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Review Article
Rho GTPases, a Potential Target for the Treatment of Neurodegenerative Disorders and the Neuroprotective Effect of Statins as a Rho GTPase Inhibitor

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Abstract

Neurodegenerative disorders (ND) such as Alzheimer's disease (AD), Parkinson's disease (PD) and associated cognitive dysfunction are major concerns that add enormous social and financial burdens on society. Growing evidence suggests that Rho GTPase is the regulator of cellular morphology and an emerging driver for neurodegenerative disorders. The superfamily of Rho GTPase offers a potential target for therapeutic intervention through Rho and Rac. In this review, the molecular mechanism of Rho GTPase in the etiology of ND via modulation of signaling cascades like PI3K/Akt, GSK-3β, MEK and ERK ½ pathway have been discussed. Further, the neuroprotective role of statins as a Rho GTPase inhibitor has been emphasized. A comprehensive literature survey was done to explicate the mechanism of Rho GTPase in neurological disorders and *in vivo*, *in vitro* and clinical studies were correlated with the inhibitory effect of statin on Rho GTPase. Based on the current review, it was hypothesized that the statins are potent inhibitors of Rho GTPase and can be efficient in the management and treatment of ND and associated cognitive dysfunction modulation of apoptosis, neuronal death, inflammatory cascade and oxidative stress that offers neuroprotection. To date, no targeted Rho inhibitor has been clinically approved. Thus, there exists a full window of opportunity for designing, leading optimization and development of Rho inhibitors. The use of techniques like molecular docking and crystal structure study to establish drug-ligand interaction between Rho GTPase and statins to increase its specificity and efficacy in the management of neurological disorders is crucial and urgent.

Key words: Geranylgeranyl pyrophosphate, Rho GTPase, neuroinflammation, pleiotropic effect, fasudil, intranasal drug delivery

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INTRODUCTION

Neurodegenerative disorders (ND) are one of the major concerns of health care professionals and considerably affect the quality of life of patients and negatively impact society[1]. The central nervous system is restricted to toxic metabolites and immunological and inflammatory cells because of the blood-brain barrier (BBB). However, sometimes circulating immune cells like neutrophils, natural killer cells (NKCs), eosinophils and dendritic cells bypass the BBB and perpetuate the immune response in the neurons and glial cells[2]. Although, these immune responses are intended to protect neurons from infections, they may cause unwanted neurological alterations[2]. Additionally, traumatic brain injury (TBI), hypoxia pollutants, high cholesterol-salt intake, microbes, toxic metabolites, autoimmunity and smoking induce neuroinflammation that involves the activation of endothelial cells, causes edema and platelets aggregations and progresses into various ND[2]. Activation of glial cells and cytokines is the common neuroimmune response to these stimuli[3]. Glial cells are innate immune cells that get activated in response to a change in cell morphology or injury[3]. Activated glial cells then stimulate the astrocytic activity, which contributes to the inflammatory cascade[3]. During neuroinflammation, there is sustained release of Interleukin-6 (IL-6), Interleukin-1β (IL-1β) and Tissue Necrosis Factor (TNF-α) that are responsible for the neurotoxicity, neuronal cell disruption and cell death[4]. Apart from these cytokines, Rho GTPase plays a decisive role in neuroinflammatory and neurodegenerative disorders, like Parkinsonism, Alzheimer's disease (AD), depression and associated cognitive impairment[5].

The Rho GTPase is a subfamily of the Ras superfamily GTPase (protein of small GTPase, also known as G-protein) that controls multiple signaling pathways. There are seven subdivisions of Rho GTPase (RhoA, Rac, Cdc42, Rnd, RhoD, RhoBTB and RhoH), out of which Rho A, Rac and Cdc42 are the most extensively studied members[6]. The three prime regulators for the activation of Rho GTPase are guanine nucleotide exchange factors (GEFs), GTPase activating protein (GAP) and guanine dissociation inhibitors (GDI). The GEFs are actively involved in the activation of Rho GTPase via the release of GDP and binding of GTP, whereas GAP stimulates or potentiates the hydrolyzed GTP to GDP and hence reverses back the activated GTPase into GDP-bound inactive state[6]. The GDI sequestered the Rho GTPase in its GDP-bound state in the cytosol (inactive state of Rho GTPase)[6]. Therefore, GAP and GDI act as a negative regulator and facilitate the inactivation of Rho GTPase as seen in Fig. 1. The Rho A, a subfamily of Rho GTPase, acts on its direct downstream

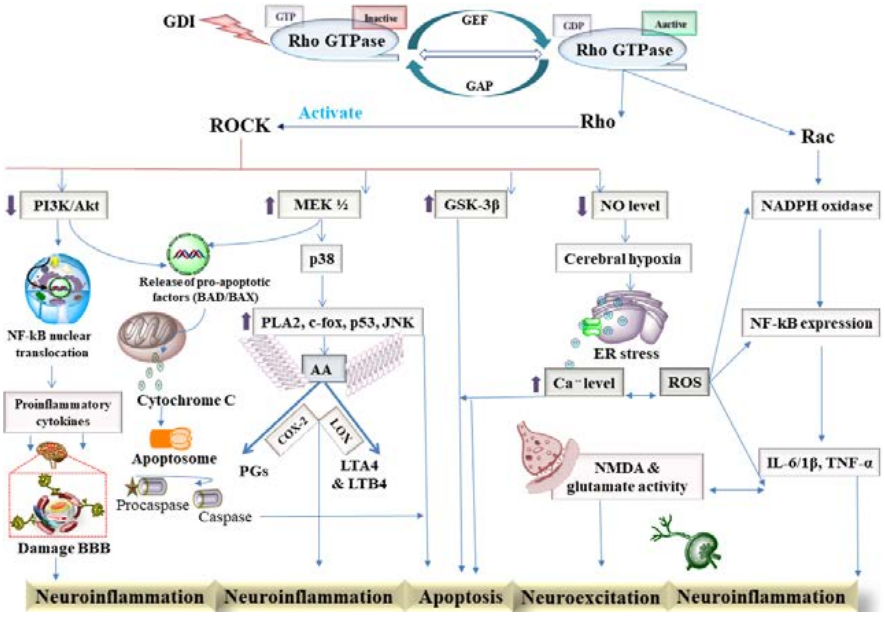

Fig. 1: Role of Rho GTPase in the pathophysiology of neuroinflammatory and neurodegenerative disorders

