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REVIEW ARTICLE

Therapeutic potentials of the Rho kinase inhibitor Fasudil in experimental autoimmune encephalomyelitis and the related mechanisms

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Abstract

Multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), and other neurodegenerative diseases of central nervous system (CNS) disorders are serious human health problems. Rho-kinase (ROCK) is emerging as a potentially important therapeutic target relevant to inflammatory neurodegeneration diseases. This is supported by studies showing the beneficial effects of fasudil, a ROCK inhibitor, in inflammatory neurodegeneration diseases. MS is an autoimmune disease resulting from inflammation and demyelination in the white matter of the CNS. It has been postulated that activation of Rho/ROCK causes neuropathological changes accompanied with related clinical symptoms, which are improved by treatment with ROCK inhibitors. Therefore, inhibition of abnormal activation of the Rho/ROCK signaling pathway appears to be a new mechanism for treating CNS diseases. In this review, we extensively discussed the role of ROCK inhibitors, summarized the efficacy of fasudil in the MS conventional animal model of experimental autoimmune encephalomyelitis (EAE), both in vivo and in vitro, and highlighted the mechanism involved. Overall, the findings collected in this review support the role of the ROCK signaling pathway in neurodegenerative diseases. Hence, ROCK inhibitors such as fasudil can be novel, and efficacious treatment for inflammatory neurodegenerative diseases.

Keywords Rho-kinase · Fasudil · Experimental autoimmune encephalomyelitis · Microglia/macrophages · Multiple sclerosis

Introduction

The Rho kinases (ROCKs) are a family of classical serine/threonine protein kinases, which consist of two homologs, ROCK1 and ROCK2 (Matsui et al. 1996; Nakagawa et al. 1996). Their kinase domains are similar, but their distribution varies. ROCK1 mainly expressed in the blood, liver, lung, testes, and the immune system, while

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ROCK2 is predominant in the brain and muscles (Hashimoto et al. 1999). ROCKs are key effectors of Rho GTPases. They play an important role in the mediation of various biological processes ranging from cytoskeletal reorganization and migration to gene expression. Therefore, ROCKs are considered to be the major orchestrator of tissue responses to injury (Julian and Olson 2014; Thumkeo et al. 2013). They also mediates actin filament stabilization and actin fibers contractility by phosphorylation of numerous downstream target proteins such as the myosin light chain (MLC), myosin phosphatase targeting protein1 (MYPT1), and LIM kinases (Amano et al. 1996; Fukata et al. 1999). ROCKs regulate fundamental cellular processes including proliferation, differentiation, movement, migration, survival, and death (Riento and Ridley 2003). ROCK activity is involved in different types of neurons in the central nervous system (CNS). Recent studies have demonstrated the role of ROCK activation in pathological conditions of the CNS, which could be associated with contraction of endothelial cells, damage of BBB, migration of inflammatory cells, reaction of glial cells, and several others pathological cascade of events (Chong et al. 2017). Therefore, the therapeutic benefits of ROCK inhibition can be achieved for various neurodegenerative diseases such as AD, PD, MS, and ALS (Hensel et al. 2015).

MS is a chronic inflammatory demyelinating and neurodegenerative disease. However, the precise pathogenesis is unclear. We hypothesized that the immune system plays a key role in the pathogenesis of MS. One possible mechanism of MS is migration of leukocytes across the damaged BBB into the CNS, causing inflammatory responses and CNS demyelination (Fig. 1). Our previous studies have clearly demonstrated the role of ROCK in MS, suggesting that ROCK inhibitors have the potential to treat MS (Hou et al. 2012; Yu et al. 2010). Fasudil (also known as HA-1077), a typical ROCK inhibitor, is a novel isoquinoline sulfonamide derivative and the only clinically available ROCK inhibitor. Several studies have shown that fasudil has multiple functions in the CNS, including activation of endogenous neural stem cells, promotion of neurotrophic factors release, inhibition of intracellular calcium release, dilation of cerebral vessels, protection of nerve cells, improvement of the nerve function, and promotion of axonal regeneration (Yu et al. 2016a; Schinzari et al. 2012; Chiba et al. 2010). Hence, fasudil has been emerging as a new powerful therapy for the treatment of CNS disorders. Experimental autoimmune encephalomyelitis (EAE) has been a well-established animal model to study MS (Bando et al. 2018). The results of our previous studies showed that fasudil treatment delayed the onset and ameliorated severity of EAE; it also improved demyelination and inhibited inflammation (Hou et al. 2012; Yu et al. 2010). These results exhibit therapeutic potential of fasudil in EAE, which is possibly mediated through the polarization of macrophages, phenotype change and the regulation of T cells. Fasudil inhibits inflammatory responses by controlling polarization in microglia/macrophages, maintains the integrity of BBB, and influences the function of astrocytes, suggesting a novel cellular therapeutic strategies to MS and EAE.

