REVIEW

Neuroprotection by immunomodulatory agents in animal models of Parkinson's disease

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Abstract
Parkinson's disease (PD) is an age-related neurodegenerative disease for which the characteristic motor symptoms emerge after an extensive loss of dopamine containing neurons. The cell bodies of these neurons are present in the substantia nigra, with the nerve terminals being in the striatum. Both innate and adaptive immune responses may contribute to dopaminergic neurodegeneration and disease progression is potentially linked to these. Studies in the last twenty years have indicated an important role for neuroinflammation in PD through degeneration of the nigrostriatal dopaminergic pathway. Characteristic of neuroinflammation is the activation of brain glial cells, principally microglia and astrocytes that release various soluble factors. Many of these factors are proinflammatory and neurotoxic and harmful to nigral dopaminergic neurons. Recent studies have identified several different agents with immunomodulatory properties that protected dopaminergic neurons from degeneration and death in animal models of PD. All of the agents were effective in reducing the motor deficit and alleviating dopaminergic neurotoxicity and, when measured, preventing the decrease of dopamine upon being administered therapeutically after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 6-hydroxydopamine, rotenone-lesioning or delivery of adeno-associated virus-α-synuclein to the ventral midbrain of animals. Some of these agents were shown to exert an anti-inflammatory action, decrease oxidative stress, and reduce lipid peroxidation products. Activation of microglia and astrocytes was also decreased, as well as infiltration of T cells into the substantia nigra. Pretreatment with fingolimod, tanshinoine I, dimethyl fumarate, thalidomide, or cocaine- and amphetamine-regulated transcript peptide as a preventive strategy ameliorated motor deficits and nigral dopaminergic neurotoxicity in brain-lesioned animals. Immunomodulatory agents could be used to treat patients with early clinical signs of the disease or potentially even prior to disease onset in those identified as having pre-disposing risk, including genetic factors.

Key Words: *Parkinson's disease; immunomodulatory agents; neuroprotection; inflammation; oxidative stress; animal models; microgliosis; astrogliosis*

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doi: *10.4103/1673-5374.237108*

Accepted: *2018-05-25*

Introduction
Parkinson's disease (PD) is an age-related neurodegenerative disease, second in prevalence to Alzheimer's disease. A range of clinical symptoms are exhibited, with the most common affecting motor function and include resting tremor, rigidity, akinesia, bradykinesia and postural instability (Winklhofer and Haass, 2010). The characteristic motor symptoms of PD appear after an extensive loss of dopamine containing neurons, the cell bodies of which are located in the substantia nigra and the nerve terminals in the striatum (Savitt et al., 2006). Pre-motor symptoms arise much earlier. A common symptom is constipation which can be experienced many years before motor dysfunction onset in PD patients (Savica et al., 2009). Computational models have been used to investigate the dopamine deficiency on PD symptoms (Daneshzand et al., 2017a, b). Characteristic of the disease is accumulation of protease-resistant α-synuclein (α-syn) in synapses and axons, formation of neuronal inclusions called Lewy bodies (LBs), and selected neuronal degeneration in the neocortex, limbic, and nigrostriatal systems, with neuroinflammation (Dickson, 2001). Recent evidence supports the view that α-syn plays a central role in the etiopathogenesis of PD (Winner et al., 2011; Lashuel et al., 2013; deSouza and Schapira, 2017). Both innate and adaptive immune responses may contribute to dopaminergic neurodegeneration and disease progression is potentially linked to these (Braak et al., 2007). While currently no proven protective treatments are available for patients with PD (Olanow et al., 2009; Athauda and Foltynie, 2015), some agents such as levodopa (L-dopa) and apomorphine can provide relief from the symptoms of PD but are less effective as the disease progresses. In addition to the loss of efficacy, these agents are associated with a range of side effects, some common such as nausea, vomiting, while others are more severe and include psychic disturbances and dyskinesia (Cotzias et al., 1970).

Studies in the last twenty years have shown an important role for neuroinflammation in PD through the degeneration of the nigrostriatal dopaminergic pathway. Characteristic of neuroinflammation is the activation of brain glial cells, principally microglia and astrocytes that release various soluble factors such as free radicals, cytokines, and lipid metabolites. Many of these factors are proinflammatory and neurotoxic and are particularly harmful to nigral dopaminergic neurons

that are also vulnerable to oxidative damage (Czlonkowska et al., 2002; Liu et al., 2003).The resident immune cells in the brain are the microglia and are sensitive to even minor disturbances in central nervous system (CNS) homeostasis. They become readily activated during most neuropathological conditions (Liu and Hong, 2003). Dopaminergic neurodegeneration is alleviated in various experimental animal models of PD by reducing neuroinflammation with anti-inflammatory drugs (Choi et al., 2005; Jin et al., 2008). We have searched the PubMed database for recent studies in years 2012–2017 aimed at downregulating immune and inflammatory processes in animal models of PD using immunomodulatory agents. These could be important in slowing the progression of PD and might be exploited as treatments in patients with PD. An ambitious yet imperative goal in research, and of paramount importance in translational medicine, is the development of new therapeutic approaches that can impede or prevent the progression of PD.

Immunomodulatory Therapies for PD

Pharmaceutical therapies

The pharmaceutical therapies were with fingolimod (FTY720), acetoside, amphetamine-regulated transcript peptide (CART), tanshinone I, tanshinone IIA, dimethyl fumarate, ginsenoside Rg1, tacrolimus (FK506), lenalidomide, thalidomide, cyclosporin, Nurr1 agonist SA00025, interferon (IFN)-β, semapimod (CNI-1493), and pycnogenol. These have all been shown to have immunomodulatory properties (FTY720: Kovarik et al., 2004; Lakshmikanth et al., 2016; acetoside: He et al., 2011; cocaine- and amphetamine-regulated transcript peptide (CART): Bik et al., 2008; tanshinone I: Lee et al., 2013; tanshinone IIA: Qin et al., 2010; dimethyl fumarate: Albrecht et al., 2012; Strassburger-Krogias et al., 2014; ginsenoside Rg1: Kenarova et al., 1990; FK506: Kaminska et al., 2004; lenalidomide: Kotla et al., 2009; thalidomide: Bodera and Stankiewicz, 2011; cyclosporin: Tajima et al., 2003; Nurr1 agonist SA00025: Maijenburg et al., 2010; IFN-β: Kasper and Reder, 2014; CNI-1493: Martiney et al., 1998; pycnegenol: Cheshier et al., 1995).