Rho GTPase activates in the presence of GEFs and GAP. Activated Rho GTPase, like Rho, when binds with ROCK, increases its activity. The Rho/ROCK and Rac are further involved in neurological disorders by modulating the PI3K/Akt/MEK ½ pathway and GSK-3β activity. The Rho/ROCK and Rac induce neuronal apoptosis, neuroinflammation and neurodegeneration through modulating NO level, cytochrome-c, caspases, AIF, COX -2, NMDA, glutamate, calcium overload, NADPH oxidase, NF-κB and ILs activity[10]

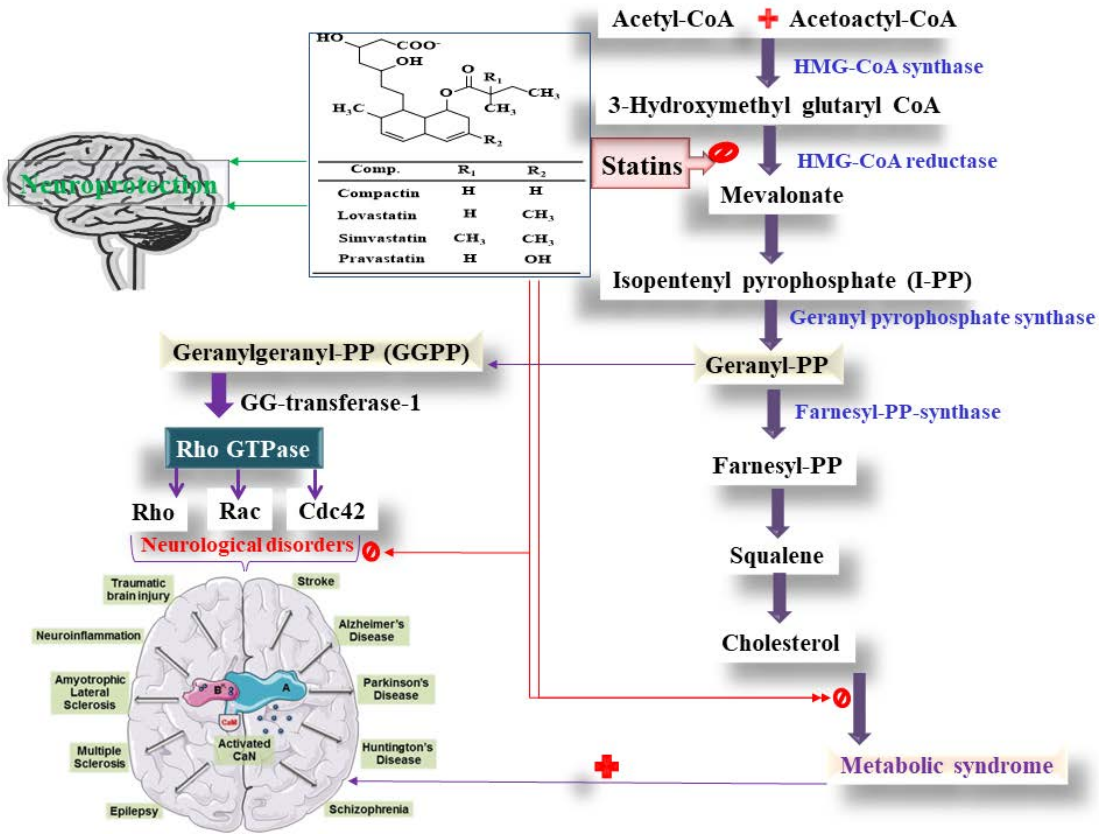

Fig. 2: Mechanism of cholesterol biosynthesis in neurological disorders and metabolic syndrome along with neuroprotective role of statin

Statins, through the inhibition of HMG-CoA reductase, inhibit mevalonate synthesis which results in downregulation of FPP, GPP and small Rho GTPase-like Rho and Rac. Inhibition of these small Rho GTPase imparts neuroprotective effects[16]

effector, ROCK (Rho-Associated-coiled-coil-containing protein kinase) which is a serine/threonine protein kinase. There are two isoforms of ROCK, ROCKI and ROCKII. The ROCKII is exclusively expressed in the brain, whereas ROCKI is present in the non-neuronal tissue, heart, lungs and skeletal muscles[5]. Activation of ROCK is reported with a diverse pathological role and its activation depends on the biochemical activation of RhoA GTPase[5,6]. In normal physiological conditions, ROCK maintains its inhibitory state by forming an auto-inhibitory loop through the back folding of the C-terminal onto the kinase domain. Biochemically activated Rho GTPase/GTP-bound Rho GTPase or Rho A when binds with the Rho Binding Domain (RBD) of ROCK, it disrupts the auto-inhibitory loop of ROCK and thus ROCK gets activated. Activated ROCK then leads to the phosphorylation of various target proteins that is responsible for neuroinflammatory and neurodegenerative disorders[5]. The ROCK inhibitors like fasudil or Y-27632 have been reported to have strong neuroprotective potentials via their anti-inflammatory, anti-apoptotic property and ability to prevent dopaminergic neuronal loss[7]. More details of various preclinical studies emphasizing the deleterious role of Rho GTPase and the neuroprotective effect of various Rho GTPase inhibitors were shown in Table 1.