In the present review, we analyzed and summarized the therapeutic potential of fasudil in different phases of the EAE model both in vivo and in vitro, in addition to exploring the possible mechanisms of its action.

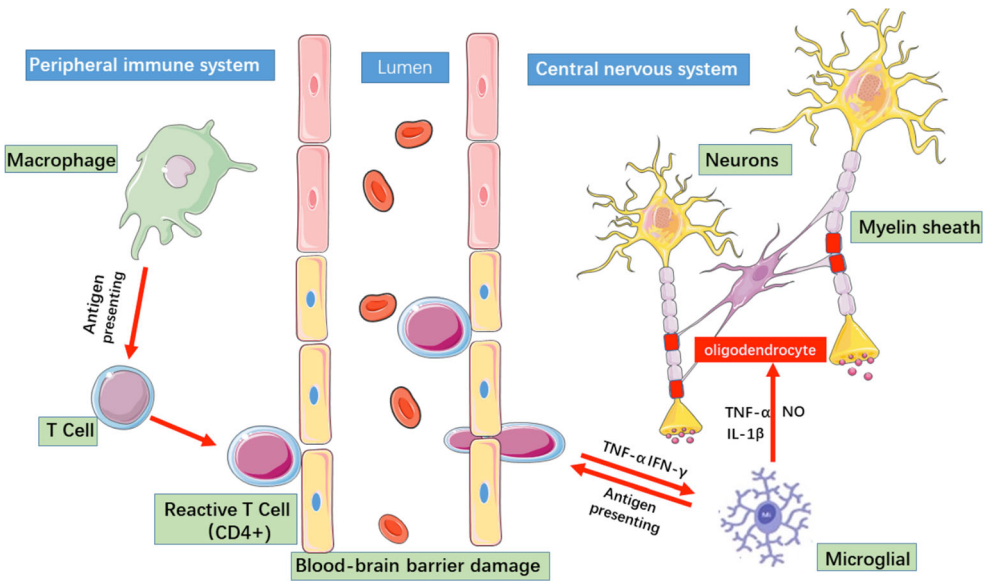

Fig. 1 Proposed mechanisms of MS. Stress or tissue injury in the peripheral nervous system are sensed by macrophages which regulates the recruitment and function of immune cells at the sites of damage, including T and B cells, as well as non-hematopoietic cells, leading to migration of leukocytes across the damaged blood-brain barrier (BBB) into the CNS. Cascade of events proceed with polarization of macrophages, phenotype change, regulation of T cells and release of inflammatory marker. This increases inflammatory responses and CNS demyelination, which contribute to the pathogenesis of MS

Fasudil inhibits the expression and activation of ROCK in both peripheral and central immune systems

ROCK is expressed in peripheral and central immune systems (Kubo et al. 2008). Animal studies suggest that ROCK dysfunction leads to the pathogenesis of autoimmune diseases (Pernis et al. 2016). Consistent with this, our previous studies demonstrate that ROCK activity is increased in the serum, spleen, and brain of MS patients, and the spinal cord of EAE mice as compared with control mice (Yu et al. 2010). Axonal loss in MS may be related to increased ROCK activity, which can be inhibited by fasudil, leading to increased synaptogenesis. Hence, fasudil may have a major role in preventing irreversible neurological disability associated with MS, which is contributed by increased ROCK activity (Chen et al. 2015). We also showed increased ROCK activity in the spleens and spinal cord in EAE mice, which was blocked by fasudil treatment (Yu et al. 2010). In addition, phosphorylated myoglobin phospholipid-targeting subunit 1 (p-MYPT1), a substrate of ROCK, has been shown to increased in brain and spinal cord tissues following fasudil treatment as compared to EAE mice (Yu et al. 2010; Garcia-Rojo et al. 2017). Interestingly, fasudil-induced inhibition of p-MYPT1 expression in the brain and spinal cord mainly occurs in early treatment stage. Based on these findings, it is reasonable to believe that ROCK in the CNS of MS is upregulated or activated, and that inhibition of ROCK activity can be a novel therapeutic strategy for MS.