The twenty animal studies utilizing these pharmaceutical agents are summarized in **Table 1**. Fourteen of these studies had used mouse models, four had employed rat models, and two had used both mouse and rat models. In the mouse studies, the ages of the animals ranged from 7 weeks to 12 months and where gender was specified had used males. The rat studies had used animals the ages of which, by reference to body weight/age growth charts, would have ranged from 6 to 13 weeks and where gender was specified had used females.

Mouse PD studies

FTY720

FTY720 treatment of 6-hydroxydopamine (6-OHDA)- or rotenone-induced PD mice reduced the deficit of motor function and the loss of TH^+ neurons in the substantia nigra, and attenuated the decrease of striatal dopamine and its metabolite levels (Zhao et al., 2017). FTY720 pretreatment of 6-OHDA-lesioned mice also reduced motor deficits and loss of nigral dopaminergic neurons, while also decreasing 6-OHDA-induced inflammation. Activation of AKT and ERK1/2 pathways and an increase in brain-derived neurotrophic factor (BDNF) expression were associated with the protective effects of FTY720 (Ren et al., 2017). Interestingly, long-term oral FTY720 reduced enteric nervous system α-syn aggregation and constipation, enhanced gut motility, and increased levels of BDNF in transgenic mice overexpressing mutant human α-syn (Vidal-Martinez et al., 2016).

Tanshinone I

Tanshinone I pretreatment of 6-OHDA-lesioned mice ameliorated dopaminergic neurotoxicity in the substantia nigra and striatum. It also protected against 6-OHDA-induced oxidative stress in the striatum by increasing glutathione (GSH) levels after 6-OHDA injection (Jing et al., 2016). In another study, tanshinone I pretreatment of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-injected mice increased retention time on the rotating rod and prevented the decrease in dopamine and its metabolites. It also alleviated the reduction in dopaminergic TH^+ neurons in the substantia nigra associated with MPTP treatment. Tanshinone I pretreatment inhibited the MPTP-induced microglial activation in the substantia nigra and striatum, attenuated the increase in the brain level of tumor necrosis factor-α (TNF-α), and preserved the increase of interleukin (IL)-10 level (Wang et al., 2015).

Tanshinione IIA

Tanshinione IIA given immediately after 6-OHDA treatment reduced apomorphine-induced contraleral rotations and alleviated 6-OHDA-induced loss of TH^+ neurons in the substantia nigra and striatum. Tanshinione IIA also attenuated the reduction of dopamine and its metabolites associated with 6-OHDA lesioning (Zhang et al., 2015). Similar findings with tanshinione IIA were reported earlier and it was also shown to decrease the number and size of CD11b-immunopositive cells in the striatum and downregulate the expression of CD11b in the striatum which was increased by MPTP treatment. Tanshinione IIA inhibited NADPH oxidase and inducible nitric oxide synthase (iNOS) in the substantia nigra which are the main sources of ROS and RNS (Ren et al., 2015).

Dimethyl fumarate

Dimethyl fumarate reduced the motor deficit, protected dopaminegic neurons in the substantia nigra against α-syn toxicity, and decreased microgliosis and astrogliosis after delivery of adeno-associated viral vector expressing human α-syn to the ventral midbrain. The protective effect was not found to occur in *Nrf2*-knockout animals (Lestes-Becker et al., 2016). An earlier study using dimethyl fumarate pretreatment showed it protected against 6-OHDA-induced ox-

Table 1 Studies of pharmacological agents with immunomodulatory properties in animal models of Parkinson's disease (PD)