Statins (3-hydroxy-3 methyl glutaryl coenzyme A reductase inhibitor) are a well-known class of drug that belongs to the group of cholesterol-lowering agents[9]. Statins, apart from the lipid-lowering effect, exert a pleiotropic effect where it suppresses the activity of Rho GTPase by blocking the synthesis of mevalonate, which is the precursor of this GTPase as shown in Fig. 2[10]. Thus, statins may act as a potent Rho GTPase inhibitor agent and can be the future therapeutic class of drug in the management of neuroinflammatory and neurodegenerative disorders. Thus, this review is focused on the pathological role of RhoA GTPase in the prognosis of neuroinflammatory and neurodegenerative disorders and based on *in vivo*, *in vitro* and clinical studies, the neuroprotective role of statins as Rho GTPase inhibitors has been discussed.

Table 1: Showing the neuroprotective effect of various Rho GTPase inhibitors in neurological disorder[8]

Drug	Type of inhibitor	Model	Outcome
Fasudil	ROCK1 and ROCK2 inhibitor	SOD1-G93A model for ALS	Improved the motor function and survival of spinal cord neurons
		MPTP-induced PD	Preserved the nigrostriatal fibers and improved the motor behavior
		EAE model for MS	Reduced the expression of iNOS, reduced astrocytic chemokines and improved demyelination
Xanthoceras sorbifolium extracts	NA	AB25-induced AD	Improved cognitive function
Y-39983	Selective ROCK inhibitor	EAE model for MS	Improved clinical symptoms and prevent the disease relapse
CNF1	Rac1 and Cdc42 activator	Animal model for PD	Enhanced the number of cellular processes
		In vitro study, treated with 6-OHDA using SH-Sy5Y cell line	Trigger autophagy and prevent oxidative stress
Flavonoids from diospyros kaki leaves	Regulator of RhoA	APP/PS1 model for AD	Improved learning and memory, reduced the Rho activities
		Olfactory bulbectomy mice	Improved cognitive dysfunction
		AB-induced AD	Protection against Aβ-induced cognitive dysfunction
		SAMP8 mice	Improved neurobehavioral attributes reduced oxidative stress and prevented aggregation of tau protein
Nobiletin	NA	Animal model for AD	Reduced brain Aβ deposition
		Animal model for AD	Improved cognitive dysfunction and reduced Aβ formation
		MPTP-induced PD	Improved motor coordination and neurobehavioral attributes
Simvastatin	Inhibitor of ROCK	Animal model for Huntington disease	Reduced ROCK activation, reduced activation of astrocytes and anti-inflammatory activities
AZA1	Rac1 and Cdc42 modulator	*In vitro* study	Inhibit the activity of Rac1 and Cdc42
ML141 (CID-2950007)	NA	*In vitro* study	Inhibit the activity of Cdc42, Rac1, Rab2 and Rab7
AZA197	Cdc42-Dbs modulator	*In vitro* study	Inhibition of Cdc42-dependent migration
ZINC08010136	Inhibitor of Rac1	*In vitro* study	Inhibition of Rac1 activity
ZINC69391		*In vitro* study	Inhibit the Rac1-GEF interaction and activity of Rac1
MBQ-167		*In vitro* study	Inhibit the Rac1 and Cdc42 activity
NSC23766		Animal model for spinal cord injury	Improved the spinal structure and reduced pain
		In vitro study for AD	Reduced the level of APP
Ibuprofen	Inhibitor of RhoA	*In vitro* neurotoxic model	Activation of PPAR-y and neuroprotection
		EAE induced MS	Improved the level of BDNF, GDNF and NT-3
			Increased expression of MAP2, reduced $CD4^+$ T cells, reduced inflammation and improved the level of BDNF, GDNF and NT-3
FSD-C10	NA	*In vitro* model using BV-2 cell line	Inhibit M1 microglial activity and improved M2 microglial activity
		APP/PS1 induced AD	Improve learning and behavioral parameters, reduced the level of Ab42, phosphorylation of tau proteins
Statins	3-Hydroxy-3- methylglutaryl coenzyme a reductase inhibitors	*In vitro* model using BV-2 cell line	Reduced iNOS expression, inhibited NADPH level, reduced Rho GTPase isoprenylation, production of ROS and inflammatory markers
EHT1864	Inhibitor of Rac1	*In vitro* model using hippocampal	Reduced the level of Rac, managed the LTP and neurons
ZCL278	Modulator of Cdc42	*In vitro* model of AD	Modulate Cdc42- related cellular processes
Loganin	NA	*In vitro* model of AD	Increased IGF-1R and GLP-1R expression resulted into leading to neurite outgrowth
Cucurbitacin	Inhibitor of RhoA	*In vitro* cell culture	Modulated the Rho phosphorylation

STATINS: DO THEY CROSS THE BLOOD-BRAIN BARRIER?

Before discussing the role of statins in neuronal disorders, it is important to understand whether the statins or their active metabolite crosses the BBB or not. Statins are administered orally and to reach the drug in the brain, the drug must cross BBB. To cross BBB, statins should be lipophilic. Indeed lipophilicity is not the only criteria for the passage of the drug in the brain the drug must be small in size and of low molecular weight with maximum bioavailability[11]. Since bioavailability is an important parameter that determines drug concentration in the brain[11]. The greater the bioavailability, the higher is the chance of reaching the drug in the brain (keeping other factors in consideration for passing BBB)[11]. Further, if the drug has reached the brain, it is not obligatory that it will exert therapeutic activity as there is a chance of drug metabolism in the neuronal tissue, as shown in Fig. 2. There are *in vitro* and *in vivo*

studies that showed atorvastatin, lovastatin, fluvastatin, pitavastatin and simvastatin cross the BBB[12-14]. Hydrophilic statins such as pravastatin and rosuvastatin also enter neuronal parenchyma[12-14]. Organic anion-transporting polypeptides (OATP) mediate the passage of statin into the brain as statins are a substrate for OATP[14,15]. Additionally, monocarboxylic acid transport is another alternate pathway for the passage of statins into the BBB.

