasudil and its derivatives have therapeutic effects in experimental models of MS, AD, and PD (Li et al., 2017b; Gao et al., 2019; Yan et al., 2019). We summarize here the role of ROCKs in immune cells and CNS-specific cells and the therapeutic potential of its inhibitor fasudil in several experimental models of neurodegenerative diseases. We also elucidate the possible cellular and molecular mechanisms of ROCKs inhibition.

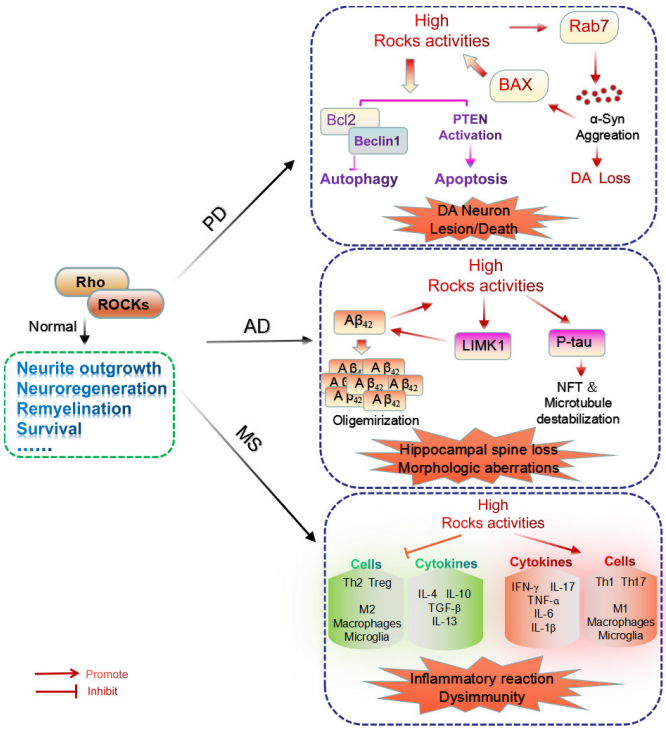

Figure 2 | **Rho/ROCK and PD, AD, and MS pathology.**
There is an activation loop between Aβ and ROCK. ROCK can phosphorylate tau (thr245 and ser409) leading to neurofibrillary tangles (NFT) and microtubule destabilization. ROCK is also involved in α-syn aggregation and impaired transmission of dopamine (DA). The aggregated α-syn can further activate ROCK and induce apoptosis of dopamine cells. ROCK is highly expressed in the central and peripheral regions of MS patients and regulates the recruitment and activation of inflammatory cells in injured and stressed sites and aggravates the inflammatory response and demyelination. AD: Alzheimer's disease; Aβ: amyloid-β; DA: dopamine; IL: interleukin; LIMK1: LIM domain kinase 1; MS: multiple sclerosis; PD: Parkinson's disease; P-tau: phospho-tauopathy; PTEN: phosphatase and tensin homolog; Rab7: Ras-related protein 7; Rho: Ras homologus; ROCK: Rho-associated kinase; TGF-β: transforming growth factor β; Th: T helper cell; TNF-α: tumor necrosis factor α; Treg: regulatory T cell; α-syn: α-synuclein.

The Effects of the Ras Homolog-Associated Kinases Signaling Pathway on Immunocytes and Central Nervous System Cells

ROCKs can be highly expressed in immune cells (T cells, B cells, macrophages, and microglia [MG]), astrocytes (AS), oligodendrocytes (OLs), and pericytes. ROCKs regulate the behavior of neurons and affect the course of neurodegenerative diseases (Koch et al., 2018; Brown et al., 2019; Rivera et al., 2019; Yan et al., 2019; Wang et al., 2021) (**Figure 3**).

The ROCKs signaling pathway regulates T lymphocytes

Activated T cells have been detected in patients with a variety of CNS neurodegenerative diseases and are the central link in the pathogenesis of neurodegenerative diseases. For example, many activated $CD4^+CD8^+$ T cells were detected in the cerebrospinal fluid and blood of patients with AD and were associated with structural magnetic resonance imaging changes and cognitive deterioration (Ferretti et al., 2016; Oberstein et al., 2018). An imbalance between type 17 helper T (Th17) cells and regulatory T (Treg) cells of the adaptive immune system may be part of the pathogenesis of PD (Bolte and Lukens, 2018; Prots and Winner, 2019). MS is an autoimmune disease mediated by abnormal activation of T cells; the main treatment strategy is to target T cells (Baecher-Allan et al., 2018). Therefore, it is very important to find molecular targets to regulate the behavior and function of T cells.

T cells need to cross the vascular endothelial barrier to enter the CNS to trigger an inflammatory cascade. This process involves adhesion with vascular endothelium, polarization of T cells, and cytokine-mediated transendothelial migration. The migration of T cells through the endothelium seems to be particularly reliant on the RhoA/ROCKs pathway, because the migration of T cells is dependent on effective T cell uropod contraction (Soriano et al., 2011). ROCKs are highly active in the cellular structures of filamentous pseudopodia and tabular pseudopodia when T cells are activated for migration and localization, which is important for T cell adhesion, migration, differentiation, proliferation, and survival, as observed in mice with inflammatory or autoimmune diseases (Paintlia et al., 2012; Pollock et al., 2014; El Azreq et al., 2016).

A recent study utilizing naive murine T cells has shown that ROCK2 is selectively activated under Th17 conditions, and T cells from heterozygous

ROCK2-deficient mice exhibit impaired Th17 differentiation, accompanied by reduced expression of transcription factors and decreased production of Th17 cytokines (Flynn et al., 2016). Furthermore, overexpression of ROCK2 in Th17 cells promotes the secretion of inflammatory cytokines such as interleukin (IL)-17 and IL-21 by phosphorylation of interferon regulatory factor 4 (Chen et al., 2018). ROCK2 can regulate the phosphorylation of signal transducer and activator of transcription 3, increase the binding rate of IL-17 and IL-21 promoters, stimulate T cells to secrete IL-10, induce T cells to polarize to Th17/Th1, and inhibit Th2/Treg differentiation (Zanin-Zhorov et al., 2014; Chen et al., 2018). RhoA deficiency in T cells also impairs Th2 differentiation, rather than Th1 differentiation, presumably by regulation of metabolic processes such as glycolysis (Yang et al., 2016). These effects may be mediated by the ROCK1 subtype, as ROCK1-deficient mice have decreased expression of the Th2 cytokines IL-5 and IL-13 (Zhu et al., 2011). Although further studies are needed to fully determine the exact roles of these two ROCKs subtypes in different Th subtypes, the above reports suggest that ROCK1 and ROCK2 may contribute to the differentiation of different Th subtypes.