Study	No. of animals, gender, ages, treatment	Comparison	Functional outcomes	Conclusion
Fingolimod (FTY720) Zhao et al. (2017)	Adult C57Bl/6L male mice, 7–8 weeks, anesthetized with ketamine/xylazine and injected with 6-hydroxydopamine (6-OHDA) (6 μg in 4 μL of normal saline with 0.02% ascorbic acid) into two separate sites of the striatum on the right side of the brain. Each mouse received 4 μL 6-OHDA at a rate of 0.5 μL/min through an infusion pump. The tendency of animals to rotate in response to s.c. administration of apomorphne (0.5 mg/kg) was measured weekly until day 21 after lesion induction. For the second model of PD, mice received rotenone (30 mg/kg suspended in 0.5% carboxymethylcellulose (CMC)) once daily by oral gavage for 28 days. Rotenone-treated mice were assessed by the rotarod test. Measurements of tyrosine hydroxylase-positive (TH^+) neurons in the substantia nigra were performed in all animals to confirm loss of dopaminergic neurons. FTY720 was dissolved in 2.5% dimethyl sulfoxide (DMSO) in saline. The sphingosine-1-phosphate receptor (S1PR) antagonist, W146, was dissolved in 0.5% ethyl alcohol in sterile water. Mice received daily injections intraperitonial (i.p.) of 0.5 mg/kg FTY720, 1 mg/kg FTY720, or 1 mg/kg FTY720 + 10 mg/kg W146. Treatments started immediately after 6-OHDA injection and lasted for 21 consecutive days, or after the first injection of rotenone and lasted for 28 consecutive days in the rotenone model. Brains from all mice were frozen and later sectioned, fixed, and used for immunostaining.	Mice injected with saline alone into two separate sites of the striatum on the right side of the brain served as controls. In the second model of PD, mice receiving 0.05% CMC alone were used as controls. Also mice received daily injections i.p. of vehicle alone.	Mice injected with 6-OHDA had unilateral lesions and aberrant apomorphine-induced rotational behavior that gradually worsened from day 7 to day 21 of treatment. In mice injected with rotenone, decreased latency to falling time in the rotarod test was observed at day 28. FTY720 treatment significantly reduced motor deficits in mice injected with 6-OHDA or rotenone. Treatment with 6-OHDA or rotenone induced a marked loss of dopaminergic neurons in both the substantia nigra and striatum. Subsequently, in mice receiving FTY720, the loss of TH^+ neurons induced by 6-OHDA or rotenone decreased markedly. In line with the loss of dopaminergic neurons induced by 6-OHDA or rotenone, the content of dopamine and its metabolites (dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)) in the striatum underwent reduction. FTY720 significantly raised the levels of dopamine, DOPAC and HVA in the striatum. FTY720 treatment mitigated the increase of cleaved caspase-3 expression in mice receiving 6-OHDA or rotenone. In contrast, FTY720 alleviated the reduction of extracellular regulated protein kinases (ERK) phosphorylation and Bcl-2 expression in both PD models. The selective S1PR antagonist, W146, abolished the beneficial effect of FTY720 in mice receiving 6-OHDA or rotenone, thereby permitting the debilitation of motor responses and loss of dopaminergic neurons in the substantia nigra. The effect of FTY720 on 6-OHDA or rotenone-induced cell apoptosis, was determined by measuring the number of TdT-mediated dUTP nick-end labeling (TUNEL)+ cells compared to controls (vehicle). The number of TUNEL+ cells in the substantia nigra of mice injected with 6-OHDA or rotenone was significantly decreased after FTY720 treatment.	Although S1PR1 expression was significantly increased in mice receiving 6-OHDA or rotenone, FTY720 treatment retained S1PR1 expression. These findings suggest the potential of S1PR modulation by FTY720 as a therapy for PD.
Ren et al. (2017)	Adult C57Bl/6 male mice, 10 weeks, were injected i.p. with 0.5mg/kg FTY720 or vehicle for 7 days prior to lesioning. On the 7th day of treatment, 1 hour after final dosing of FTY720, mice were injected with 6 μg 6-OHDA (in 2 μL normal saline with 0.02% ascorbic acid) into two different sites of the right striatum. Mice were euthanized at different time points following 6-OHDA injection for histochemical or biological assessment. Apomorphine-induced rotations were monitored over a period of 3 weeks starting from 1 week post 6-OHDA lesioning. Apomorphine was injected s.c. into mice at a dose of 0.1 mg/kg, with mice placed individually in plastic beakers and videotaped from above for 30 minutes. Animals were anesthetized with sodium pentobarbital at 3 weeks after 6-OHDA administration, perfused with normal saline followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Brains were dissected out, postfixed and cryopreserved. Frozen brains were then coronally sectioned for immunohistochemistry. Striatal tissues were removed at 3 weeks following 6-OHDA administration and striatal dopamine (DA) and its metabolites DOPAC and HVA measured using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method.	Mice injected with saline alone into two separate sites of the striatum on the right side of the brain served as controls.	Evaluation of tissues collected 21 days after 6-OHDA injections showed that the 6-OHDA-induced neurodegeneration in the substantia nigra and striatum was attenuated by FTY720. Immunoblots of striatal extracts showed higher TH protein levels in FTY720 treated mice. Mice were monitored for apomorphine-induced rotation at 7, 14 and 21 days after 6-OHDA lesioning. Apmorphine-induced asymmetrical rotations contralateral to the 6-OHDA injection site were significantly reduced by FTY720 treatment compared to mice treated with 6-OHDA alone. There was a profound reduction in striatal DA and its metabolites after 6-OHDA lesioning that was attenuated by FTY720 pretreatment, which produced a significant elevation in striatal DA and the metabolites DOPAC and HVA at 21-day post 6-OHDA lesioning. 6-OHDA administration markedly increased markers of inflammation such as reactive astrocytes and microglia compared to vehicle-treated control animals where only a few faintly immunoreactive astrocytes and microglia were observed in the substantia nigra and striatum. FTY720 administration resulted in a significant decrease in 6-OHDA-induced astrogliosis and microgliosis.	Administration of FTY720 significantly increased the phosphorylation of protein kinase B (AKT), extracellular receptor-stimulated kinase (ERK)1/2 and P-cAMP response element binding protein (CREB) in mouse striatal tissues. FTY720 treatment also significantly increased brain-derived neurotrophic factor (BDNF) protein levels in 6-OHDA lesioned mice. FTY720 may hold promise as a PD therapeutic acting through AKT/ERK1/2/P-CREB-associated BDNF expression.