PROTECTIVE ROLE OF STATINS AGAINST Rho GTPase-MEDIATED NEURODEGENERATIVE DISORDER

Rho GTPase, Parkinson's disease and statins: Parkinsonism is among the common most diagnosed neuroinflammatory and neurodegenerative disorders manifested as compromised function of dopaminergic neurons found in the midbrain[17]. Apart from the role of dopaminergic neurons in the etiology of PD, growing evidence suggests the role of neuroinflammation, apoptosis and oxidative stress in the pathophysiology of Parkinsonism[17]. It has been reported that the activated glial cell in Parkinsonism releases pro-inflammatory mediators such as COX-2, IL-1β, IL-6, TNF-α and interferon-γ whereas, Rho GTPase plays a central role in its pathophysiology through activation of these inflammatory mediators, apoptosis and oxidative stress as shown in Fig. 3[17,18]. The Rho promotes the expression of COX-2 which amplifies the inflammatory pathway in PD[19,20]. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and steroidal anti-inflammatory drugs (SAIDs) have been reported to exert antiparkinsonian effect and improve locomotor coordination[21]. Apart from the inflammation, the downregulation of the PI3K/Akt signaling cascade also progresses the etiology of PD as the downregulated PI3K/Akt pathway enhances apoptosis and stimulates the activity of GSK-3β[22]. The Rho/ROCK has been reported with downregulated Akt activity, enhanced GSK-3β and disruption of autophagy in the brain of PD patients[23]. Further, Rho-mediated activation of ROCK is reported with axonal collapse and retrograde degeneration of dopaminergic axons, whereas ROCK inhibitors like fasudil or Y-27632 exert an axonal stabilizing effect, promote the growth of dopaminergic neurons and exhibit the antiapoptotic effect in animal models of PD[7]. In a pre-clinical study, significant activation of Rho/ROCK signaling cascade and increased expression of ROCK II mRNA was observed after 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MTTP) injections (MPTP is a well-established chemical intoxicant for inducing parkinsonism)[24]. A similar activity of MTTP-induced ROCK activation was observed in the *in vitro* study[24]. This suggests a strong correlation between the involvement of the Rho/ROCK pathway and the etiology of PD[24].

In an experiment, Barcia *et al.*[25] reported that MTTP induced microglia cell polarization and microglia-dopaminergic neuron association (A process that stimulates the pathogenesis of PD)[25]. Additionally, Rho/ROCK signaling cascade disrupts BBB and causes microvascular endothelial hyperpermeability that results in the accumulation of metabolites and toxins, which exacerbate the severity of PD[26]. The RhoA/ROCK cascade also interacts with Vascular Endothelial Growth Factor (VEGF) and upregulates the level of VEGF[27]. Levated VEGF then disrupts the BBB, induces hyperpermeability of BBB, reduces blood circulation and induces hypoxia, edema and dopaminergic neuron degeneration[25]. In fact, a low level of VEGF has been reported with neuroprotective effects, but elevated VEGF stimulates the severity of PD[28]. Some studies suggest that the NADPH oxidase-related superoxide activates the NF-κB pathway that further activates Rho/ROCK cascade[18]. The ROCK-NADPH oxidase interaction then induces dopaminergic neuron death either by increasing the level of ROS or by stimulating dopamine neuron degeneration via an inflammatory pathway[18]. Apart from the apoptotic and neuroinflammatory pathway, the Rho/ROCK cascade and NADPH oxidase interact with angiotensin and participate in dopaminergic cell death[26]. Angiotensin II (AT-II), an essential peptide of the renin-angiotensin system (RAS), exerts its effect by AT-I and AT-II receptor. The AT-I and AT-II receptors are present in dopaminergic neurons where hyperactivity of AT-II via AT-I receptors induces dopaminergic neuroinflammation and degeneration[29]. Administration of ROCK inhibitor, fasudil or Y-27632, was found to reduce microglial activation and dopaminergic cell death by minimizing the pernicious effect of AT-II via AT-I receptor[30]. In an *in vitro* study, MTTP stimulated the effect of AT-II on dopaminergic cell apoptosis and this damage was antagonized by ROCK inhibitor[26]. Therefore, there exists a profound role of Rho/ROCK cascade and NADPH oxidase in dopaminergic cell death via AT-II mediated by A-I receptors. Thus, it can be inferred that the Rho/ROCK-mediated pathway plays a crucial role in the pathophysiology of PD, whereas their inhibitors appear to be a potent therapeutic moiety in the treatment and symptomatic management of PD.

There are epidemiological studies that showed the reduced risk of PD in patients who are on statin therapy[31]. Wolozin *et al.*[32] performed the study using a sample size of