Effects of fasudil on immunoregulation

The RhoA-ROCK pathway is a critical coordinator of tissue responses to injury (Ricker et al. 2016) (Fig. 2). It also regulates non-hematopoietic cells. Increasing evidence supports that the RhoA-ROCK pathway acts as an important regulator for the recruitment and function of immune cells at the sites of damage and stress, including T and B cells (Ricker et al. 2016). ROCK inhibition decreases T cell receptor-mediated actin polymerization and lipid raft formation, suggesting its potential to impair immune responses in vitro and in vivo (Tharaux et al. 2003). The RhoA-ROCK pathway activates the downstream chemokine receptors, causing activation of T cells and subsequent polarization and migration mediated by chemokine (Vicente-Manzanares et al. 2002; Heasman and Ridley 2010). RhoA-ROCK signaling also plays vital role in the regulation of T cell activation, migration, and differentiation. It has been demonstrated that the proliferation of T cells is regulated by ROCK (Tharaux et al. 2003; Aihara et al. 2003), more specifically, TH17, but not TH0, TH1, or TH2 conditions. Interestingly, TGFβ drives ROCK2 activation in T cells and promotes TH17 differentiation (Biswas et al. 2010). Our recent study showed that BMSCs combined with Fasudil treatment significantly decreased the number of Rock-II+ cells /mm2 in the spleen of APP/PS1 double transgenic animals, as compared with Fasudil (approx. 2 folds) or BMSCs (approx. 7 folds) alone. This provided promising demonstration for that BMSCs potentiates the inhibitory effect of Fasudil on expression of ROCK-II (Yu et al. 2018). These findings support the role of ROCKs in the pathogenesis of autoimmune disorders. Since, MS is a T cell-mediated autoimmune disease of the CNS, ROCK inhibitors can be used for treatment of MS.

As a potent ROCK inhibitor, fasudil affects the incidence and development of EAE by controlling inflammatory reaction. It inhibits the function of Th1 and Th17 cells and M1 macrophages/microglia, while increases the expression of regulatory T cells and M2 macrophages/microglia in the peripheral and central immune systems (Liu et al. 2016). In addition, fasudil increases the proportion of CD4 + CD25+ T cells and

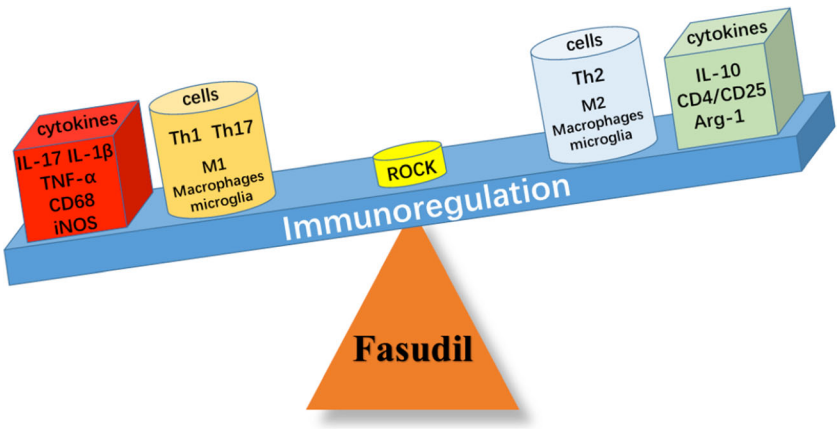

Fig. 2 The RhoA-ROCK pathway participates in immunoregulation. ROCK plays a pivotal role in immunoreaction by regulation of cytokines, T cell activation, migration, and differentiation. Inhibition of ROCK by fasudil reduces proliferation and differentiation of T cells (TH17, TGFβ) and produces inhibitory effects on M1-type macrophages/microgliocytes (iNOS in CD68, iNOS). It inhibits the function of Th1 and Th17 cells and M1 macrophages/microglia. On contrary, fasudil increases 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 reduces the proportion of IL-17 in CD4+ T cells