Vidal-Martinez et al. (2016)	A53T αSyn (B6;C3-Tg-Prnp/SNCA*A53T/83Vle/J) mice were used to generate a cohort of mice from A53T heterozygous breeders, which produced both wild type (WT) and transgenic (Tg) mice. The Tg mice included heterozygous and homozygous offspring that overexpress one or two copies of A53T mutant human α-syn. FTY720 was dissolved in ethanol at a concentration of 29 mM. Mice received FTY720 (0.5 mg/kg) twice weekly by voluntary oral dosing. This involved preparing tablets from bacon softies and mouse chow mixed with Splenda in sterile water to form a paste from which tablets (0.5 cm diameter) were made. Mice in home cages were individually pretrained to eat an entire tablet in 1 minute or less. Tablets in 24-well tissue culture plates were inoculated with the correct volume of FTY720. For Trk-B receptor inhibitor ANA-12, littermate mice received daily oral dosing of ANA-12 dissolved in DMSO (0.5 mg/kg) mixed with 10 μL sesame oil and delivered by pipette. FTY720 (0.5 mg/kg) alone or in combination with ANA-12 was dissolved in DMSO and given twice weekly in sesame oil. ANA-12 experiments included the following treatment groups: vehicle ($n = 4$), FTY720 ($n = 4$), ANA-12 ($n = 3$), FTY720 + ANA-12 ($n = 7$). Behavioral assessment included fecal water content, colonic motility, whole gut transit time. Mice were euthanized, the gut was flushed of fecal contents, bisected along the longitudinal axis, and divided into samples for assessment.	Mice received an equivalent amount of ethanol (vehicle) twice weekly by voluntary oral dosing with prepared tablets inoculated with the correct volume for each mouse.	Tg mice overexpressing mutant human α-syn developed enteric nervous system (ENS) pathology by 4 months. The responses of Tg mice and their WT littermates to FTY720 or vehicle control were evaluated from 5 months of age. Long term oral FTY720 in Tg mice reduced ENS αSyn aggregation and constipation, enhanced gut motility, and increased levels of brain-derived neurotrophic factor (BDNF) but produced no significant change in WT littermates. A role for BDNF was assessed in a cohort of young A53T mice given vehicle, FTY720, ANA-12, or FTY720 + ANA-12 from 1 to 4 months of age. ANA-12 treated Tg mice developed more gut α-synuclein (α-syn) aggregates as well as constipation, whereas FYT720-treated Tg mice had reduced αSyn aggregation and less constipation, occurring in part by increasing both pro-BDNF and mature BDNF levels.	The data from young and old Tg mice showed FTY720-associated neuroprotection and reduced α-syn pathology, suggesting that FTY720 may also benefit PD patients and others with synucleinpathy.
Acetoside Yuan et al. (2016)	Adult Sprague-Dawley rats, 200–300 g, were randomly divided into 3 groups: control, rotenone model ("model"), or rotenone + acetoside treatment ("treatment") ($n = 10$ rats/group). The later two groups received rotenone injections. Rotenone was initially dissolved in DMSO 0.1% and then in olive oil (0.2 mg/mL). Rotenone subcutaneous (s.c.) injection (1 mg/kg/day) was performed in the "model" and "treatment" rats for 42 consecutive days. For the "treatment" group, acetoside (30 mg/kg/day, oral gavage for 42 days) was also administered. The clinical signs of rats were observed daily, and if the criteria of humane endpoints were met, animals were euthanized. The open field test (OFT) was applied to evaluate the rats' behaviors. The observed indicators included gait instability, muscle tremors, slowed activity, the number of times the rats entered into the adjacent area, the duration in which the forelimbs were off the ground by more than 1 cm, and the duration that rats stayed in central area.	Control group was injected with the same amount of olive oil.	Starting from 1 week of daily rotenone injection, symptoms of PD were evident. The majority of the rats exhibited increases in saliva secretion, piloerection, hypotonia, respiratory frequency, as well as sensitivity to environmental stimuli and eating difficulties. The OFT revealed that the rotenone rats exhibited significantly increased time in the central area, along with reduced movement and standing times. Such parkinsonism symptoms were largely alleviated with co-administration of acetoside. The expression profiles of three key PD-associated proteins were tested in the rats. Immunohistochemistry (IHC) staining revealed that expression of α-syn was significantly increased in substantia nigra regions of rotenone rats. Further, caspase-3 level tested by Western blot assay was also increased in substantia nigra regions of rotenone rats, while MAP2 IHC intensity was decreased in the substantia nigra regions of the PD rats. Such changes were largely attenuated with co-administration of acetoside. The potential binding between caspase-3 and acetoside in several hydrogen bonds was shown by molecular docking and verified in the molecular dynamics stimulation method. This indicated that casspae-3 could be a potential target of acetoside. No apparent binding between acetoside and α-syn was observed.	Acetoside binds to caspase-3 and exerts neuroprotection in the rotenone rat model of PD.