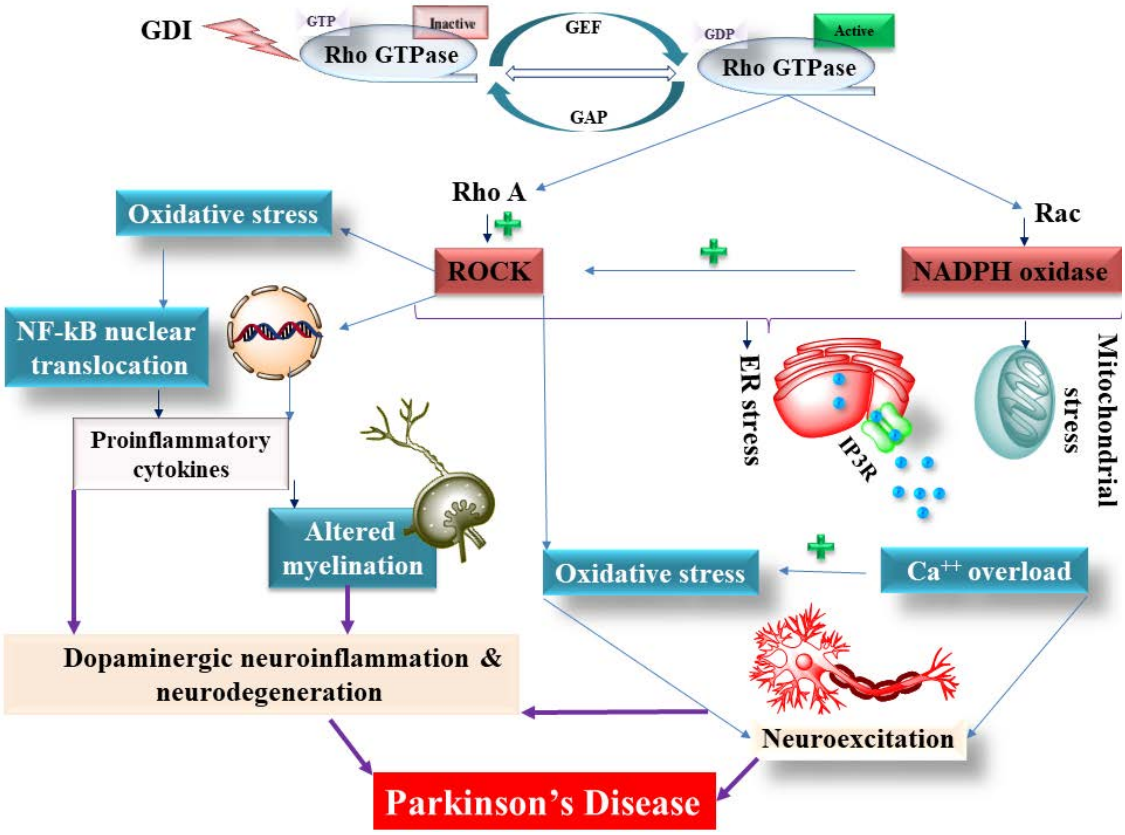

Fig. 3: Pathological role of Rho GTPase in the etiology of Parkinson's disorder. Rho/ROCK cascade and Rac are involved in the pathogenesis of PD

Reduced Akt, increased GSK-3β and calcium overload are involved in apoptosis, whereas NADPH oxidase, NF-κB, COX-2 activity, ROS and AT-II are involved in altered myelination, dopaminergic neuroinflammation and dopaminergic neurodegeneration. The increased activity of VEGF under the influence of the Rho/ROCK cascade alters the BBB permeability and is responsible for dopaminergic cell death[17,18]

4.5 million people in which 700,000 patients were on simvastatin and 50000 patients were on atorvastatin. The outcome of this study concludes that only simvastatin showed marked control on dementia and Parkinson symptoms, whereas atorvastatin was only marginally effective[32]. Mutez *et al.*[33] conducted a case-control study to determine the effect of statins on PD and found that the use of statins increased the onset of PD by nine years as compared to the patients who were not on statins. Further, the investigator found that the use of statin also increases the bioavailability of levodopa[33]. Lin *et al.*[34] conducted a large prospective study using one million patients from the National Health Institute (NHI) database. Parkinson's patients having diabetes were also included in the study. Interestingly, the finding of this study showed a lower risk of PD in statin users than non-statin. All statins except lovastatin showed a dose-dependent protective effect on the incidence of PD[34]. Shalaby and Louis[35] conducted a meta-analysis systemic review of epidemiologic studies and found the protective effect of statins in PD. In an *in vitro* using 6-Hydroxydopamine (6-OHDA) treated the PC12 cell, simvastatin at a dose of 1.5 μM exhibited a significant reduction in inflammatory markers such as TNF-α, IL-6, COX-2 and apoptotic marker caspase-3[36]. In another study by Kumar *et al.*[37] the administration of simvastatin (30 mg/kg) and atorvastatin (20 mg/kg) for 14 days resulted in a significant reduction in 6-OHDA-induced inflammatory markers such as TNF-α, IL-6 and oxidative stress. In the chemically induced Parkinson's model, administration of simvastatin (1 mg/kg) after 90 min of MPTP injection inhibits activation of $p21^{ras}$ and NF-κB in microglial cells[38]. Therefore, statins, by their ability to block GTPase activity, i.e., block Rho/ROCK cascade, could be a future therapy for PD.

Rho GTPase, Alzheimer's disease and statins: The Alzheimer's disease (AD) is a neurodegenerative disorder