The ROCKs signaling pathway regulates B cells

B cells mediate humoral immunity and exhibit effector functions that depend on the nature and affinity of the signal and the immune environment (Natkanski et al., 2013). Major histocompatibility complex class II-positive B cells induce activation of myelin-specific T cells and subsequent demyelination and axonal loss in MS and experimental autoimmune encephalomyelitis (EAE); these processes are reversed upon B cell depletion or if mice are B cell deficient (Häusler et al., 2018). B cells also promote stroke-associated dementia in animal models and patients even in the decade following the stroke (Doyle and Buckwalter, 2017).

As an important signaling molecule that regulates actin, ROCKs not only regulates the remodeling of the B cell cytoskeleton but also promotes the development, proliferation, and differentiation of B cells. Some studies demonstrated that the number of peripheral B cells in CD19-Cre mice was significantly reduced after ROCKs inhibition. This reflects the imbalance of B cell-activating factor/B cell-activating factor receptor-mediated signal transduction after inhibition of B cell-activating factor receptor expression by deletion of ROCKs (Zhang et al., 2012; Ricker et al., 2016, 2020). This suggests that the B cell-activating factor-mediated B cell survival partially relies on ROCKs activation. One important function of the RhoA/ROCKs pathway is the control of phosphatidylinositol 3-kinase (PI3K)/AKT signaling via the regulation of PTEN. Activated RhoA/ROCKs/PTEN signaling promotes lymphocyte niche confinement by counterbalancing the PI3K/AKT signaling induced by chemokines such as stromal-derived factor 1α and CXC motif chemokine 13 (Bouafia et al., 2019). Knock down of ROCK1 expression using a ROCK1-specific siRNA can significantly abolish PTEN-mediated inhibition of PI3K activity and thereby the extent of B cell activation (Li et al., 2014). ROCK2 can regulate the formation and maintenance of B cells in the germinal centers by inhibiting AKT activation and promoting a forkhead box O1-dependent transcriptional program, which promotes the appropriate location and metabolic adaptability of B cells in the germinal center (Jeong et al., 2019; Ricker et al., 2020). In addition, CD40, a key T cell-derived signaling molecule, can strongly induce the activity of ROCK2 in B cells, which instructs the fate of B cells and promotes formation of the germinal center (Jeong et al., 2019; Ricker et al., 2020). Therefore, the involvement of ROCKs in the regulation of B cell responses could have immediate and broad therapeutic implications.

The ROCKs signaling pathway regulates macrophages/microglia

MG, immune cells of the CNS, continuously monitor the CNS microenvironment and are activated in the early stages of brain injuries; thus, they have a dual role in neuroprotection and nerve injury (Hickman et al., 2018; Song and Colonna, 2018). Caldeira et al. (2017) studied AD patients and found both inflammatory M1 and anti-inflammatory/neurotrophic M2 MG and significant morphological changes when MG migrate to the Aβ plaque area and phagocytize the myeloid structure. Many studies have since focused on the regulation of MG by ROCKs and have found that ROCKs are highly expressed in activated MG, which have an M1 phenotype, increased cell size and number of filopodia, and increased phagocytic/secreting domains, and are neurotoxic (Barcia et al., 2012; Xu et al., 2020). Using an *in vitro* coculture system of neurons and MG, almost all activated ROCKs signals were located in activated MG, and RKIs protected against neuronal injury in this system (Xu et al., 2019). Y-27632, a RKI, also protected dopaminergic neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced toxicity but only in the presence of MG (Zhang et al., 2016). The increased expression of RhoA and ROCK2 were also detected after oxygen-glucose deprivation/reoxygenation in BV2 MG cells, and the cells subsequently polarized to the M1 phenotype. The effects of RhoA and ROCKs on hypoxia-induced microglial polarization may involve the profilin-1 gene (Lu et al., 2020a). Expression of proinflammatory cytokines, such as IL-6, IL-1β, and tumor necrosis factor α (TNF-α), as well as release of reactive oxygen species and stimulation of phagocytosis have been implicated as downstream effectors of the ROCKs pathway in MG (Zhao et al., 2015b). The ROCKs/p38 mitogen-activated protein kinase/nuclear factor-κB pathway can be activated in response to the stimulation of P2Y and P2Y receptors, which leads to the occurrence and maintenance of MG inflammation (Tatsumi et al., 2015; Liu et al., 2017; Jing et al., 2019).

In addition, migration and phagocytosis of MG cause the reorganization of stress fibers and focal adhesions, as well as the rearrangement of filopodia. A previous study found that RhoA and ROCKs are necessary for complement receptor 3 (also known as CD11b/CD18)-related microglial cell phagocytosis (Liu et al., 2020). Complement receptor 3 belongs to the β2 integrin family and is mainly expressed on phagocytes involved in microglial phagocytosis of myelin debris. Studies of complement receptor 3-mediated myelin debris phagocytosis have focused on chronic pathogenic processes, including MS and amyotrophic lateral sclerosis (ALS) models (Ramaglia et al., 2012; Liu et al., 2020). Therefore, blocking motility using RKIs represents a potential strategy to diminish microglial-mediated inflammatory responses, thus specifically protecting neurons from microglial phagocytosis and preventing their complete elimination.