Martinez B, Peplow PV (2018) Neuroprotection by immunomodulatory agents in animal models of Parkinson's disease. Neural Regen Res 13(9):1493-1506. doi:10.4103/1673-5374.237108

Table 1 Continued

Study	No. of animals, gender, ages, treatment	Comparison	Functional outcomes	Conclusion
Amphetamine-regulated transcript peptide (CART) Upadhya et al. (2016)	Adult male Sprague-Dawley rats (230–250 g) were stereotaxically cannulated targeting left lateral ventricle and right substantia nigra. At 7 days after i.c.v. or intra-substantia nigra cannulations, the rats were allocated to different groups (n = 6–9 rats/group) for treatment. 6-OHDA (8 μg) was dissolved in 2 μL vehicle (0.1% ascorbate in 0.9% saline) and administered unilaterally in the right substantia nigra to produce lesions in the nigrostriatal tract. While apomorphine (0.3 mg/kg, n = 7) or levodopa (5–20 mg/kg, n = 6) was given by i.p. route, cocaine- and amphetamine-regulated transcript peptide (CART, 25–100 ng/rat, n = 8) or its antibody (1:500 dilution, 5 μL/rat, n = 8) was administered by intracerebroventricular (i.c.v.) route on day 15 in 6-OHDA pre-treated (PD-like condition induced) rats. In addition to the above, CART (25–100 ng/rat, i.c.v., n = 7) or CART antibody (1:500 dilution, 5 μL/rat, i.c.v., n = 7) was injected prior to apomorphine (0.3 mg/kg, i.p.) and the effect on apomorphine-induced rotations was monitored. In a separate group of rats, CART (25–100 ng/rat, i.c.v., n = 8) or CART antibody (1:500 dilution, 5 μL/rat, i.c.v., n = 8) was injected prior to levodopa (20 mg/kg, i.p.) and the effect on levodopa induced rotation was evaluated. A separate group of rats were administered CART (100 ng/rat, i.c.v., n = 7) or CART antibody (1:500 dilution, 5 μL/rat, i.c.v., n = 7), 15 minutes prior to 6-OHDA (intra-substantia nigra). At 15 days later, these rats were challenged with apomorphine (0.3 mg/kg, i.p.) and the effect on rotational movement was investigated. Each rat was subjected to the rotation test 15 minutes after the last i.c.v. and 30 minutes after the last i.p. treatment. The brains from sham (n = 5) and 6-OHDA injected rats, treated with artificial CSF (aCSF) or CART (n = 5 each group) were isolated and processed for immuno-histochemical labeling with TH antibody.	The sham-operated animals were injected with the vehicle (0.1% ascorbate in 0.9% saline intra-substantia nigra).	Considerable TH-immunoreactivity was observed in the substantia nigra of aCSF injected control rats which was significantly decreased following 6-OHDA treatment. However, treatment with CART (100 ng/rat, i.c.v.) prior to 6-OHDA significantly restored the TH-immunoreactive content. The rats treated with 6-OHDA directly into the substantia nigra to induce PD-like condition, were treated with apomorphine hydrochloride (0.3 mg/kg, i.p.) on day 15 and the rotation pattern monitored. Apomorphine produced contralateral rotations and the counts were significantly greater than those in the naïve rats. Prior treatment with CART (50 or 100 ng/rat, i.c.v.) significantly decreased apomorphine-induced contralateral rotations compared to those of aCSF pre-treated rats, but lower dose (25 ng/rat) had no effect. CART antibody (dilutions 1:250 and 1:500) failed to influence apomorphine-induced rotation. To evaluate neuroprotective effect of CART, rats were treated with aCSF, nonimmune serum, CART (100 ng/rat, i.c.v.) or CART antibody (dilution 1:500, i.c.v.) prior to 6-OHDA. After 15 days, animals were challenged with apomorphine (0.3 mg/kg, i.p.) and the effect on the number of contralateral rotations was considered as an index of the severity of damage. Rats administered with CART showed significant decrease in apomorphine-induced rotations compared to those in aCSF-treated rats. CART antibody significantly increased the number of contralateral rotations following apomorphine. CART treatment in the PD-like condition induced rats significantly produced ipsilateral rotations in a dose-dependent manner compared to that in the aCSF-treated group. CART at 50 or 100 ng/rat doses showed significant ipsilateral rotations, but at lower dose (25 ng/rat) CART did not alter the number of rotations compared to that in the aCSF-treated rats. CART antibody at dilutions 1:250 and 1:500 did not cause any change in the rotations. Levodopa at 10, 15 or 20 mg/kg doses caused significant contralateral rotations, but at lower dose 5 mg/kg it caused no change in rotations compared to saline-treated rats. CART treatment (50 or 100 ng/rat, i.c.v.) significantly decreased the levodopa (20 mg/kg) induced contralateral rotations, but at lower dose (25 mg/kg) it failed to produce any effect. CART antibody (dilutions 1:250 and 1:500) had no effect of the levodopa-induced rotation.	While CART-immunoreactivity in the arcuate nucleus, paraventricular nucleus, striatum, substantia nigra, ventral tegmental area and locus coeruleus was reduced in the PD-induced rats, levodopa treatment restored the expression of CART-immunoreactivity in these nuclei. Endogenous CART might closely interact with the dopamine containing substantia nigra-striatal pathway. CART may be a potential therapeutic agent in the treatment of PD.
Tanshinone I Jing et al. (2016)	Adult C57Bl/6 male mice, 8 weeks, were injected i.p. with tanshinone I (10 mg/kg) for 3 days prior to lesioning. On the 3rd day of treatment, 1 hour after final dosing, anesthetized mice were injected stereotaxically with 6 μg 6-OHDA (in 2 μL saline with 0.02% ascorbic acid) into two different sites of the striatum on the right side of the brain separately. Mice were euthanized at different time points following 6-OHDA for biochemical or histological assessment. For immunostaining, mice were anesthetized with sodium pentobarbital at 3 weeks after 6-OHDA administration, transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Brains were dissected out, postfixed and cryopreserved. Frozen brains were then coronally sectioned.	Mice injected with vehicle for 3 days prior to lesioning. Mice were injected stereotaxically with saline.	6-OHDA induced significant increases in reactive oxygen species (ROS) formation in the striatum at 1 and 7 days after the toxin injection. Tanshinone I pretreatment significantly decreased 6-OHDA-induced ROS formation. Tanshinone I pretreatment also exhibited a consistent protection against the 6-OHDA-induced oxidative stress in the striatum by increasing glutathione (GSH) levels at 1 and 7 days after 6-OHDA injection. In brains collected at 21 days after 6-OHDA lesioning, immunohistochemical staining showed that tanshinone I treatment remarkably attenuated the 6-OHDA-induced loss of TH-positive neurons in the striatum and substantia nigra. These findings were supported by immunoblots of striatal extracts evaluated using an anti-TH antibody, which showed higher TH protein levels in tanshinone I-treated mice.	Tanshinone I treatment significantly attenuated 6-OHDA induced striatal oxidative stress and ameliorated dopaminergic neurotoxicity in 6-OHDA-lesioned mice.
Wang et al. (2015)	Adult C57Bl/6 male mice, 8–10 weeks, were divided into groups. For MPTP group, mice received four injections i.p. of MPTP hydrochloride (20 mg/kg) in saline in consecutive 2 hours intervals. For tanshinone I treatment, mice were administered tanshinone I (5, 10 mg/kg per day) by gavage in 0.5% (w/v) carboxymethylcellulose suspension for 7 days beginning at 24 hours before the first MPTP injection. Mice were evaluated for their motor performance by a rotarod apparatus. Each mouse was individually examined in three consecutive trials (30-minute intertrial intervals) with an initial rotation of 4 r/min slowly increasing to 40 r/min over 5 minutes. The time latency to fall was measured. For measurement of dopamine and its metabolites in striatum, mice were euthanized at 6 days after the last injection. Immunohistochemical staining of the substantia nigra and striatum was performed. For measurement of brain cytokines, animals were euthanized 72 hours after the MPTP injection.	Control group treated with vehicle.	Behavioral analysis showed a significant decrease in the retention time on the rotating rod for MPTP-treated mice compared to controls. Administration of tanshinone I at 5, 10 mg/kg in MPTP-injected mice significantly improved the retention time in a dose dependent manner. MPTP treatment reduced striatal levels of dopamine, DOPAC and HVA, while treatment with tanshinone I prevented the decrease of dopamine and its metabolites dose-dependently. Moreover, MPTP treatment led to a marked decease in the number of dopaminergic TH^+ neurons in substantia nigra. However, in treatment with tanshinone I (10 mg/kg) daily, there was less reduction in TH^+ neurons in substantia nigra. Numerous immunoreactive Iba-1^+ activated microglia with large cell bodies were observed in striatum and substantia nigra at 2 days after the last injection of MPTP. By contrast, in mice treated with MPTP + tanshinone I only a few faintly immunoreactive microglia with small cell bodies and thin processes were observed. Brain levels of TNF-α and IL-10 were significantly increased in MPTP mice compared to controls. Tanshinone I treatment significantly attenuated the increase in TNF-α but preserved the increase of IL-10 level.	Tanshinone I could provide neuroprotection by modulating the response of microglia.

