

Glial Cells as Possible Targets of Neuroprotection through Neurotrophic and Antioxidative Molecules in the Central and Enteric Nervous Systems in Parkinson's Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. The loss of nigrostriatal dopaminergic neurons produces its characteristic motor symptoms, but PD patients also have non-motor symptoms such as constipation and orthostatic hypotension. The pathological hallmark of PD is the presence of α -synuclein-containing Lewy bodies and neurites in the brain. However, the PD pathology is observed in not only the central nervous system (CNS) but also in parts of the peripheral nervous system such as the enteric nervous system (ENS). Since constipation is a typical prodromal non-motor symptom in PD, often preceding motor symptoms by 10–20 years, it has been hypothesized that PD pathology propagates from the ENS to the CNS via the vagal nerve. Discovery of pharmacological and other methods to halt this progression of neurodegeneration in PD has the potential to improve millions of lives. Astrocytes protect neurons in the CNS by secretion of neurotrophic and antioxidative factors. Similarly, astrocyte-like enteric glial cells (EGCs) are known to secrete neuroprotective factors in the ENS. In this article, we summarize the neuroprotective function of astrocytes and EGCs and discuss therapeutic strategies for the prevention of neurodegeneration in PD targeting neurotrophic and antioxidative molecules in glial cells.

Key words: Parkinson's disease, astrocyte, enteric glial cell, neurotrophic factor, antioxidative molecule

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. The characteristic pathology is the loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) and the appearance of α -synuclein (α -Syn)-containing cytoplasmic inclusion Lewy bodies in the surviving neurons. When the number of nigral DA neurons is reduced by 30%, motor symptoms such as akinesia/bradykinesia, tremor, rigidity, and postural instability appear [1]. In addition, PD patients also exhibit non-motor symptoms such as constipation, hyposmia,

orthostatic hypotension, depression, anxiety, hallucinations, and sleep disorders, which often precede motor symptoms [2]. Braak *et al.* reported that Lewy pathology in the central nervous system (CNS) appears first in the brainstem and olfactory bulb, and then spreads progressively through the SN and amygdala to reach the cerebral cortex [3]. Furthermore, several studies have reported that PD pathology was detected early in the peripheral nervous system including Auerbach's and Meissner's plexuses in the gut [4,5]. Therefore, it is hypothesized that PD pathology propagates from the enteric nervous system (ENS) to the CNS, in part, via the vagal nerve [6]. Indeed, epi-

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miological studies reported that vagotomy reduced the risk of PD [7]. Clearly, it is desirable to find approaches that inhibit the progression of neurodegeneration in both the CNS and ENS.

Astrocytes are the most abundant glial cells in the CNS and play an important role in the maintenance of the neuronal environment: specifically, regulation of extracellular ion concentration and energy supply [8]. Astrocytes secrete neurotrophic and antioxidative factors and prevent neurodegeneration. Indeed, various studies have demonstrated the neuroprotective effects of astrocytes in models of neurodegenerative diseases. We recently reported astrocyte-targeting dopaminergic neuroprotection in PD models [9,10]. In the ENS, astrocyte-like enteric glial cells (EGCs) play important roles in protecting enteric neurons by secretion of neurotrophic and antioxidative factors [11-13]. In this review, we summarize the neuroprotective functions of astrocytes and EGCs in the CNS and ENS, respectively, especially focusing on neurotrophic and antioxidative molecules.

Astrocyte-Mediated Dopaminergic Neuroprotection in the CNS

Neuroprotection by neurotrophic factors secreted by astrocytes. Astrocytes secrete neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), mesencephalic astrocyte-derived neurotrophic factor (MANF), and cerebral dopamine neurotrophic factor (CDNF) [9,14]. GDNF is a protein consisting of 134 amino acids that belongs to the transforming growth factor (TGF)- β superfamily [15,16]. It is found in cultured cells derived from mesencephalic neurons and is a potent dopaminergic neuroprotective factor [17]. GDNF is expressed mainly in neurons in the normal condition; conversely, astrocytes express and secrete the protein in diseased brains [11]. It was reported that GDNF overexpression increased the number of dopaminergic neurons in the SNpc and dopaminergic nerve terminals in the striatum of mice [18]. Intracerebroventricular injection of GDNF up-regulated DA content in neurotoxin 6-hydroxydopamine (6-OHDA)-treated PD model rats [19]. In addition, GDNF restored DA levels in the midbrain and ventral tegmental area and improved motor symptoms in rhesus monkeys of PD model induced by injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(MPTP) [20]. Thus, GDNF could be a potent neuroprotectant in PD. However, GDNF cannot pass through the blood-brain barrier (BBB); therefore, research on GDNF delivery using viral vectors is underway. Lentiviral vector-based delivery of GDNF showed neuroprotection against 6-OHDA-induced neurotoxicity [21]. In addition, it is reported that several DA D1 and D2 agonists up-regulate the expression of GDNF in astrocytes [22,23].

