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

**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 eukaryotic cells 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 modifications. 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 expression patterns 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 Ca2+/calmodulin-activated dependent MLC kinase and is dephosphorylated by MLC phosphatase (Figure 1) (Kobayashi et al., 2016; Shimokawa et al., 2016; Narumiya et al., 2016). 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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**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.

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- Liu et al., 2008
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- Shimokawa et al., 2016
- Thumkeo, 2018
- Kobayashi et al., 2016
- Tajouri et al., 2005
- Yan et al., 2019
- Ibrahim et al., 2020

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

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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, uncontrollable 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). Rock is highly expressed in the central and peripheral regions of MS patients 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 Aβ aggregates but also promotes the formation of Aβ deposits. ROCK activity is present in both the CNS and peripheral blood of AD patients. ROCKs activity is also involved in the pathology of AD, PD, and MS, revealing that ROCKs are involved in the progression of neurodegenerative diseases and are the central link in the pathogenesis of these diseases.

**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 [MGs]), astrocytes (ASs), 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 ROCKs/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; Polloco et al., 2014; El Aziz 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 significantly increases the production of inflammatory cytokines such as 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, induce T cells to fully polarize to the 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 lipogenesis (Yang et al., 2018). 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 ROCK subtypes in different settings, it is clear 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 (National, 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 (Hauser et al., 2018). B cells also promote stroke-associated demyelination and affect patients even in the decade following the stroke (Doyle and Buckwalter, 2017).

As an important signaling molecule that regulates actin, ROCKs not only regulate the remodeling of the B cell cytoskeleton but also promote the development, proliferation, and differentiation of B cells. Some studies demonstrated that the number of peripheral B cells in CD19−/− mice is significantly reduced after ROCKs inhibition. This reflects the imbalance of B cell-activating factor (BAFF) production, which is the major cytokine expressed by follicular helper T cells stimulating B cells to differentiate into antibody-secreting plasma cells (Kehler et al., 2017).

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 play a crucial role in neuroprotection and neuroinjury recovery (Hickman et al., 2018; Song and Colonna, 2018). Caldeira et al. (2017) studied AD patients and documented that MG are needed to fully determine the exact roles of these two ROCK subtypes in different settings, it is clear that ROCK1 and ROCK2 may contribute to the differentiation of different Th subtypes.

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ROCK is highly expressed in immune cells (T cells, B cells, and macrophages/microglia), astrocytes (A5), pericytes, and OPCs, which then interact with neurons to regulate the behavior of these cells and affect the course of disease. S-HTAR: Serotonin receptor 4; A1: protein kinase; B3: Arg/3; Arg/3; complex; FGFR: fibroblasts growth factors receptor; CR3: complement receptor 3; SytLZ2: syntolienin 2 receptors; DDR1: discoidin domain receptor 1; ERK: extracellular signal-regulated kinase receptor; Gia13: alpha13, IRF-4: interferon regulatory factor 4, LAR: leucine common antigen-receptor 1-1, ROCK: Rho-associated protein kinases, LPA1-RK: lysophospholipase function-associated antigen-1 receptor; MLCK: myosin light chain 2; MLCP: myosin light chain phosphatase; mTOR: mammalian target of rapamycin; NDRG2: N-myc downstream-regulated gene-2; NFKB: nuclear factor-kappa B; NRG: neuregulin receptor; OPC: oligodendrocyte progenitor cell; PI3K: phosphatidylinositol 3-kinase; P2Y2: P2Y6 G-protein-coupled receptors; P38: MAPK3: p38 mitogen-activated protein kinase 3; PTEN: phosphatase and tensin homolog; PTPα: receptor type protein tyrosine phosphatase-α; ROCK: Rho-associated kinase; TGF-β: transforming growth factor-beta receptor; TNF-α: tumor necrosis factor-α receptor; VE-cadherin: vascular endothelial-cadherin; VAP: Yes-associated protein.