Table 1 Continued

Study	No. of animals, gender, ages, treatment	Comparison	Functional outcomes	Conclusion
Dimethyl fumarate Lastes-Becker et al. (2016)	Colonies of $Nrf2^{-/-}$ mice and $Nrf2^{+/+}$ littermates were established. Each experimental group comprised 5–8 animals. An adeno-associated pseudotype 6 (rAAV6) viral vector was used to express human α-Syn under the neuron-specific human Synapsin 1 promoter. Animals received a 2 μL unilateral intracerebral injection of viral particles into the right substantia nigra stereotaxically. Dimethyl fumarate (DMF; 100 mg/kg or 300 mg/kg) was suspended in 0.8% methocel and given by oral gavage. Mice were subjected to an elevated body swing test. The animal was held in the vertical axis and a swing was recorded whenever the animal moved its head out of the vertical axis to either side. Immunohistochemistry in mice was performed on coronal brain sections. Dopamine and its metabolites were determined in hemi-brains frozen on dry ice until use. Immunofluorescence of mouse tissues was also performed.	A control rAAV6 expressing GFP was used. Mice received vehicle (VEH) by oral gavage.	At 4 hours after oral administration of 100 mg/kg or 300 mg/kg DMF, NRF2 protein levels were slightly increased in the cortex of $Nrf2^{+/+}$ mice, whereas Nrf2 mRNA levels were unchanged. An *in vivo* multifactorial model of PD was used, based on stereotaxic delivery of a rAAV6-α-syn viral vector to the ventral midbrain (VMB) of $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice. A control rAAAV6 expressing GFP did not have any significant effect on neuron viability, gliosis, or inflammation. DMF (100 mg/kg) was administered daily by oral gavage for 1 and 3 weeks, and every other day for 8 weeks after stereotaxic injection. One day before euthanizing, mice were evaluated for motor asymmetry based on the elevated body swing test. During the whole time course, vehicle-treated $Nrf2^{-/-}$ mice presented increased contralateral body torsion compared with $Nrf2^{+/+}$ mice. DMF prevented this behavioral alteration in $Nrf2^{+/+}$ mice. After 1 week from infection, the substantia nigra of all animals expressed human α-syn, and the expression in DMF-treated $Nrf2^{+/+}$ mice was the strongest. At 3 and 8 weeks, only DMF-treated $Nrf2^{+/+}$ mice still supported a significant number of neurons expressing α-syn. Parallel series of coronal sections were processed with anti-TH antibody to assess loss of dopaminergic neuron bodies in substantia nigra and fibers in striatum. Stereological counting indicated that TH^+ neurons of $Nrf2^{+/+}$ VEH, $Nrf2^{-/-}$ VEH, and $Nrf2^{-/-}$ DMF were partially lost at postinfection week 1 (~50%) and progressed through weeks 3 and 8 (~75–80%). Similarly, α-syn overexpression induced a depletion of TH^+ terminals in the striatum, in a time-dependent fashion in vehicle- and DMF-treated $Nrf2^{-/-}$ mice. In the α-syn-expressing side of the striatum (ipsilateral side), the dopamine and DOPAC levels were significantly decreased at 3 weeks in vehicle-treated $Nrf2^{+/+}$ mice but DMF attenuated these changes. A distinctive hallmark of PD is the presence of low-grade inflammation that is characterized by microgliosis and astrogliosis at the basal ganglia. Regarding astrogliosis, α-syn toxicity correlated with a significant increase in glial fibrillary acidic protein $(GFAP)^+$ astrocytes at the ipsilateral VMB side of $Nrf2^{+/+}$ mice, with a maximum at 3 weeks after infection, and an astrocyte scar remained visible at 3 and 8 weeks. DMF reduced astrogliosis in $Nrf2^{+/+}$ mice, with a slight effect in $Nrf2^{-/-}$ mice. Similarly, the astrogliosis observed in the ipsilateral striatum for vehicle-treated $Nrf2^{+/+}$ mice was abolished in the DMF-treated $Nrf2^{+/+}$, without any effect in $Nrf2^{-/-}$ mice. Regarding microglia, Iba1 immunohistochemistry showed that α-syn expression induced microgliosis at the ipsilateral side of vehicle-treated $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice after 1 week, reached a maximum after 3 weeks, and returned close to basal levels after 8 weeks. DMF greatly ameliorated the microgliosis of $Nrf2^{+/+}$ and had a minor effect on $Nrf2^{-/-}$ mice.	The dopaminergic neuron loss was greatly attenuated in $Nrf2^{+/+}$ mice treated with DMF (about 75% of neurons preserved). Targeting NRF2 with DMF may be a therapeutic strategy to reinforce endogenous brain defense mechanisms against PD-associated synucleinpathy.
Jing et al. (2015)	Adult C57Bl/6 male mice, 8 weeks, were treated with DMF (50 mg/kg) by oral gavage for 7 days prior to lesioning. On the 7^{th} day of treatment, 1 hour after final dosing, mice were injected stereotactically with 6-OHDA (6 μg in 2 μL saline with 0.02% ascorbic acid) into two different sites of the striatum on the right side of the brain separately. Apomorphine-induced rotations were monitored over 3 weeks starting 1 week after 6-OHDA lesioning. Apomorphine (0.1 mg/kg) was injected s.c., mice placed individually in plastic beakers and videotaped from above for 30 min. Mice were euthanized at different time points following 6-OHDA for biochemical or histological assessment.	Control mice received vehicle by oral gavage. Mice injected stereotactically with saline.	The levels of ROS and GSH at 7 days after 6-OHDA lesioning were measured to test if the *in vivo* activation of Nrf2/ARE signaling by DMF protects against 6-OHDA-induced oxidative stress. 