MANF and CDNF are the known neurotrophic factors secreted by astrocytes [24,25]. Under normal conditions these proteins are primarily localized in the endoplasmic reticulum (ER) and are secreted in response to ER stress [26]. MANF and CDNF reduce ER stress and exert pro-survival effects on neurons. It has been reported that overexpression or chronic treatment with CDNF or MANF prevents dopaminergic neurodegeneration in 6-OHDA-injected PD models [27-29]. As a potential therapeutic target, CDNF has the advantage of a wide distribution in the brain [30].

Neuroprotection by antioxidative molecules produced by astrocytes. Astrocytes are also known to produce antioxidative molecules such as glutathione (GSH) and metallothionein (MT)-1,2 [31,32]. GSH is a well-known intrinsic antioxidant against reactive oxygen species. Previous studies reported that GSH levels were decreased in the SN of PD patients [33,34]. Therefore, up-regulation of GSH could be a neuroprotective treatment for PD. GSH is a tripeptide composed of glutamate, cysteine, and glycine. Neurons need to take up cysteine for GSH synthesis. However, extracellular cysteine is readily oxidized to the oxidized homodimer cystine [35], and neurons lack the cystine/glutamate exchange transporter (xCT), the main protein for bringing cystine into cells [36,37]. Astrocytes do express xCT for cystine uptake, and reduce it to cysteine for the synthesis of GSH [38]. Astrocytes release the synthesized GSH into the extracellular space via multidrug resistance-associated protein 1 (MRP1) [39,40]. The extracellular GSH is then reduced by disulfide-exchanging or peptidase reactions to produce cysteine, which is taken up by neighboring neurons and used for GSH synthesis in neurons [9]. Thus, GSH synthesis in neurons is dependent on cystine uptake by astrocytes and their synthesis and release of GSH [9,41]. We previously reported that the antiepileptic drugs, zonisamide and levetiracetam, upregulated xCT expression followed by GSH synthesis in astrocytes and

protected dopaminergic neurons in parkinsonian models [42,43]. It is also reported that stimulation of D2 receptors on astrocytes promotes GSH synthesis via activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and protects neurons against MPTP-induced dopaminergic neurotoxicity *in vitro* and *in vivo* [44]. Nrf2 is a master transcription factor to produce various phase II-detoxifying enzymes and antioxidative molecules including GSH and MT-1,2 [45,46].

Metallothioneins bind to heavy metals such as copper, cadmium, and zinc, and function in metal homeostasis and detoxification [47,48]. MTs contain 20-cysteine residues and exert antioxidative properties [49]. There are four isoforms, MT-1, -2, -3 and -4; MT-1,2 are expressed in most organs including the brain, and the proteins are mainly produced by astrocytes [50]. MT-1,2 expression is increased in astrocytes in the SN of PD patients [51]. Our previous studies demonstrated that astrocytes produced MT-1,2 in response to oxidative stress, and extracellular MT-1,2 prevented dopaminergic neurotoxicity [45]. Furthermore, we previously reported that stimulation of serotonin (5-HT) 1A receptors on astrocytes promoted astrocyte proliferation and expression/secretion of MT-1,2 in/from astrocytes [52]. Taken together, upregulation of MT-1,2 and 5-HT1A stimulation in astrocytes could be a target of neuroprotection in PD.