IL-10 in CD4+ T cells, while reduces the proportion of IL-17 in CD4+ T cells (Liu et al. 2013). In our recent study, we observe that Fasudil combined with BMSCs significantly increased CD4+/CD25+ suggesting that the combination therapy exhibits more effective regulation of T-cells. This suggest the direct role of ROCK in regulation of T cells (Yu et al. 2018).

Macrophage phenotypes are usually divided into M1 and M2 based on the functional status. Fasudil shifts M1 type of peripheral macrophage to M2. M1 is the classic activation type, which expresses high oxidative stress products and pro-inflammatory factors and causes inflammatory lesions of tissues. M2 type is selective activation, which inhibits immune inflammatory responses and promotes tissue repair (Ghosh et al. 2016). M1 macrophages primarily express iNOS, TNF, and IL-12α, while M2 macrophages mainly express Arg-1 and IL-10 (Zhang et al. 2015). Fasudil in either early or late treatment partially reverses the transfer of the M1 phenotype to M2, i.e., the expression of M1 is reduced, while the expression of M2 is increased (Liu et al. 2016).

Recent studies have demonstrated that iNOS and Arg-1 are the most important and representative markers of macrophage M1 and M2 phenotypes, respectively (Lisi et al. 2017). Treatment with fasudil at the early and late stages reduces the levels of iNOS in the spleen macrophages, while fasudil at the early treatment stage increases the expression of Arg-1. These results suggest that fasudil inhibits the expression of M1 macrophages and increases the generation of M2 macrophages, which are non-antigen-dependent (Liu et al. 2013, 2016; Yu et al. 2016b).

Fasudil regulates the response of the central immune system. CD68 is a surface marker of microglia/macrophages. We have demonstrated that EAE mice display increased infiltration of inflammatory CD68 positive cells in the spinal cord, which were blocked by fasudil. Fasudil inhibits the expression of iNOS in CD68 positive macrophages/microglia cells; in contrast, it does not affect the expression of Arg-1 (Liu et al. 2013; Kushiyama et al. 2013; Gao et al. 2015), suggesting that fasudil has an inhibitory effect on M1-type macrophages/microgliocytes with inflammatory characteristics.

Fasudil reduces BBB permeability and inhibits infiltration of peripheral immune cells in the CNS

The immune system in the CNS is entirely different from the peripheral immune system. There is no immune response when the BBB is maintained intact. However, pathological conditions cause disruption of the BBB and prevention of peripheral immune cell migration into the CNS, leading to occurrence of diseases. Therefore, BBB dysfunction is considered as the bottle neck event in MS/EAE pathological processes (Guo et al. 2014; Zhang et al. 2017). Abnormality of the BBB causes the preconditioning and activation of immune cells of peripheral to CNS, which leads to subsequent immune attack and nerve injury.

Activation of ROCK in astrocytes causes retraction and affects the BBB (Niego et al. 2012), in which ROCK-2 is the predominant isoform and acts as the key factor in the function and maintenance of BBB (Niego et al. 2017). Our previous studies have revealed the potent efficacy of fasudil and BMSCs in the CNS, which is involved in prevention BBB leakage and prevention of A-beta deposition.

Fasudil retains the integrity by reducing the permeability of the BBB. Activation of Rho/ROCK causes destruction of occludin, an integral plasma-membrane protein located at the tight junction between endothelial cells, which leads to increased BBB permeability (Niego et al. 2017; Huang et al. 2011). ZO-1 is one of the important tight junction proteins. When ZO-1 is destroyed, the function of BBB's tight junction changes (Kim et al. 2016; Liu et al. 2011; McRae et al. 2018). Fasudil treatment increases the expression of occludin and ZO-1 in vascular endothelium of the tight junction and prevents the permeability of BBB, leading to inhibition of peripheral inflammatory cells invasion in the CNS. Thus, fasudil is effective in treatment of EAE and improves the clinical symptoms (Kubo et al. 2008; Fujii et al. 2012)(Fig. 3).