6-OHDA treatment stimulated a significant increase in striatal ROS formation at 7 days after injection. DMF pretreatment significantly decreased the 6-OHDA-induced striatal ROS formation. DMF pretreatment also protected against 6-OHDA-associated oxidative stress by increasing GSH striatal levels at 7 days after 6-OHDA injection. 6-OHDA administration markedly increased markers of inflammation such as reactive astrocytes and microglia, whereas in vehicle-treated animals, only a few faintly immunoreactive astrocytes and microglia were observed in the striatum. DMF administration significantly decreased 6-OHDA-induced astrocyte and microglial activation. In brains collected at 21 days after 6-OHDA lesioning, IHC staining showed that the 6-OHDA-induced loss of TH^+ neurons in the striatum and substantia nigra was markedly attenuated by DMF treatment. Immunoblots of striatal extracts evaluated using an anti-TH antibody showed that DMF treatment resulted in significantly higher TH protein levels in 6-OHDA-lesioned mice. There was a profound reduction in striatal dopamine and its metabolites after 6-OHDA lesioning that was attenuated by DMF treatment that caused significant increase in striatal dopamine, DOPAC and HVA at 21 days after 6-OHDA lesioning. Apomorphine-induced asymmetrical rotations contralateral to the 6-OHDA injection site, monitored at 7, 14 and 21 days after 6-OHDA lesioning, were significantly reduced by DMF treatment.	DMF may be beneficial for the treatment of PD.
Ginsenoside Rg1 Zhou et al. (2015)	Adult C57Bl/6J male mice, 6–8 weeks, after adaptive feeding for 1 week, were randomly divided into groups (n = 5/group): Rg1 20 mg/kg group, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 30mg/kg group, MPTP 30 mg/kg + Rg1 5 mg/kg group, MPTP 30 mg/kg + Rg1 10 mg/kg group, MPTP 30 mg/kg + Rg1 20 mg/kg group. Groups with MPTP were injected i.p. with MPTP hydrochloride 30 mg/kg in saline for each mouse for 5 consecutive days. Groups with Rg1 were treated i.p. with Rg1 2 hours before MPTP injection and with Rg1 for another 10 days of post treatment. Group with Rg1 only was treated with Rg1 for 15 consecutive days. All of the mice were anesthetized and 0.5–0.6 mL peripheral blood was drawn through the angular vein. Serum was separated from the whole blood by centrifugation and stored at –80°C until ELISA analysis. Peripheral blood mononuclear cells (PBMCs) were isolated with lysate, and incubated with fluorescently labeled antibodies against $CD3^+$, $CD4^+$, $CD8^+$ T cells and $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells. All of the mice were anesthetized and rapidly perfused through the aorta with saline for 10 minutes, followed by 4°C precooled 4% paraformaldehyde for 10 minutes. The brains were rapidly removed and postfixed and sequentially dehydrated with 20% and 30% sucrose in 0.1 M PBS for immunofluorescence to examine the number of TH^+ neurons.	Control group injected i.p. with 0.3 mL saline for 15 consecutive days.	When MPTP was combined with Rg1 treatment, Rg1 inhibited MPTP-induced dopaminergic neuronal loss in the substantia nigra, the number of TH^+ neurons and protein expression levels of TH being significantly higher compared with the MPTP group. The number of TH^+ neurons and protein expression levels of TH in the Rg1 group was similar to that in the control group. The percentage of $CD3^+$ $CD4^+$ T cells in the peripheral blood was significantly lower and the percentage of $CD3^+$ $CD8^+$ T cells was significantly higher in MPTP-treated mice than in controls. The ratio of $CD3^+$ $CD4^+$ to $CD3^+$ $CD8^+$ T cells was significantly lower in MPTP-treated mice than in controls. The percentage of $CD3^+$ $CD4^+$ T cells was significantly higher and the percentage of $CD3^+$ $CD8^+$ T cells was significantly lower in MPTP + Rg1 groups than in the MPTP group. The ratio of $CD3^+$ $CD4^+$ to $CD3^+$ $CD8^+$ T cells was significantly higher in MPTP + Rg1 groups than in the MPTP group. The percentage of $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells was significantly lower in MPTP-treated mice than in controls. By contrast, the percentage of $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells was significantly higher in MPTP + Rg1 groups than in the MPTP group. In the serum, the concentrations of tumor necrosis factor-α (TNF-α), interferon (IFN)-γ, interleukin (IL)-1β and IL-6 were all significantly increased in the MPTP-treated group compared with the control group. However, the concentrations of TNF-α, IFN-γ, IL-1β and IL-6 in the MPTP + Rg1 groups were all significantly decreased compared with the MPTP group. The concentrations of TNF-α, IFN-γ, IL-1β and IL-6 in the Rg1 group did not differ significantly from the control group. Microglia in the control and Rg1 group were non-activated. In the MPTP-treated group, microglial cells were activated and dopaminergic neurons were significantly reduced. In MPTP + Rg1 groups, the microglial cells were not as activated and tended to return to a resting stage, and dopaminergic neurons were not as reduced compared with MPTP group. In addition to microglial activation, the infiltration of T cells into the brain is considered to be correlated with neurodegenerative processes. $CD3^+$ is a known marker for T lymphocytes. The number of $CD3^+$ cells in the substantia nigra was significantly increased in the MPTP-treated mice compared with control group. However, the number of infiltrated $CD3^+$ cells in the substantia nigra region was significantly decreased in MPTP + Rg1 groups compared to MPTP group.	Rg1 may be a promising drug for the treatment of PD *via* the regulation of peripheral and central inflammation.