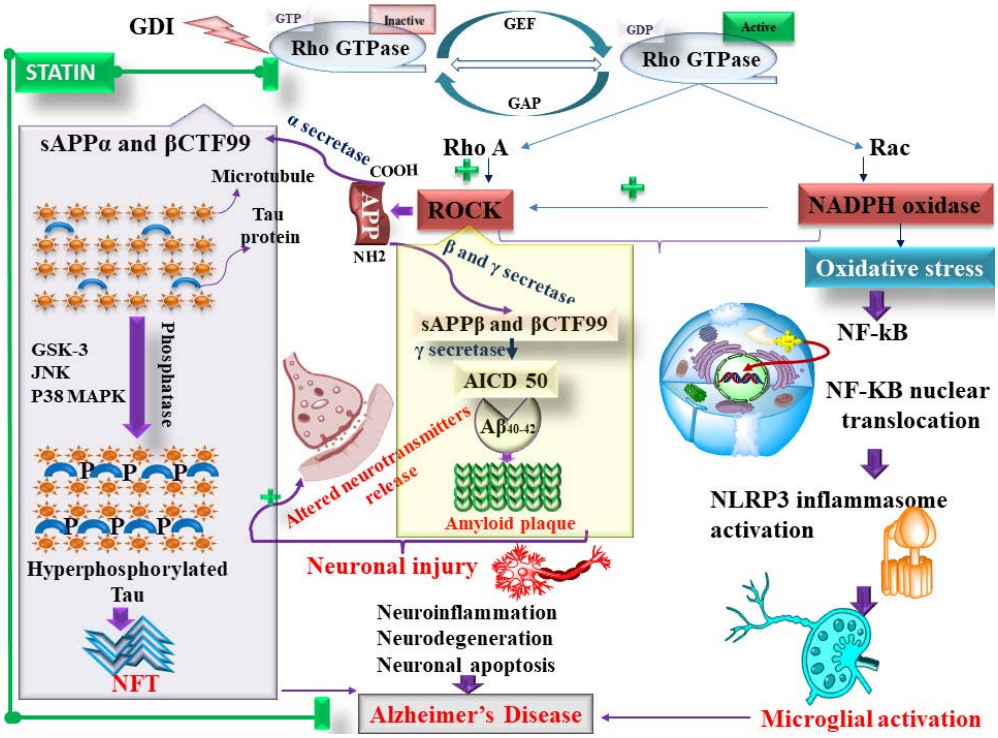

Fig. 4: Pathological role of Rho GTPase in the etiology of Alzheimer's disease

Aβ is directly involved in the pathogenesis of Alzheimer's disease. Apart from βa, Rho GTPase directly stimulates the βa, as well as Rho GTPase-like Rac1, involved in inflammatory cascade through NADPH oxidase, NF-κB and TNF-α activity that results in apoptosis, neuronal death and loss of memory. The RhoA and ROCK increase oxidative stress and reduce the Akt/PI3k signaling cascade, which in turn increases the activity of GSK-3β. The GSK-3β then induces neuronal inflammation and, along with the combined activity of hexokinase and VDAC phosphorylation, forms hyperphosphorylated tau protein and fibrillary tangles[67]

characterized by dementia, cognitive dysfunction and loss of memory[39]. The AD is a major concern for public health and age progression is a major risk factor in its pathogenesis[40]. Common risk factors for AD include hypercholesterolemia, cerebrovascular disorder and coronary artery disease. Neuroinflammation, neurodegeneration, inflammatory microglia and hyperphosphorylated neurofibrillary tangles of tau protein (NFTs) are seen in patients with AD[40]. The presence of extracellular amyloid (beta-amyloid or Aβ) and NFTs are common clinical diagnostic criteria for evaluation of AD[41]. It is hypothesized that the Amyloid Beta Protein Precursor (AβPP), which is 677-770 transmembrane amino acid is the precursor of Aβ. The AβPP is acted upon by 2 enzymes, β-secretase and γ-secretase resulting in the formation of Aβ isoform as shown in Fig. 4[39]. The α-secretase, on the other hand, acts through the non-amyloidogenic pathway, cleavage AβPP and produces Secreted AβPP (sAβPP) and carboxy-terminal fragment alpha (CTFα), which then mitigate AβPP generation[39]. Apart from the role of Aβ, neuroinflammation and neurodegeneration play a pivotal role in the pathology of AD, which is evident from the post-mortem of the AD-affected brain[41]. The Aβ is reported to activate the inflammatory cascade via the binding of microglia and astrocytes to Toll-Like Receptors (TLRs) and stimulate microglia to produce NADPH oxidase and TNF-α[39]. Although, the role of Aβ and tau protein in the pathogenesis of AD is well-established, it is important to point out that this review is focussed on the pathological role of Rho GTPase in neurological disorder and in line with that, Rho GTPase has been found to play a pivotal role in the pathology of AD[42]. The Aβ directly stimulates the activity of Rho GTPase as well as Rho GTPase independently modulates the etiology of AD by inducing an alteration in the cytoskeleton structure and loss of synaptic plasticity[43]. In the brain of AD patients, Rho A was found to be increased and localized in hyperphosphorylated tau aggregates[42]. The Aβ also increases the activity of Rho/ROCK in the AD, which is evident from an *in vitro* study where oligomeric $A\beta_{1-42}$ administration stimulates the activity of Rho/ROCK in PC12 cells[44]. In another interesting experiment, treatment with $A\beta_{1-42}$ activated the Rho/ROCK pathway, whereas intraventricular injection of ROCK inhibitor Y-27632 reversed this change. This study further strengthens the pathological role of Rho/ROCK in the progression of AD[45]. Activated Rho/ROCK reduced the activity

of the PI3K/Akt pathway and the downregulated Akt pathway increased the GSK-3β activity that induces inflammation and apoptosis[46]. Downregulation of the Akt/PI3K pathway in AD is evident from a study where wortmannin (PI3K inhibitor) induced tau hyperphosphorylation, similarly, activation of the PI3K/Akt pathway attenuated the Aβ induced synaptic disorder, cognitive impairment and Aβ induced hyperphosphorylation[46]. The increased GSK-3β activity also stimulates the expression of pro-inflammatory cytokines like IL-6, IL-1β and tumor necrosis factor and promotes NFT formation through hexokinase and voltage-gated anion channel (VDAC) phosphorylation[47]. In the AD, inhibition of Akt/PI3K and activation of GSK-3β results in the phosphorylation of VDAC, due to which hexokinase cannot bind with VDAC and detaches from mitochondria[48]. This process limits the hexokinase for access to glucose metabolism (glycolysis), mitochondrial ATP production and apoptosis in the neurons of AD[49]. Along with the Akt/PI3K mediated activation of GSK-3β signaling, Aβ, calpain and calcineurin directly activate the GSK-3β signaling leading to AD pathogenesis[47,48]. Apart from the involvement of Rho/ROCK, Rac1 also plays a crucial role in the pathogenesis of AD[50]. The Rac1, which is the small GTPase molecule, was also found to be upregulated in AD and this phenomenon causes the formation of NFTs[50]. There is an *in vitro* study that suggests the pathological role of Rac1 in the formation of Aβ from AβPP whereas Rac1 inhibitor NSC23766 reduces the AβPP level and apoptosis in the hippocampal neuron[50,51.] The Rac1 is also associated with increasing the activity of NADPH oxidase, which in turn mediates the neuroinflammatory cascade with the help of NF-κB and TNF-α[52-55]. Notably, there is clear, unambiguous mechanistic involvement of Rho GTPase (Rac1 and RhoA) in the etiology of AD through the induction of oxidative stress, neuroinflammation, apoptosis and NFTs formation. As of now, few ani-Alzheimer's drugs are approved by FDA but they have potential role in the symptomatic management of the disease. In recent times, novel formulation approaches such as formulation and development of galantamine (approved anti-Alzheimer's drug) *in situ* gel have been studied for potential anti-Alzheimer effect[56].