The ROCKs signaling pathway regulates astrocytes

AS are the most abundant glial cell type and are distributed evenly throughout the brain. They maintain the stability of the CNS by participating in the formation of myelin sheaths, which maintain the integrity of the CNS so that AS can respond to various forms and degrees of damage, including minor disturbances, by a process called reactive AS proliferation (Li et al., 2019; Linnerbauer et al., 2020). Reactive AS modify disease progression by regulating the expression and secretion of chemokines, cytokines, adhesion molecules, antigen-presenting molecules, and receptors, thus affecting AS-to-neuron communication in AD, MS, and PD patients (Vardjan et al., 2012; Liddelow and Hoyer, 2016; Hinkle et al., 2019; Nutma et al., 2020). ROCKs can be expressed in the cytoplasm and plasma membrane of reactive AS, but ROCKs expression in healthy and resting AS *in situ* is significantly lower than in adjacent neurons (Renault-Mihara et al., 2017; Renault-Mihara and Okano, 2017). Thus, when the CNS is damaged, such as in stroke patients, AS in the ischemic hemisphere of rodents and humans are activated, which is accompanied by increased intracellular ROCKs activity. This implies that reactive AS–mediated inflammatory responses, neurotoxicity, and the growth, migration, and formation of glial scars are closely related to ROCKs activation (Renault-Mihara et al., 2017; Renault-Mihara and Okano, 2017). For example, ROCKs activation is related to a decrease in glutamate transporter expression in AS. Studies have shown that exposure of primary AS to thrombin can lead to the downregulation of AS glutamate-aspartate transporter expression, resulting in compromised glutamate uptake (Pajarillo et al., 2019; Peterson and Binder, 2020). Fasudil can reverse these changes (Piao et al., 2019). In a mouse model of spinal cord injury, increased ROCKs activity in reactive AS, followed by upregulation of PTEN expression, disrupted synaptic plasticity by inhibition of posttraumatic synaptogenesis and destruction of newly formed synapses (Povysheva et al., 2018). Thus, it is apparent that ROCKs inhibition results in reduced AS activation and secretion of inflammatory-related factors, such as matrix metalloproteinase-2 and-9 (Guo et al., 2006; Kim et al., 2020).

The morphological changes associated with reactive astrogliosis are caused by changes in actin dynamics, which are controlled by the actin-related protein 2/3 complex or by oligophrenin-1 signaling, which both regulate ROCKs activity in reactive AS (Murk et al., 2013; Pillet et al., 2020). AS cell cultures that adopt a reactive morphology following actin-related protein 2/3 complex inhibition have a higher proportion of active GTP-bound Rho than inactive GDP-bound Rho, and these morphological changes are reversed upon ROCKs inhibition. Oligophrenin-1 is a negative regulator of Rho GTPases that stimulates the GTPase activity of RhoA to its GDP-bound inactive form and turns off signaling from RhoA to ROCKs and MLC2 (Pillet et al., 2020). Knockout of oligophrenin-1 in AS leads to lower migratory ability of AS, showing that the ROCKs pathway is essential for reactive astrogliosis.

The ROCKs signaling pathway regulates oligodendrocytes

OLs are derived from OL progenitor cells (OPCs) in the CNS. Their plasma membranes extend to envelop axons to form insulating myelin sheath structures. In MS patients, the myelin sheath is attacked by the immune system, leading to OL death, demyelination, axon exposure, and impaired neuron function (Dulamea, 2017). Remyelination of demyelinating lesions can occur, but its effectiveness is too low to resist disease progression. Therefore, sufficient OPCs must be recruited to the lesion site to remyelinate effectively; these OPCs differentiate into OLs that rewrap the exposed axons (Stone et al., 2017; Jarrell et al., 2018; Nasrabady et al., 2018). The morphology of OPCs changes dramatically during their migration and differentiation, which requires the correct spatial and temporal polymerization of tubulin and actin and the assembly of activated nonmuscle myosin with actin fibers (Ulc et al., 2019). Because ROCKs activation seems to be necessary to stabilize the actin cytoskeleton, the migration, proliferation, and differentiation of OPCs may be regulated by ROCKs (Sokol et al., 2019). In OPCs, inhibition of actin polymerization leads to increased process elongation and differentiation to OLs, which is conducive to myelin formation. When siRNA is used to selectively inactivate ROCKs, myelin protein expression was upregulated by 55–65% (Pedraza et al., 2014). The guanine nucleotide exchange factor Vav3, an activator upstream of ROCKs, was knocked out in OLs in mice, resulting in a significant increase in cell differentiation, characterized by longer internodes and an increased number of myelin sheaths formed per cell (Ulc et al., 2019). Migration and proliferation of OPCs and OLs may be also regulated by downstream signaling molecules of ROCKs, including phosphorylated MLC, which requires the polymerization of actin and tubulin subunits (Sokol et al., 2019; Ulc et al., 2019). Therefore, inhibition of ROCKs signaling molecules leads to OPC differentiation and results in morphological and functional cell maturation and myelin formation. In addition, increased activation of the ROCKs signaling pathway correlates with increased proliferation and survival of OPCs and a slowed cell cycle in OPCs. ROCKs activity is negatively regulated by receptor-type tyrosine-protein phosphatase F alpha-Fyn signaling (Wang et al., 2012a). Chondroitin sulfate proteoglycans also can regulate OL differentiation and apoptosis in the injured adult spinal cord by activation of both receptor-type tyrosine-protein phosphatase F and receptor-type

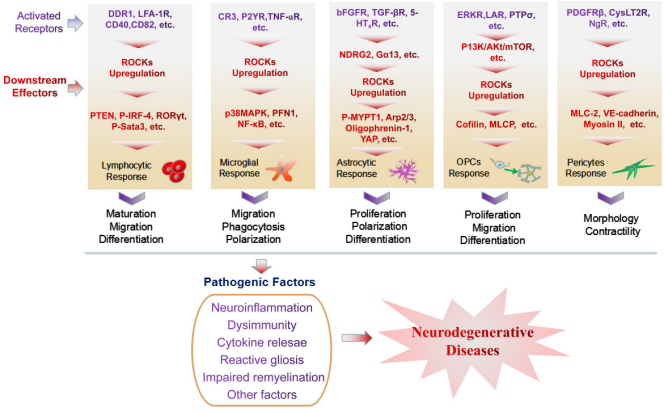

Figure 3 | **ROCK acts on many receptors and uses many downstream effectors in different cell types.**