Novel Neuroprotective Agents Targeting Astrocytic 5-HT1A Receptors

Rotigotine, a DA agonist, is used to treat PD. Rotigotine binds to DA D1-D5 receptors, with a particularly high affinity for D2, D1 and D3 receptors, in that order [53]. In addition, rotigotine possesses 5-HT1A partial agonistic properties [54]. Our recent study demonstrated dopaminergic neuroprotective effects of rotigotine via 5-HT1A receptors in PD models [55]. Rotigotine treatment promoted astrocyte proliferation and MT-1,2 expression in astrocytes. The rotigotine-induced upregulation of MT-1,2 in astrocytes was mediated by Nrf2 activation. Pretreatment with conditioned medium from rotigotine-treated astrocytes inhibited 6-OHDA-induced dopaminergic neurotoxicity via astrocytic 5-HT1A receptors and secretion of MT-1,2. Furthermore, daily subcutaneous administration of rotigotine increased MT-1,2 expression in astrocytes and prevented the reduction of dopaminergic neurons in the SNpc of 6-OHDA-injected PD model

mice. These effects were blocked by co-administration with a 5-HT1A receptor antagonist. These results suggest that rotigotine exerts neuroprotective effects by upregulation of MT-1,2 expression in astrocytes via 5-HT1A receptors.

Mirtazapine is a noradrenergic and specific serotonergic antidepressant (NaSSA). It increases extracellular levels of noradrenaline and 5-HT by blocking pre-synaptic α_2 adrenergic receptors on noradrenergic and serotonergic nerve terminals. In addition, mirtazapine inhibits 5-HT2 and 5-HT3 receptors to indirectly stimulate 5-HT1A receptors. We have demonstrated the neuroprotective effects of mirtazapine by upregulation of MT-1,2 expression in astrocytes via 5-HT1A receptors [56]. A previous study demonstrated that 5-HT1A receptor agonists improved motor dysfunction and L-dopa-induced dyskinesia in PD model animals [57]. Mirtazapine also improves motor- and non-motor symptoms in PD patients [58,59]. These results suggest that mirtazapine could be a possible disease-modifying agent for PD [56].

As mentioned above, rotigotine also upregulated antioxidative molecules in astrocytes and ameliorated dopaminergic neurodegeneration via 5-HT1A receptors [55]. Taken together with these experimental results, targeting the 5-HT1A receptor on astrocytes may be a possible strategy for neuroprotection in PD [9].

Role of Glial Cells in the ENS

EGCs are counterparts of astrocytes in the CNS. EGCs express GFAP and represent the morphological features of astrocytes [60]. The astrocytic glial cells that support neurons and nerve fibers in the ENS were first described by Dogiel in 1899 [61]. EGCs are classified into 1-4 subtypes based on their morphology and distribution [60]. EGCs interact with neurons and epithelial cells by secreting neurotrophic factors and bioactive molecules [62]. Calcium imaging studies demonstrated EGC activation in response to neuronal activity in the ENS [63]. These Ca^{2+} -mediated interactions between EGCs and neurons can modulate intestinal motility [64]. Furthermore, it is reported that EGCs are activated in response to the release of neurotransmitters such as acetylcholine (ACh) and 5-HT [65-67]. Collectively, these reports suggest that the interaction between EGCs and enteric neurons may regulate intestinal motility. It is also reported that EGCs are involved

in maintaining the mucosal lining and repairing barrier function by secreting several mediators [62,68]. A study of EGC-knockdown mice showed that their intestinal cell permeability was increased, although intestinal motility was significantly decreased [69]. These results indicate that EGCs work to maintain intestinal permeability. EGCs are activated by inflammation and secrete GDNF to improve the barrier function of the intestine [70]. In addition, EGCs express major histocompatibility complex II (MHCII), respond to noxious inputs via toll-like receptors (TLR)-2 and -4, and regulate immune function in the ENS via the release of pro-inflammatory cytokines [71]. Furthermore, EGCs themselves function as antigen-presenting cells for both innate and acquired immune systems [72]. Thus, EGCs have an important role in intestinal motility, mucosal lining, and immune responses in the ENS. As mentioned above, accumulation of α -Syn has been observed in the ENS of PD patients with gastrointestinal disorders such as constipation. Moreover, the proportion of EGCs expressing GFAP was increased in the ENS [73]. EGCs could be involved in the pathogenesis of neurodegenerative diseases, but their role is rather complex.