Some recent works have investigated the role of ROCKs in immune cell function. ROCKs signaling regulates a variety of processes in immune cells, including adhesion, migration, and cytokine production. ROCKs inhibition can modulate immune cell function, potentially leading to improved therapeutic outcomes in various immune-mediated diseases. ROCKs inhibition may represent a promising therapeutic strategy for neuroimmune diseases.
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, ROCK inhibitors applied in clinical applications include fasudil (Rikita 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 ROCKs that act on both ROCK1 and ROCK2 include 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 slice culture of EAE mice, thereby maintaining BBB integrity and maintaining the function of the BBB (Gu et al., 2017). Failure to maintain BBB integrity and maturation of OPCs is common in progressive MS patients, which is related to the lack of sufficient neuroprotective factors in the CNS (Zhang et al., 2009; Vondran et al., 2010; Yang et al., 2014). Fasudil significantly increased brain-derived neurotrophic factor and neurophin-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 recovery of damaged tissues. Previously published experiments showed that fasudil could modify the evolution of encephalic Th1 and Th17 cells and anti-inflammatory Th2 cells in the spleen of EAE mice on day 9 postimmunization. On day 9 postimmunization, splenocytes treated with fasudil to which did not induce a Th1 response to a Th2 response. Based on an EAE model compared with control cells (Liu et al., 2015; Guo et al., 2019). The encephalic splenocytes modified by fasudil in vitro also reduced clinical symptoms and delayed the occurrence of CNS inflammatory 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 function and damage to synaptic transmission. Synaptic loss is a major cause of AD-related cognitive impairment, and dynamic regulation of action 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 double-transgenic mice (E3 cotm1) in the hippocampus (Gu et al., 2017). Fasudil significantly increased brain-BBB integrity and function. Fasudil can upregulate the expression of anti-inflammatory Treg cells and Th2 cells in the CNS (Yang et al., 2014). Fasudil also protected the 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., 2017). Fasudil can also increase the number of dendrites 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 pyramid 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 collagen-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 expression of antioxidants and by activation of 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 a more active brain nitric oxide synthase 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 (Dorssey et al., 2007). The clinical symptoms of PD are induced by degeneration 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 (Segal et al., 2002). The pathogenesis of PD relates to higher expression of pathological α-syn abnormal 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 (Saai 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 line and in vivo in a 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 (Zha 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 (MPTP)-treated PD model (Zhao et al., 2020). It efficiently protected against 6-OHDA-induced 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, cyclooxygenase 2, and matrix metalloproteinase 9, thus reducing motor symptoms and improving motor ability (Zhao et al., 2015a). Fasudil treatment also induced a marked increase in the levels of phosphorylated adducin, elevated activation of PTEN, and reduced levels of phosphorylated adducin, elevated activation of PTEN, and reduced levels of phosphorylated adducin, elevated activation of PTEN, and reduced levels of phosphorylated adducin, elevated activation of PTEN.

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 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 reduced 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). Decreased levels 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 reduced the expression of astrocytes and microglia for a shorter 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, inhibition of ROCKs may reduce the expression of α-syn and 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 actions of cellular and molecular mechanisms of the pathological processes 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.

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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.

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

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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 Giorgi 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 (Kolare 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 promotes cell survival and activation of neural 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 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 survival of neural stem cells in vitro (Tang et al., 2020). 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 or prolong survival 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 actions of cellular and molecular mechanisms of the pathological processes 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.

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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 | 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 |
|                |             | -Protection and therapy in EAE |            |
|                |             | -Increased dendrite branching in APP/PS1 mice |            |
|                |             | -Inhibited 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 |            |
|                |             | -Inhibited oligomeric tau protein |            |
|                |             | -Combined intervention with MSCs attenuated EAE |            |
|                |             | -Reduced oligomeric tau protein |            |
|                |             | -Reduced oligomeric tau protein |            |
| Y-27632        | ROCK1/2     | -Increased cerebral blood flow and stroke protection; | Rikitake et al., 2005; Zhang et al., 2006; Borrajo et al., 2014; Zhang et al., 2019; Hamano et al., 2020 |
|                |             | -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 |            |
| H-1152         | ROCK1/2     | -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.