ROCK is highly expressed in immune cells (T cells, B cells, and macrophages/microglia), astrocytes (AS), pericytes, and OPCs, which then interact with neurons to regulate the behavior of these cells and affect the course of disease. 5-HT4R: Serotonin receptor 4; Akt: protein kinase B; Arp2/3: Arp2/3 complex; bFGFR: b-fibroblast growth factors receptor; CR3: complement receptor 3; CysLT2R: cysteinyl leukotrienes 2 receptor; DDR1: discoidin domain receptor 1; ERKR: extracellular signal-regulated kinase receptor; Gα13: galpha13; IRF-4: interferon regulatory factor 4; LAR: leukocyte common antigen-related; LFA-1: lymphocyte function-associated antigen-1; LFA-1R: lymphocyte function-associated antigen-1 receptor; MLC2: myosin light chain 2; MLCP: myosin light chain phosphatase; mTOR: mammalian target of rapamycin; NDRG2: N-myc downstream-regulated gene 2; NF-κB: nuclear factor-κB; NgR: Nogo receptor; OPC: oligodendrocyte progenitor cell; PI3K: phosphatidylinositol 3-kinase; P2YR: P2Y G-protein-coupled receptors; P38MARK: p38 mitogen-activated protein kinase; PDGFRβ: platelet-derived growth factor receptor β; PFN1: profilin 1; P-IRF-4: phosphorylated Interferon-regulatory factors; P-MYPT1: phosphorylated myosin phosphatase target subunit-1; P-Sata3: phosphorylated signal transducer and activator of transcription 3; PTEN: phosphatase and tensin homolog; PTPσ: receptor type protein tyrosine phosphatase-sigma; ROCK: Rho-associated kinase; TGF-βR: transforming growth factor-beta receptor; TNF-αR: tumor necrosis factor-α receptor; VE-cadherin: vascular endothelial-cadherin; YAP: Yes-associated protein.

tyrosine-protein phosphatase S and the downstream Rho/ROCKs pathway (Dyck et al., 2019). Taken together, these findings indicate that ROCKs activation inhibits the proliferation, migration, and differentiation of OPCs, whereas ROCKs inhibition contributes to myelin regeneration, suggesting that RKIs should be further investigated as stimulators of myelin regeneration.

The ROCKs signaling pathway regulates pericytes

Pericytes, an understudied cell type located on capillaries, are of crucial importance in regulating cerebral blood flow, maintenance of the blood-brain barrier (BBB), and control of vascular development and angiogenesis (Brown et al., 2019; Hirunpattarasilp et al., 2019). Pericytes can also facilitate neuroinflammation and possess stem cell-like properties (Hirunpattarasilp et al., 2019). Therefore, aberrant pericyte function and/or pericyte deficiency accompanied by vascular dysfunction is increasingly recognized as a contributor to the progression of vascular diseases such as stroke and neurodegenerative diseases such as AD (Cheng et al., 2018). ROCKs are key regulators of pericyte shape and contractility (Anastasia et al., 2014; Hirunpattarasilp et al., 2019). ROCKs inhibit MLC phosphatase, leading to increased phosphorylation of MLC by Ca^{2+}/calmodulin-activated dependent MLC kinase, thereby increasing pericyte contractility. A study showed that pericytes overexpressing ROCKs were hypercontractile, with numerous actin-enriched projections surrounding a centrally contracted cytoplasmic mass, and had a 1.5-fold greater frequency of a contractile, substrate-deforming phenotype compared with control pericytes (Kutcher et al., 2007). Wang et al. (2012b) discovered that polymorphonuclear neutrophil-pericyte contacts could induce relaxation rather than contraction of pericyte cytoskeletons by inhibition of the ROCKs signaling pathway in pericytes. Pericyte cytoskeleton relaxation contributes to the opening of the gaps between pericytes in the vascular basement membrane, facilitating polymorphonuclear neutrophil extravasation (Wang et al., 2012b). This raises the possibility that pericytes may be targeted in therapies aimed at regulating inflammation. In addition, alterations in ROCKs signaling also caused significant abrogation of pericyte-mediated endothelial growth arrest (Kutcher et al., 2007). These studies strongly suggest that ROCKs inhibition may be a therapeutic strategy for decreasing the pericyte-mediated constriction of capillaries that occurs in neurodegenerative diseases.

Neurobiology of Ras Homolog-Associated Kinase Inhibition

Increased ROCKs activity leads to the abnormal behavior of T cells, B cells, MG, AS, OLs, and pericytes that induces the common pathological features of CNS neurodegenerative diseases, such as axonal degeneration, protein clearance dysfunction, neuroinflammation, and immune imbalance (Koch et al., 2018). A treatment that targets just one of these disease features is unlikely to outpace all other pathways and halt disease progression. Therefore, drugs that can affect several of these processes would have a clear advantage. Because the ROCKs signaling pathway is important in all these processes, it is expected to be a promising target for new therapeutic approaches.

Immunomodulation and neuroinflammatory inhibition by Ras homolog-associated kinase

Immune imbalance and neuroinflammation are involved in the pathophysiology of various neurological disorders and are currently considered as prime targets for therapy development. Several animal studies have investigated the efficacy of different immunomodulatory approaches in patients with neurological diseases (De Virgilio et al., 2016; Morshedi et al., 2019; Zhang et al., 2020). Immune system trials have focused on reducing MG/macrophage activation and inhibiting lymphocyte migration and polarization (Faridar et al., 2020; Kuo et al., 2020). The increased activity of ROCKs in these cells, on the one hand, maintains the proinflammatory phenotype of immune cells and promotes their migration; on the other hand, increased ROCKs activity promotes the secretion of inflammatory factors and adhesion molecules that promotes the deterioration of immune imbalance and neuroinflammation.

Pharmacological ROCKs inhibition with fasudil or Y-27632 leads to immune regulation and inhibition of neuroinflammation, including the induction of MG/macrophages to polarize to the M2 phenotype, regulation of the Th17/Treg and Th1/Th2 balance, and reduction of the secretion of inflammatory factors (Zhao et al., 2019; Zhou et al., 2019). For example, fasudil can restore the balance of Th1/Th2/Th17/Treg subsets in peripheral blood mononuclear cells of patients with myasthenia gravis *in vitro*. Briefly, fasudil inhibited $CD4^+$ T cell differentiation to Th1 and Th17 cells by reducing phosphorylation of signal transducer and activator of transcription 1-alpha/beta and signal transducer and activator of transcription 3; fasudil promoted Treg cell differentiation by increasing phosphorylation of signal transducer and activator of transcription 5 (Chen et al., 2018; Song et al., 2019). Y-27632 can also decrease IL-17 and IL-21 production in purified T cells from systemic lupus erythematosus patients or from Th17 cells (Rozo et al., 2017). Fasudil led to a decreased M1 and increased M2 phenotype of MG and induction of reactive oxygen species in BV-2 MG induced by advanced glycation end products (Chen et al., 2017). Inhibition of ROCKs activity by Y-27632 reduced the release of TNF-α from MG and reduced the death of dopaminergic neurons in primary mesencephalic cultures and in mice treated with the catecholaminergic neurotoxins 1-methyl-4-phenylpyridiniumion or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, respectively. Taken together, immune imbalance and neuroinflammation can be reversed by RKIs.