Fasudil inhibits the activation of astrocytes and the chemokines of peripheral immune cells

In the demyelination plaque of MS/EAE, the reactive astrocytes are mostly glial cells. Since, glial hyperplasia formation is one of the major pathological changes, astrocytes are majorly involved in the pathogenesis of MS/EAE (Lau et al. 2011).

Fasudil not only inhibits the activated astrocytes, oxidative stress and inflammatory reactions (Li et al. 2014), but also decreases the expression of chemokines in astrocytes (Li et al. 2017). In brain tissues, CCL20 is one of the chemokines mainly expressing in astrocytes. Its expression is increased in the brain of EAE mice, which is inhibited by fasudil treatment. In astrocytes (in vitro study), LPS increases the mRNA levels of MIP-1α, RANTES, CCL20, and MCP-1, which are all inhibited by fasudil (Guo et al. 2014).

The astrocyte foot processes can be attached to the endothelial cells of the vascular endothelial cells (O'Shea et al. 2015). Therefore, the expression of chemokines in astrocytes has been considered to facilitate the T cells invasion into the CNS; fasudil inhibits astrocyte activation and chemokine expression, leading to inhibition of the infiltration of peripheral MNCs in the CNS (Kesherwani et al. 2014; Lau et al. 2012; Li et al. 2012).

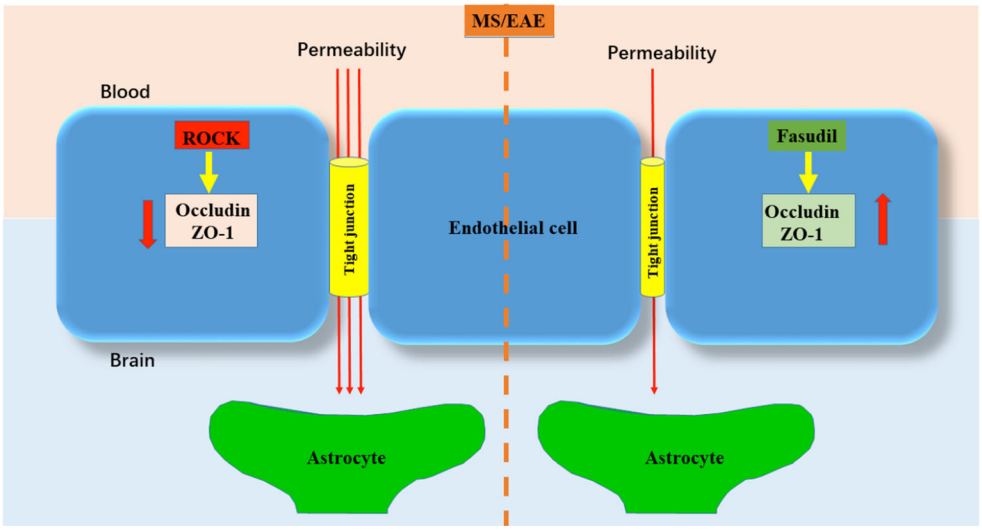

Fig. 3 Mechanism of fasudil to maintain BBB integrity and reduce permeability. Fasudil prevents the retraction in astrocytes and provides maintenance of BBB by blocking the destruction of occludin, an integral plasma-membrane protein located at the tight junction between endothelial cells, and ZO-1, a tight junction protein. Fasudil treatment increases the expression of occludin and ZO-1 in vascular endothelium of the tight junction, maintain the integrity of the cells and reduces the permeability of BBB, leading to inhibition of peripheral inflammatory cell invasion in the CNS

Fasudil affects the phenotype and function of microglia

The Rho-kinase (ROCK) signaling pathway is the major regulator of microglial activity. The ROCK pathway in microglial cells regulates migration, phagocytosis, and release of inflammatory cytokines, and finally mediates the microglial phenotype (Borrajo et al. 2014; Yan et al. 2012). It has been well established that ROCK is increased in microglial cells in MS (Chen et al. 2014).