Enteric Neuroprotection Mediated by EGCs in PD Models

EGC-mediated enteric neuroprotection in the rotenone-injected PD model. The ENS-neuroprotective roles of EGCs have been studied from several angles. Abdo *et al.* demonstrated that the neuroprotective effects of EGCs against oxidative stress occur via the release of reduced GSH [74]. In addition, the pesticide rotenone causes loss of the enteric plexus and decreased gastrointestinal motility [75]; thus, we examined changes in MT-1,2 expression in the colonic myenteric plexus of rotenone-injected PD model mice [76]. Subcutaneous administration of rotenone (50 mg/kg/day) for 6 weeks increased MT expression and activated glial cells not only in the striatum but also in the colonic myenteric plexus. Furthermore, rotenone-induced neuronal loss of the myenteric plexus was aggravated in MT-1,2 knockout mice compared to wild-type mice, suggesting the neuroprotective effects of MT expression in the EGCs [76]. In addition, upregulation of antioxidative molecules, such as GSH and MT, in EGCs can provide neuroprotection in the ENS in PD. Our previous study demonstrated that administration with caffeic acid (CFA) or chlorogenic acid (CGA), polyphenol

components of coffee, inhibited the reduction in MT-1,2 expression in EGCs and prevented the loss of enteric plexus in low-dose rotenone-treated PD mice [77]. Several studies have reported that CFA and CGA activate the Kelch-like ECH-associated protein 1 (Keap1)-Nrf2 pathway [78,79]. Therefore, activation of the Nrf2 signaling pathway could be a target of neuroprotection in the ENS. Finally, CDNF is also important as a neuroprotectant in the ENS. CDNF knockout mice exhibited loss of intestinal Meissner's plexus, but not of nigral dopaminergic neurons [80].

Further investigation of the effects of rotenone found that it activates GFAP-positive glial cells in the ENS to cause impairment of intestinal motility [81]. In addition, in the rotenone model using *C. elegans*, dysfunction of mitochondrial complex I activated the innate immune response pathway via the p38 MAPK/ATF-7 pathway and prevented dopaminergic denervation in the intestine [82]. Neuroprotection by p38 MAPK/ATF-7 requires the autophagy mechanism and PINK-1, suggesting that p38 MAPK-mediated immunity activates mitophagy and results in neuroprotection. These reports suggest that EGCs are activated by inflammatory responses in the intestine and may be involved in the protection of enteric neurons and regulation of intestinal motility in the intestine.

Involvement of gut microbiota in the protection of enteric plexus. Recent studies have reported interactions between gut microbiota and neurodegenerative diseases such as multiple sclerosis, Alzheimer's disease, and PD [83]. The mammalian gut microbiota is composed of bacteria, viruses, yeasts, and bacteriophages [84]. The gut microbiota plays important roles in food digestion, vitamin synthesis, angiogenesis, epithelial cell maturation and development, education of the host immune system, and protection against pathogens. The gut microbiota synthesizes neurotransmitters such as ACh, γ -aminobutyric acid (GABA), and 5-HT, which can directly and indirectly affect physiological function in the CNS [85-87]. In addition, short-chain fatty acids (SCFAs) produced by the gut microbiota in the presence of indigestible fiber maintain intestinal homeostasis by promoting the intestinal lining and mucus production as a barrier, and promoting a pro-inflammatory permissive response; they also migrate to the CNS to restore the integrity of the BBB [88]. In PD patients, the composition of SCFAs was changed due to a decrease in butyrate acidophilic bacteria [89].

Transplantation of microbiota from PD patients into α -Syn-overexpressing mice enhanced motor disability compared with transplantation of microbiota from healthy human donors [90]. These findings suggest that gut bacteria can influence motor function in mice and that alterations in the human microbiome could be a risk factor for PD. Recently, it was reported that the addition of restraint stress (RS) to rotenone treatment enhanced rotenone-induced oxidative stress and increased expression of α -Syn, GFAP, and IL-1 β in the intestine, as well as dopaminergic neuronal loss and microglial activation in SN [91]. In this experiment, changes in the gut microbiota were also examined; RS decreased *Lactobacillus* populations and RS-plus-

rotenone treatment increased *Akkermansia* populations. RS with or without rotenone also showed a decreasing trend of butyric acid content in feces, which correlated with the data of PD patients. These reports clarified the relationship between PD and intestinal inflammation, or PD and EGC activation. However, the direct connection between intestinal inflammation and EGC activation in PD is still obscure.