Neuroprotective effects of Ras homolog-associated kinase

In neurodegenerative diseases, pathological and physiological changes in synapses, neuronal morphology, neurite growth, neuronal apoptosis, and survival are closely associated with decreased cognitive, learning, and motor abilities. Dynamic polymerization and depolymerization of actin filaments are required for neuron growth and synaptic plasticity, in which ROCKs activity plays an essential role (Koch et al., 2018). Following CNS injury, axons and synapses are exposed to growth-inhibitory myelin debris and to infiltrating MG and reactive AS that predominantly activate the ROCKs signaling pathway. Increased ROCKs activity leads to retraction forces at the axon terminal and presynaptic contraction, which contributes to regenerative failure and an imbalance in synaptic plasticity (Fujita and Yamashita, 2014; Deguchi et al., 2016). The RKI Y-27632 can counteract axon retraction and can restore synaptic and behavioral plasticity (Lingor et al., 2007; Deguchi et al., 2016). Similar results were observed using rodent spinal cord injury models, in which animals were treated locally with Y-27632 or fasudil for 2 weeks after hemisection. RKIs can induce axons to extend to the site of injury and accelerate the recovery of mobility dysfunction of rodents' hind limbs (Nishio et al., 2006; Luo et al., 2021). When cultured mouse cortical neurons were treated with serum from MS patients, neurite length and synapse formation were significantly reduced. Conversely, addition of fasudil to the culture promoted axonal branching and synaptic formation, partially rescuing mobility dysfunction (Chen et al., 2015a).

In addition, ROCKs are involved in regulating neuronal apoptosis and survival. Some studies support the idea that increased ROCKs activity induces neuronal responses to noxious stimuli and that inhibition of ROCKs activity reduces neuronal apoptosis, leading to neuroprotection (Gao et al., 2019; Li and Liu, 2019; Wei et al., 2021). ROCKs levels were shown to increase prior to neuron death in an Aβ-induced toxicity assay of primary mouse hippocampal neurons. However, inhibition of ROCKs activity by fasudil led to higher neuron survival. Fasudil caused these effects by inhibiting the mitogen-activated protein kinase kinase kinase 5/c-Jun N-terminal kinase signaling pathway and reducing calcium overload and mitochondrial membrane potential (Gao et al., 2019). Reduced hippocampal apoptosis was also detected in the amyloid-beta precursor protein/presenilin-1 transgenic AD mouse model treated with fasudil (Wei et al., 2021). In the middle cerebral artery occlusion-induced cerebral ischemia injury rat model, the inhibitory effect of Y-27632 on ROCKs promoted the survival of neurons and the recovery of motor function after nerve injury (Li and Liu, 2019).

Collectively, the abovementioned studies highlight that inhibition of ROCKs activity promotes nerve regeneration and repair of synaptic and behavioral plasticity and interferes with the induction of neuronal cell death; thus, it is a promising neuroprotective strategy.

Application of the Ras Homolog-Associated Kinase Inhibitor Fasudil in Neurodegenerative Diseases

The activation of ROCKs in neurodegenerative diseases indicates that ROCKs may affect the occurrence and development of diseases (Xu et al., 2021). Inhibition of ROCKs has become an effective strategy for the treatment of neurodegenerative diseases (Feng et al., 2016) (**Additional Table 1**). Currently, RKIs approved in clinical application include fasudil (Rikitake et al., 2005; Sun et al., 2006; Couch et al., 2010; Song et al., 2013; Zhao et al., 2015c; Yu et al., 2016a; Guo et al., 2019, 2020; Hamano et al., 2020) for the treatment of cerebral vasospasm and netarsudil and ripasudil for the treatment of glaucoma, which are more selective than fasudil (Tanna and Johnson, 2018). Other RKIs that act on both ROCK1 and ROCK2 include Y-27632 (Rikitake et al., 2005; Zhang et al., 2006, 2019; Borrajo et al., 2014; Hamano et al., 2020), H-1152 (Zhang et al., 2006; Hamano et al., 2020), Wf-536, Y-39983, AMA-0076, GSK-269962A, SB-772077-B, SAR-407899, and RKI-1447 (Berrino and Supuran, 2019). Currently, only KD-025 is ROCK2-specific (Berrino and Supuran, 2019). Studies have shown that treatment with fasudil in MS patients can repair the BBB, promote neuroprotection and remyelination, and significantly relieve clinical symptoms in patients with neurodegenerative diseases (Yu et al., 2010; Huang et al., 2011). However, clinical application of fasudil shows high toxicity and a narrow safety window, so fasudil can only be used for a short time, which limits its application. In addition, fasudil can only be given intravenously in the clinic, owing to poor oral bioavailability. Therefore, the research and development of new RKIs has been a hot topic in the past 10 years (Yu et al., 2010; Berrino and Supuran, 2019). Here, we summarize the therapeutic effects and mechanisms of RKIs for the treatment of neurodegenerative diseases and illustrate the potential value and the problems to be solved for clinical application of RKIs (**Figure 4**).