Fasudil treatment affects the phenotype of BV-2 cells/primary microglial cells (Zhang et al. 2013). LPS treatment activates microglial expression of iNOS, but it does not affect the expression of Arg-1. However, fasudil treatment inhibits the expression of iNOS and increases the level of Arg-1, suggesting that fasudil can partially reverse the M1 to M2 anti-inflammation microglia (Yu et al. 2017; Zhang et al. 2012).

NF-κB is an inflammatory reaction of nuclear factor, the M1 model induced by LPS microglia produces more inflammatory cytokines such as IL-1, IL-6 and TNF-alpha. Treatment with fasudil shifts the microglial phenotype into type M2 and produces less inflammatory cytokines (Zhang et al. 2013; Yang et al. 2016; Higashi et al. 2017). Fasudil is also involved in the mediation of the TLR-4-NF-κB signaling pathway by inhibiting the inflammatory response of immune cells (Li et al. 2014; Song et al. 2011; Chen et al. 2017), suggesting its major role in phenotype and function of microglia.

Interestingly, Huangqi glycoprotein, a natural plant glycoprotein derived from Astragalusm Embranaceus, also exhibits anti-inflammatory and immunity-enhancing effects in treatment of EAE, as demonstrated in our previous study. (Zhao et al. 2017)

Fasudil promotes remyelination

MS is characterized by degeneration of chronically demyelinated axons. In response to demyelination, oligodendrocyte precursor cells (OPCs) replace lost oligodendrocytes by a process called remyelination due to maturation of new myelin-producing cells (Franklin and Ffrench-Constant 2008). However, failure of OPCs differentiation causes the lack of myelin sheath regeneration, which is commonly observed in patients with progressive MS. It has been demonstrated that inhibition of ROCK2 produces OPCs differentiation (Li et al. 2017). Inhibition of ROCK by a fasudil derivate increases the number of OPCs in EAE, OPC survival, and the arborization/maturation of oligodendrocytes by shifting M1 to M2 phenotype in vitro. It also induces remyelination of demyelinated organotypic cultures. Therefore, ROCK signaling is a vital process of remyelination in MS (Li et al. 2017). In addition, inhibition of ROCK signaling increases reactive oxygen species (ROS) generation and mitochondrial membrane depolarization, thereby protecting oligodendrocytes against Th1 and Th17 cytokine toxicity, as demonstrated in a mouse model of MS (Paintlia et al. 2013). Inhibition of ROCK signaling contributes to the OPC differentiation or survival. Therefore, ROCK can be a potential target for treatment of MS.

Fasudil protects neurons through PI3K/Akt and Wnt/β-catenin signaling pathways

Fasudil may protects neurons through PI3K/Akt and Wnt/β-catenin cell signaling pathways in the mouse model of PD (Zhao et al. 2015). It modulates the K+ channel Kv7.4, leading to modulation of the excitability of DRN 5-HT neurons (Zhao et al. 2017). Fasudil also inhibits the activation of JNK and p38 MAPKs induced by LPS, whereas the ERK1/2 pathway remains unaffected (Liu et al. 2014). Protection of neurons in the brain against ischemia injury by fasudil is mediated by decreasing neuronal apoptosis through the PTEN/Akt pathway (Wu et al. 2012). Aberrant Wnt signaling in Alzheimer's disease neuropathology indicates that fasudil could be a possible therapeutic target for treatment of Alzheimer's disease (Sellers et al. 2018).

Conclusions

The studies highlighted above demonstrate that fasudil is effective in treatment of EAE. Anti-inflammatory activity likely is the major mechanism because fasudil reduces EAE inflammatory cells infiltration and improves demyelination. Early treatment of fasudil is more potent.

The role of fasudil as an anti-inflammatory and immune regulation agent may be the result of reduced Th1, Th17 cell differentiation and increased Th2 cell differentiation. Fasudil drives inflammatory macrophages/microglia cells from the M1- to M2-type, inhibits the chemotactic function of astrocytes, decreases TLR-4 /NF-κB signaling, and down regulates inflammatory cytokine levels.

Overall, fasudil improves the function of vascular endothelial cells in the CNS, maintains BBB integrity, reduces the central infiltration of peripheral immune cells and inflammatory pathological lesions of the CNS, and increases remyelination. Fasudil protects neurons via multiple pathways.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests. None of the authors has any potential financial conflict of interest related to this manuscript.

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