Conclusion

Astrocytes in the CNS and EGCs in the ENS provide neuroprotection by secretion of neurotrophic and anti-oxidative molecules (Fig. 1). Therefore, up-regulation

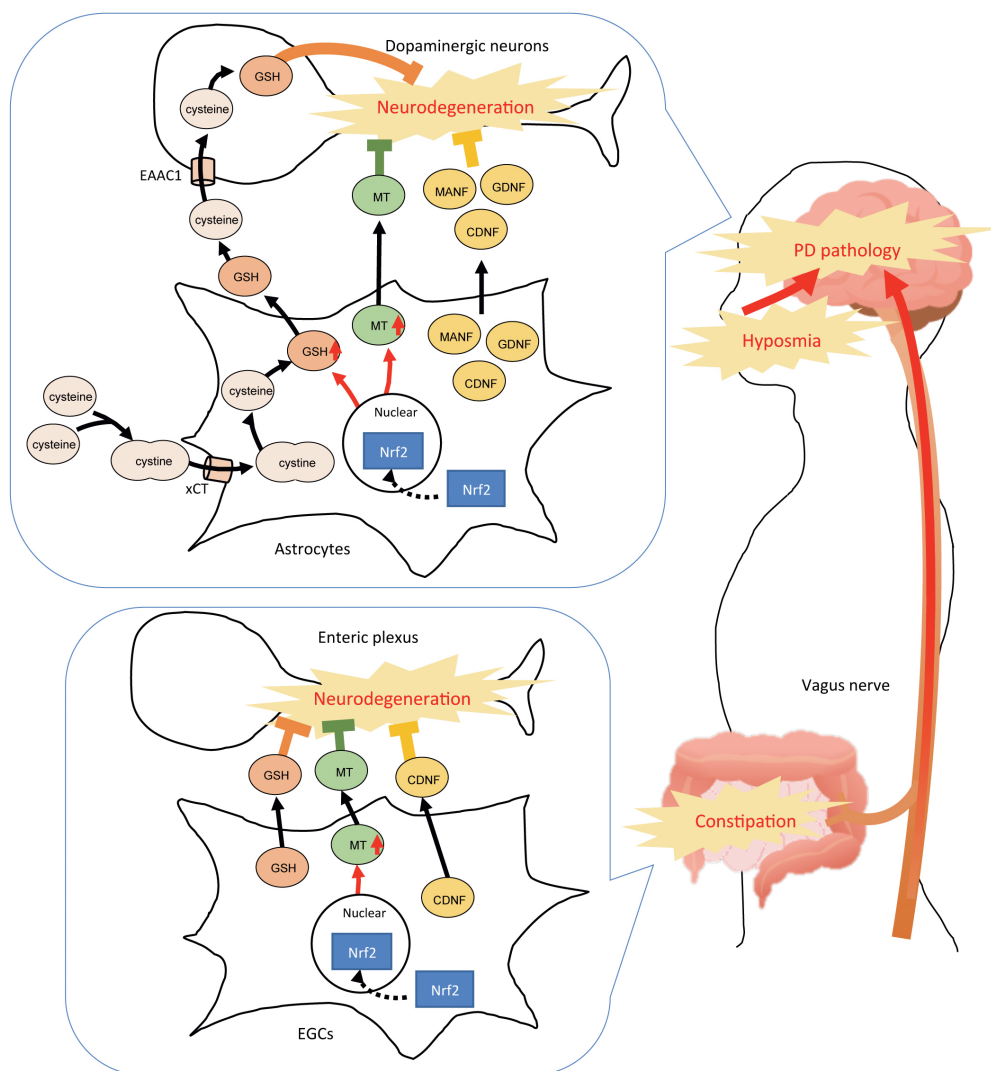


Fig. 1 Neuroprotection by glial cells in the CNS and ENS. Astrocytes in the CNS and EGCs in the ENS provide neuroprotection by secretion of neurotrophic and antioxidative molecules.

of these molecules in astrocytes or glial cells could be a possible target of neuroprotection. Since enteric manifestations of PD often precede motor dysfunction by 10-20 years, a neuroprotective approach in the ENS could be a potent therapeutic strategy to inhibit the initial enteric pathogenesis and progression of PD.

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