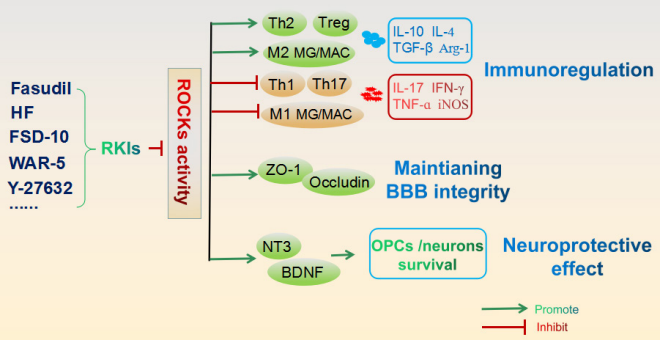

Figure 4 | **Mechanisms of RKIs in the treatment of neurodegenerative diseases.**
RKIs can be used to treat neurodegenerative diseases by immunomodulation, BBB repair, and neuroprotective effects. They inhibit the function of Th1 and Th17 cells and M1 macrophages/microglia. In contrast, they increase the generation of M2 macrophages (Arg-1 and IL-10) and the proportion of $CD4^+CD25^+$ T cells and IL-10 in $CD4^+$ T cells, while reducing the proportion of IL-17 in $CD4^+$ T cells. RKIs maintain the BBB by blocking the destruction of occludin, an integral plasma membrane protein located at the tight junctions between endothelial cells, and ZO-1, a tight junction protein. RKIs also induce the secretion of neurotrophic factors, which increases the number of OPCs and promotes neuron survival in EAE. Arg-1: Arginase-1; BBB: blood-brain barrier; BDNF: brain-derived neurotrophic factor; EAE: experimental autoimmune encephalomyelitis; FSD-C10: fasudil-C10; HF: hydroxyfasudil; IFN-γ: interferon-γ; IL: interleukin; iNOS: inducible nitric oxide synthase; MAC: macrophage; MG: microglia; NT-3: neurotrophins-3; OPC: oligodendrocyte progenitor cell; RKI: ras homologus kinase inhibitor; ROCK: ras homologus-associated kinase; TGF-β: transforming growth factor β; Th: T helper cell; TNF-α: tumor necrosis factor α; Treg: regulatory T cell; ZO-1: zonula occludens-1.

Multiple sclerosis

EAE is a classical animal model of MS. Previous studies have shown that fasudil treatment of EAE mice can reduce its incidence, prolong its onset time, alleviate its severity, improve neuroinflammation and demyelination, and protect the integrity of the BBB (Yu et al., 2010, 2016b). In addition, hydroxyfasudil, an active metabolite of fasudil, and derivatives of fasudil, such as WAR-5 and FSD-C10 (Li et al., 2017a), also inhibit neuroinflammation, improve demyelination, and reduce the incidence and severity of EAE in mice (Xin et al., 2015). It is important that hydroxyfasudil and FSD-C10 not only exhibit good therapeutic effects but also have lower cytotoxicity and side effects when compared with fasudil.

Our previously published results showed that fasudil can treat EAE through immune regulation, inflammation inhibition, BBB protection, and neurotrophic effects. Fasudil reduced the infiltration of peripheral macrophages and T cells in the CNS by inhibiting the expression of the chemokines C-C motif chemokine 2, C-C motif chemokine 3, C-C motif chemokine 5, and C-C motif chemokine 20 (Guo et al., 2014). Fasudil also reduced the proportion of Th1 and Th17 cells in EAE mice, while increasing the expression of anti-inflammatory Treg cells and Th2 cells in the periphery and CNS (Liu et al., 2013, 2016), thus modifying the immune imbalance. In addition, fasudil can reduce the number of M1 macrophages/MG in the spleen and spinal cord, increase the polarization to the M2 phenotype, inhibit the secretion of inflammatory factors, and increase the release of neurotrophic factors (Liu et al., 2013, 2016; Borrajo et al., 2014). An intact BBB can block infiltration of peripheral immune cells into the CNS and prevent peripheral immune attack. Tight junctions between brain endothelial cells are important structures to maintain BBB integrity and function. Fasudil can upregulate the expression of the tight junction proteins occludin and zonula occludens protein 1 in brain tissue of EAE mice, thereby protecting the integrity and maintaining the function of the BBB (Gu et al., 2017). Failure of differentiation and maturation of OPCs is common in progressive MS patients, which is related to the lack of sufficient neurotrophic factors in the CNS (Zhang et al., 2009; Vondran et al., 2010; Yang et al., 2014). Fasudil significantly increased brain-derived neurotrophic factor and neurotrophin-3 in the spinal cord of EAE mice, protecting and promoting myelin regeneration (Stone et al., 2017). We also found that other RKIs had the same therapeutic effect in EAE mice and had similar cellular and molecular mechanisms of action to fasudil (Yu et al., 2010).

In recent years, cellular immunotherapy has become a research hotspot in the field of neurology. This therapeutic method has multiple advantages, including easy sampling, isolation, and culture; autologous transplantation; no allograft rejection; low cost and high safety profiles; and no ethical problems. Additionally, there is no tumorigenicity concern because immune cells are terminally differentiated and only survive for a limited time *in vivo*. Immune cells have both inflammatory and anti-inflammatory/restorative effects. Anti-inflammatory and restorative effects can improve immune tolerance and even modify and regulate the inflammatory microenvironment, contributing to the repair of neurons. Our previously published experiments showed that fasudil could modify the evolution of encephalitic Th1 and Th17 cells and anti-inflammatory Th2 and Treg cells in the spleen of EAE mice on day 9 postimmunization. On day 9 postimmunization, splenocytes treated with fasudil *in vitro* did not induce C57BL/6 mice to establish a passive-transfer EAE model compared with control cells (Liu et al., 2015; Guo et al., 2019). The encephalitic splenocytes modified by fasudil *in vitro* also reduced clinical symptoms and improved the CNS inflammatory environment and demyelination in active immune-induced EAE mice. Therefore, we believe that immune cells modified by fasudil *in vitro* are an effective prospect for MS treatment in the future.

Alzheimer's disease

It is well known that AD is characterized pathologically by the accumulation of Aβ plaques and neurofibrillary tangles, which leads to progressive loss and changes in synaptic potency and damage to synaptic terminals. Synaptic loss is a major cause of AD-related cognitive impairment, and dynamic regulation of actin aggregation plays a key role in morphological changes in dendritic spines. Furthermore, studies have shown that the expression of ROCKs protein is significantly increased in prefrontal cortex homogenates of patients with AD. Taken together, the ROCKs signaling pathway has become one of the important targets of AD therapy.

One experimental strategy to halt progression of AD is to mitigate the levels of pathogenic Aβ and neurofibrillary tangles in the brain. Fasudil and its derivative FSD-C10 significantly improved the cognitive dysfunction of APP/PS1 mice by decreasing brain levels of $A\beta_{42}$, phosphorylated tau protein, and beta-site amyloid precursor protein cleaving enzyme 1 in the hippocampus (Yu et al., 2017; Guo et al., 2020). Further observation demonstrated that fasudil reduced the degeneration and loss of cholinergic neurons (Yu et al., 2018). Fasudil can also promote the proliferation of endogenous neural stem cells in the dentate gyrus and subventricular area of the hippocampus, increase the number of cholinergic neurons in the hippocampus, promote the regeneration of axons, and maintain morphological integrity (Gao et al., 2019). In addition, neuropathological studies showed that the number of dendrites decreased significantly in AD patients treated with fasudil in the brain regions that support cognitive function (Rakic et al., 2018; Sun et al., 2019). Fasudil injection into the ventricle can increase the number of dendrite branches of CA1 pyramidal neurons in APP/PS1 mice, promote the extension of dendrite branches, and improve the spatial learning and working memory ability of mice (Yu et al., 2017; Guo et al., 2020). In addition, fasudil also protected against Aβ-induced neurotoxicity by reducing cofilin-1 phosphorylation, acute synaptic damage, and synaptic toxicity in primary cortical neurons (Rush et al., 2018).