Statins are reported with a pleiotropic mode of action in PD. Statins directly inhibit the formation of Aβ and statins, by inhibiting Rho GTPase, exert a protective role in PD[57]. Treatment with atorvastatin has been associated with the suppression of Aβ peptides induced by β-secretase in cultured microglia from the cortex, whereas simvastatin attenuated the production of inflammation induced by Aβ[58-61]. It was inferred that the statin inhibits GGPP and FPP and exerts protection in AD. Another *in vitro* study reported that lovastatin and simvastatin downregulated the production of Aβ and on behalf of this study, the author proposed the protective mechanism of statins involving the inhibition of Rho GTPase[55,60-62]. Based on the published study, it can be inferred that statins restore the BBB and enhance clearance of Aβ, stimulate the release of α-secretase that cleavages the AβPP to produce secreted sAβPPα and membrane-associated carboxy-terminal alpha, inhibit the dimerization of β and γ secretase which reduces the formation of sAβPPβ and CTFβ[57,63,64]. This subsequently suppresses the Aβ production and inhibits the Rho GTPase that results in the inhibition of β and γ secretase activities and reduces the expression of proinflammatory cytokines (IL-β1, IL-6 and TNFα), NF-κB expression and suppress the Rac1 mediated oxidative stress and NADPH oxidase activities[57,63-65]. Thus, statins, through the inhibition of Rho GTPase, upregulate the Akt/PI3K cascade that directly reduces the expression of pro-apoptotic mediators like caspase 3 and caspase 9. The Akt/PI3K upregulation or activation inhibits the activity of GSK-3β and hexokinase-VDAC phosphorylation which were responsible for the formation of NFTs. Statins thus exert a protective effect in the treatment and management of Alzheimer's disease via Rho GTPase inhibitory property[55,57-61,63-66].

Statins were found to be associated with a reduction in AD severity by 70%, whereas a cross-sectional study performed by Wolozin *et al.*[68] signifies that only lovastatin and pravastatin were able to reduce the symptoms of AD, whereas simvastatin was ineffective. Eckert *et al.*[69] for the first time, established that the product of isoprenyl, i.e., GGPP and FPP, is elevated in grey matter and white matter of Alzheimer's patients and not cholesterol. Contrary to the findings of Wolozin *et al.*[68] this study showed that simvastatin, when administered for 21 days at a dose of 50 mg/kg to C57BL/6J mice, reduces the level of FPP and GGPP[69]. Additionally, it has been reported that the administration of simvastatin and atorvastatin inhibits the ROCK pathway and mitigates the level of the amyloid precursor protein (APP) in N2a/Swe neuroblastoma cell[70]. There is further evidence that atorvastatin when administered at a dose of 80 mg/kg for 14.5 months, results in a marked reduction in neuroinflammation and controls the progression of Alzheimer's disease[71]. There is also a significant impact of the duration of therapy and the age of the patient in the treatment of Alzheimer's disease[72]. Simvastatin, when administered at the dose of 40 mg/kg for 3-6 months, completely restored the memory dysfunction in 6-month-aged transgenic mice, but there was no effect of this dose on a similar strain of mice at the age of 12 months[72]. Therefore, statins' cumulative effect is to inhibit Aβ formation, inhibit

Table 2: Clinical evidence of statin in AD[73]

Type of study	Findings	Author
Observational study	Long-term use of statin positively affects clinical outcomes in cognitive dysfunction and dementia patient	Bitzur *et al.*
Narrative review	Use of statin has a negative effect on cognitive dysfunction in patients	Schultz *et al.*
Randomized controlled clinical trial	Use of statin has a neutral effect	Power *et al.*
Cochrane review	Use of statin in older patients has no preventive effect on AD or dementia	McGuinness *et al.*
Clinical trial	Atorvastatin has a preventive effect on cognitive dysfunction	Posvar *et al.*
Observational study	Lipophilic statins have a positive effect on cognitive dysfunction	Sahebzamani *et al.* and Li *et al.*
Case-control study	Use of statins lowers the AD	Jick *et al.*
Prospective study	Statin users have improved cognitive scores as compared to non-statin users	Sierra *et al.*
Population-based study	Early use of statin has lower progression of AD	Lin *et al.*
Prospective study	Use of statin shave protective effect on AD	Haag *et al.*
Cross-sectional study	Use of statin shave protective effect on AD	Rockwood *et al.*
Systemic review and meta-analysis	Statin use has a neutral effect on AD	Olmastroni *et al.*
Systemic review and meta-analysis	Use of statin has a positive effect on AD	Xuan *et al.*
Observational study and Meta-analysis	Use of statin has reduced the risk of AD	Wood *et al.*
Systemic review and meta-analysis	Use of statin reduced all types of dementia	Chu *et al.*
Observational study and Meta-analysis	Statin use has a protective effect on post-stroke dementia	Yang *et al.*

neuroinflammation-neurodegeneration, prevent apoptosis and prevent the hyperphosphorylated tau protein. Thus, from all the above-mentioned studies, it can be concluded that statins exert neuroprotection in Alzheimer's disease. Nevertheless, a more detailed and mechanistic-based approach is a prerequisite for better evaluation of statins in Alzheimer's disease, as shown in Table 2.