The protective effect of inhibition of ROCKs in AD models may also be realized by inhibiting neuroinflammation and oxidative stress. Fasudil influences neuroinflammation in amyloid-beta precursor protein/presenilin-1 transgenic mice, mainly by shifting the phenotype of MG from M1 to M2 and by reducing the inflammatory Toll-like receptor 2/4-myeloid differentiation factor 88-nuclear factor-κB axis and production of IL-1β, IL-6, and TNF-α, thereby improving the inflammatory environment in the CNS (Guo et al., 2020). For the oxidative stress response of AD mice, fasudil can increase the concentration of antioxidant substances and reduce lipid peroxides by activating the nuclear factor erythroid 2-related factor 2 signaling pathway (Wei et al., 2021). In addition, fasudil can also ameliorate AD symptoms by reinstating PI3K-mediated upregulation of endothelial nitric oxide synthase and control over brain nuclear factor-κB activity, achieving anti-inflammatory and antioxidant effects (Kumar and Bansal, 2018). In view of these data, fasudil has a potential therapeutic effect on AD, which is worth studying in more detail.

Parkinson's disease

PD is the second most common neurodegenerative disease after AD. It is estimated that more than 8 million people in the world will suffer from PD in the next 10 years (Dorsey et al., 2007). The clinical symptoms of PD are induced by degeneration and death of dopaminergic neurons in the substantia nigra compacta, degeneration of the dopaminergic pathway in the substantia nigra striatum, and the subsequent significant decrease of dopamine content (Song et al., 2020). The pathogenesis of PD relates to higher expression of pathological α-syn abnormally activated by ROCKs (Li et al., 2017b). A follow-up study illustrated that ROCK2 was highly expressed on AS and MG by analyzing the postmortem brains of patients with PD, which further suggests that ROCKs may be involved in the occurrence and development of PD (Saal et al., 2017). Therefore, inhibition of ROCKs may reduce the expression of α-syn and protect dopaminergic neurons.

Because α-syn aggregation is a major hallmark in the pathogenesis of PD, the antiaggregation potential of fasudil was evaluated. The results showed that fasudil treatment significantly reduced α-syn aggregation *in vitro* in an H4 cell culture model, as well as in a cell-free assay (Tatenhorst et al., 2016). *In vivo* experiments also demonstrated that fasudil improved the survival rate of dopaminergic neurons and inhibited the inflammatory response in the brain by clearing α-syn, which significantly improved the motor ability of PD mice (Zhao et al., 2015a; Tatenhorst et al., 2016). The main clearance mechanism of fasudil is its ability to induce macroautophagy, which was shown to be mediated by the beclin-1 and AKT/mammalian target of rapamycin pathways (Yang et al., 2020).

Fasudil demonstrated a multitarget neuroprotective effect in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of PD, which improved the motor function of mice by anti-inflammatory and antioxidant effects and by promoting the secretion of neurotrophic factors. Specifically, fasudil inhibited the expression of IL-1β, TNF-α, Toll-like receptor 2, inducible nitric oxide synthase, and gp91-phox and enhanced the level of antioxidant factors such as nuclear factor erythroid 2-related factor 2 and heme oxygenase 1, as well as neurotrophic factors including glial cell line-derived neurotrophic factor (Zhao et al., 2015a). These mechanisms may be related to the activation of the PI3K/phosphorylated AKT and wingless-type MMTV integration site/frizzled-1/catenin beta-1 cell signaling pathways (Zhao et al., 2015a). These studies highlight the potential of fasudil as a disease-modifying agent for the treatment of PD.

Amyotrophic lateral sclerosis

ALS is an incurable and fatal neurodegenerative disease characterized by age-related progressive degeneration of both upper and lower motor neurons (Takata et al., 2013). Mutations in the gene coding for the copper-zinc superoxide dismutase 1 (SOD1) enzyme represent a known main cause of ALS. Thus, the SOD1-G93A transgenic mouse is a classical animal model for studying ALS that exhibits enhanced ROCKs activity leading to increased levels of phosphorylated adducin, elevated activation of PTEN, and decreased activity of AKT (Takata et al., 2013). Oral fasudil inhibited these effects, thus reducing motor neuron loss, improving motor symptoms, significantly delaying disease progression, and prolonging survival (Takata et al., 2013). Detection of neuroinflammation showed that fasudil inhibited abnormal activation of AS and MG and reduced the release of proinflammatory cytokines and chemokines, such as TNF-α, IL-6, C-C motif chemokine 2 (CCL2), CCL3, and CCL5 (Tönges et al., 2014). However, in symptomatic mice, fasudil only improved the motor function of the male mice for a short time, and the survival time of the mice was not significantly prolonged (Günther et al., 2017). The reasons for this are unclear, and fasudil needs to be studied in more detail as a promising treatment for ALS.

Huntington's disease

Huntington's disease (HD) is a devastating, incurable neurodegenerative disease, which is characterized by a severe loss of neuronal cells in the striatum and cortex. It is caused by an expanded polyglutamine tract in huntingtin (Li et al., 2013; Ahmed et al., 2016). Therefore, the inhibition and degradation of huntingtin is one of the important strategies for the treatment of HD. Profilin-1 can promote huntingtin aggregation through ROCKs-dependent phosphorylation, so ROCKs inhibition could serve as a mechanism for HD therapy (Bauer et al., 2009). In the 3-nitropropionic acid-induced HD mouse model, fasudil can improve motor performance, protect mitochondrial function, and reduce expression of markers of oxidative stress, inflammation, and apoptosis. Its mechanism is related to the activation of the AKT/endothelial nitric oxide synthase signaling pathway by fasudil (Ahmed et al., 2016). Interestingly, the intravitreal application of fasudil to target retinal neurons more directly in the R6/2 mouse model of HD was more efficient compared to systemic administration of fasudil. In this study, fasudil significantly inhibited the phosphorylation of profilin-1, leading to partial recovery of retinal neuron dysfunction (Li et al., 2013). These data suggest that fasudil holds therapeutic potential in HD models, but the underlying mechanisms are not fully understood and require further study.

The Combination of Fasudil and Cell-Based Therapy in Neurodegenerative Diseases

Neurodegenerative diseases often lead to different degrees of neural dysfunction. At present, it is difficult to cure such diseases with ordinary interventions, which can result in limited therapeutic effect, poor prognosis, and a high disability rate. In recent years, it has been demonstrated that embryonic stem cells and mesenchymal stem cells can be differentiated into neurons or glial cells that can be used to treat neurodegenerative diseases, promote the regeneration of neurons and the myelin sheath, and improve neural function (Sugaya and Vaidya, 2018; Staff et al., 2019; De Gioia et al., 2020). However, this cell-based therapy has shortcomings, such as its detrimental effect on the microenvironment, low efficiency of survival and migration of neural stem cells into the brain, tumorigenesis risk, and relatively low proliferation and differentiation rates (Kolagar et al., 2020).

Recent studies suggest that fasudil promotes the mobilization of neural stem cells from the subventricular zone *in vivo* and promotes the differentiation of the C17.2 cerebellar neural progenitor line and primary neural stem cells *in vitro* (Chen et al., 2015b; Nizamudeen et al., 2018). In addition, fasudil provides an ideal environment for survival and activity of transplanted stem cells by inhibiting the inflammatory response and promoting the production of neurotrophic factors (Li et al., 2017b; Hu et al., 2019). Therefore, fasudil combined with cell-based therapy using neural stem cells or bone marrow stromal cells in AD, PD, and EAE achieved a better effect than neural stem cell therapy or fasudil alone (Yu et al., 2016a, 2017; Tang et al., 2020). The pretreatment of bone marrow stromal cells with fasudil *in vitro* can accelerate the proliferation of bone marrow stromal cells and promote the secretion of neurotrophic factors. Thus, fasudil-pretreated bone marrow stromal cells in MPTP-PD mice had stronger therapeutic potential (Tang et al., 2020).

Conclusion

So far, most drugs that target neurodegenerative diseases are limited in their ability to improve clinical symptoms and do not prevent disease progression. Abnormal activation of ROCKs under pathological conditions mediates neuroinflammation, oxidative stress, energy metabolism disorder, and neural inhibition; thus, ROCKs are effective targets for the treatment of neurodegenerative diseases. Fasudil, the first ROCKs inhibitor to be used clinically, is mainly used for the short-term treatment of vasospasm after subarachnoid hemorrhage. It has a good therapeutic effect on neurodegenerative diseases, but its relatively narrow safety window and lack of an orally administered formulation limit its long-term clinical application. Fasudil has limited selectivity with respect to ROCKs and may also act on other kinases at therapeutic concentrations. However, inhibition of other kinases may also contribute to the beneficial effects of fasudil, particularly in CNS disorders. In addition to target selectivity, the chemical structure of the drug may be important. For example, Y-27632 and fasudil have similar effects on actin remodeling and axonal growth, but only fasudil can attenuate the aggregation of α-syn owing to its specific protein-binding properties, which are separate from its ability to inhibit ROCKs. RKIs have been widely studied over the past few decades in order to develop RKIs with high safety and efficacy profiles. This review mainly focuses on the effects of fasudil, but it is also meaningful to compare the efficacy and molecular mechanisms of different Rho kinase inhibitors.

The etiologies and courses of neurodegenerative diseases are complex and involve many signaling pathways for which the cellular and molecular mechanisms are not always clear; thus, suppression of the ROCKs signaling pathway is not sufficient for disease treatment. It is necessary to further clarify the cellular and molecular mechanisms of the pathogenesis of neurodegenerative diseases and test combinations of drugs to achieve therapeutic effects by blocking different signaling pathways. Therefore, the collaborative application of RKIs and other drugs is an important future research direction.

Author contributions: *Manuscript design and draft: QW, LJS, ZBD, ZC; coordinate manuscript draft: CGM; manuscript revision: JZY, CGM, BGX. All authors approved the final version of the manuscript.*
Conflicts of interest: *The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.*
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Additional file:
Additional Table 1: *Trials of RKIs in neurodegenerative diseases.*

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C-Editor: Zhao M; S-Editors: Yu J, Li CH; L-Editors: Yu J, Song LP; T-Editor: Jia Y

Additional Table 1 Trials of RKIs in neurodegenerative diseases.

ROCK-inhibitor	Target spot	Subject of Trail	References
Fasudil	ROCK1/2	-Increased cerebral blood flow and stroke protection -Protection and therapy in EAE -Increased dendrite branching in APP/PS1 mice -Inhited Aβ-induced hippocampal neurodegeneration -Protected dopamine neurons via activation of PI3K/p-Akt and WNT1/Fzd1/β-catenin; -Combined intervention with MSCs attenuated EAE -Nasal delivery of fasudil-modified immune cells attenuated EAE -Inhibited the neurotoxic activation of microglia and astrocytes in APP/PS1 Tg mice -Reduced oligomeric tau protein	Rikitake et al., 2005; Sun et al., 2006; Couch et al., 2010; Song et al., 2013; Zhao et al., 2015c; Yu et al., 2016a; Guo et al., 2019; Guo et al., 2020; Hamano et al., 2020
Y-27632	ROCK1/2	-Increased cerebral blood flow and stroke protection; -Induced neurite outgrowth in PC-12 cells -Protected dopaminergic cell via inhibiting microglial response -Improved symptoms of PD by inhibiting Drp1-mediated aberrant mitochondrial -Reduced oligomeric tau protein	Rikitake et al., 2005; Zhang et al., 2006; Borrajo et al., 2014; Zhang et al., 2019; Hamano et al., 2020
H-1152	ROCK1/2	-Induced neurite outgrowth in PC-12 cells; -Reduced oligomeric tau protein	Zhang et al., 2006; Hamano et al., 2020

APP: Amyloid precursor protein; Aβ: beta-amyloid; Drp1: dynamin related protein 1; EAE: experimental autoimmune encephalomyelitis; Fzd1: frizzled-1; MSC: mesenchymal stem cell; p-Akt: phosphorylated protein kinase B; PD: parkinson's disease; PI3K: phosphatidylinositol 3-kinase; PS1: presenilin-1; RKI: ras homologus kinase inhibitor; ROCK: ras homologus-associated kinase; Tg: transgenic.