Rho GTPase, cognitive dysfunction and statins: Cognitive impairment is the result of many neurological disorders like depression, AD, PD, multiple sclerosis (MS) and epilepsy, cerebral ischemia, traumatic brain injury and stroke[88]. In cognitive dysfunction, the role of increased intracellular calcium concentration, glutamate activity, apoptosis, NADPH oxidase, oxidative stress and expression of inflammatory markers have been reported by Raz *et al.*[89]. Reduced levels of nitric oxide, Brain-Derived Neurotrophic Factor (BDNF) and vascular endothelial growth factor (VGDF) are also associated with cognitive dysfunction[90,91]. The Rho GTPase plays a pivotal role in the progression of cognitive dysfunction by mediating all the pathological factors[7,92]. Statins, by their GTPase inhibitory activity, are emerging as a future therapy for improvement in cognitive impairment[93,94]. There are two published meta-analysis studies conducted by Wolozin *et al.*[68] and Swiger *et al.*[95] where, the outcome established the fact that the use of statins is not associated with any adverse effect on dementia and cognitive function, preferably the author concluded that the positive effect of statins in cognition and dementia[68,95]. Statins, when administered, act on HMG-CoA reductase and inhibit cholesterol metabolism, thus by blocking the action of HMG-CoA reductase, there is downregulation of molecules such as Geranylgeranyl Pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) which are the product of isoprenoid. Downregulation of FPP and GGPP-associated GTPase like Rho and Rac is supposed to be a protective pathway in cognition[92,94,96]. Simvastatin exerts a protective effect on cognition and upon administration of FPP, this protection was abolished[97]. Administration of statins is found to be associated with upregulation of VEGF and BDNF which result in improved cognition outcomes[98,99].

LIMITATION IN THE DEVELOPMENT OF Rho GTPase INHIBITORS AND USE OF STATINS

The development of Rho GTPase inhibitors has been a challenging task for researchers because of their picomolar nucleotide affinity[100]. Apart from the aim to develop specific Rho GTPase or RhoA/Rac1 inhibitors, ROCK-II selective inhibitors could be a better alternative[101]. A major problem encountered in the development of ROCK inhibitors is the presence of two isoforms of ROCK, i.e., ROCK-I and ROCK-II[102]. A challenge that exists in the development of a Rho GTPase/ROCK inhibitor is teratogenicity, as, Y-27632, a Rho GTPase inhibitor, has been reported with teratogenic side effects in an animal model[103]. No doubt, stains are one of the most widely prescribed drugs and are useful in the prevention of cardiovascular and cerebrovascular disorders. There are findings which show some side effects of statins that limit their use[104]. Statins increase the hepatic transaminase level asymptomatically, causing myositis rhabdomyolysis and tubular proteinuria[104]. Moreover, the interaction of statins with antibiotics such as erythromycin or clarithromycin increases the toxicity[104]. A published meta-analysis using 32752 participants showed the increased risk of diabetes in a patient who takes a higher dose of statins, as compared to the patients who take lower or moderate doses of statins[105].

Therefore, keeping in view the limitation of fasudil, Y-27632 and statins, the use of nanoformulation could be the better therapeutic approach. Nanoformulation will reduce the dose of statins significantly and eventually, will minimize the hepatotoxic effect. In line with this, pravastatin-naringenin nanotransferosomes was designed and developed to improve bioavailability and to minimize hepatic side effects[106]. The nanoformulation approach has been also used in various other diseased conditions such as oral candidiasis, in relieving pain and inflammation and also against ocular fungal disease[107-109].

CONCLUSION

The current manuscript highlighted the deleterious effect of Rho GTPase on Alzheimer's disease, Parkinsonism and associated depression and cognitive dysfunction. In Japan, fasudil, a RhoA/ROCK inhibitor, has been marketed since 1995 for the treatment of cerebral spasms after haemorrhage. Fasudil, after oral administration, metabolizes into hydroxy fasudil, which has poor brain penetration. To date, only fasudil, Y-25632 and H-1152 have been studied *in vivo* models and no selective Rho GTPase inhibitor has been clinically approved by the US FDA. Considering the therapeutic importance of Rho GTPase, various small molecules are drugs under different phases of development such as ITX3 (selective GEF inhibitor), sacrament (NSC23766) and CASIN and ML141 (cdC42 inhibitor) and EHT 1864 (Rac inhibitor). Henceforth, there exists an unmet need for novel Rho GTPase inhibitors or a search for existing molecules that may act as Rho GTPase inhibitors. Additionally, there exist the use of techniques like docking and NMR, ultra-high field magnets, robotic autosamplers and cryogenic probes and surface plasmon resonance for quicker identification and development of hits into a specific lead molecule with Rho GTPase inhibitory activities. Moreover, nanoformulation of statin class of drugs or fasudil, precisely if used intranasally could overcome the limitations of these drugs and can also exhibit potent neuroprotective effects. Furthermore, more extensive studies and clinical practice guidelines are a prerequisite for the use of statins in neurological disorders as Rho GTPase inhibitor.

SIGNIFICANCE STATEMENT

Alzheimer's disease (AD) and Parkinson's disease (PD) are commonly diagnosed neurological disorders (NDs). Studies have shown the pathological role of Rho GTPase in NDs and hence, their inhibitors have been developed as neuroprotective agents. Herein, the possible multi-factorial pathogenic-mechanistic role of Rho GTPase in NDs, along with the neuroprotective role of stains as a Rho GTPase inhibitor, is discussed. Further, the limitations and challenges in the development of their inhibitors were also discussed. Additionally, it was concluded that the development of nano-formulation-based drug delivery of stains or another inhibitor would be an innovative approach to the management of NDs. Hence, the manuscript will be a valuable addition for the researchers to rationally design and develop Rho GTPase inhibitors for the NDs.

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