Table 1 Continued

Study	No. of animals, gender, ages, treatment	Comparison	Functional outcomes	Conclusion
Tanshinone IIA Zhang et al. (2015)	Adult C57Bl/6 male mice, 10 weeks, were randomly assigned to groups (n = 10/group): 6-OHDA model group, and 6-OHDA + 10 mg/kg tanshinone IIA group. Mice were anesthetized and 6 μg 6-OHDA (in 2 μL saline containing 0.02% ascorbic acid) was injected stereotaxically at 0.2 μL/min into two different sites of the striatum separately. Mice were given 10 mg/kg tanshinone IIA (in PBS including 1% DMSO) i.p. in 6-OHDA + Tan IIA group immediately after 6-OHDA treatment. Mice were euthanized at different time points between 1 and 21 days following 6-OHDA injection and tissues collected for biochemical and histological assessment. Apomorphine-induced rotation test was performed for 30 minutes before 6-OHDA lesion, 14 days and 21 days after the lesion. Mice were injected s.c. with apomorphine (0.1 mg/kg in saline), then placed individually in plastic beakers and videotaped from above for 30 minutes. Analysis of completed (360°) rotation was made. Animals were anesthetized with sodium pentobarbital at 3 weeks after 6-OHDA administration, then perfused with saline, followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Brains were dissected out, fixed and cryopreserved. Frozen brains were coronally sectioned and dopaminergic neurons identified with antibody against TH. The striatum of mice was removed at 3 weeks following 6-OHDA administration and dopamine, DOPAC and HVA measured by HPLC-MS/MS.	Control group treated with vehicle only (PBS including 1% DMSO).	Apomorphine-induced contralateral rotation was significantly reduced with Tan IIA treatment compared with 6-OHDA treatment alone. In brains collected at 21 days after 6-OHDA injection, Tan IIA significantly alleviated 6-OHDA-induced loss of TH^+ neurons in the striatum and substantia nigra. The TH protein level was higher in the Tan IIA-treatment group than that in the group treated with 6-OHDA alone. At 21 days after 6-OHDA administration, striatal dopamine and its metabolites DOPAC and HVA were analysed. Tan IIA treatment attenuated the reduction of dopamine and its metabolites by 6-OHDA lesion.	Tan IIA may be beneficial for the treatment of PD. Using SH-SY5Y cells treated with 6-OHDA, Tan IIA abolished the enhanced expression of miR-153. It had been predicted that miR-153 targets 3′-UTR of Nrf2 and miR-153 reduced the Nrf2 level in the nucleus and cytoplasm of SH-SY5Y cells.
Ren et al. (2015)	Adult C57Bl/6 male mice, 3–4 months, were randomly divided into groups. Mice in group B (n = 6) were injected i.p. with MPTP hydrochloride (20 mg/kg) in PBS 4 times at 2 hours intervals, and then with PBS including 1% DMSO as in group A mice. Mice in group C (n = 6) were injected with MPTP hydrochloride as in group B mice and also received Tan IIA (25 mg/kg) in PBS including 1% DMSO at 12 hours after the last injection of MPTP, and once a day for the following 6 days. Mice in group D (n = 6) were injected i.p. with PBS as in group A mice and also received Tan IIA as in group C mice. Mice were euthanized at the chosen time points and the ventral midbrains dissected out. Rotarod test was used to measure the dyskinesis of mice. Before MPTP administration, mice were trained on the rotarod at 20 r/min for 5 minutes, 4 times per day for 7 consecutive days. Mice were chosen that did not fall off during the training for the next step. At 7 days after the last MPTP administration, mice receiving several different treatments were tested at 20 r/min for 20 minutes. The time that mice stayed on the rod before falling was recorded. After the rotarod test, mice were euthanized and the content of dopamine in the striatum was measured by HPLC. Mice of each group were euthanized at 3 days and 7 days after the final injection of MPTP.	Mice in group A (n = 6) were injected i.p. with PBS 4 times at 2 hours intervals, and then injected i.p. with PBS including 1% DMSO at 12 hours after the last injection of PBS, and once a day for the following 6 days. They served as controls.	MPTP is completely converted to MPP+ and largely cleared within 12 hours after injection. MPTP reduced the number of TH immunopositive cell bodies in the substantia nigra and fibers in the striatum compared to those treated with vehicle. MPTP treatment markedly decreased the expression level of TH in the substantia nigra. Tan IIA alone had no effect on the number of TH immunopositive cell bodies in the substantia nigra and fibers in the striatum, or the expression level of TH in the substantia nigra. MPTP treatment reduced the number of Nissl-stained neurons in the substantia nigra compared to the vehicle control group. Tan IIA treatment largely reduced the loss of Nissl-stained neurons in the substantia nigra. After MPTP treatment, the time that mice stayed on the rod was much shorter than that of the vehicle-treated group, and the content of dopamine in the striatum was much less than that of the vehicle-treated group. Tan IIA treatment recovered the behavioral dysfunction of mice as well as the content of dopamine in the striatum. Tan IIA alone had no effect on motor performance or striatal dopamine content. Anti-CD11b-antibody was used to detect microglia. After MPTP treatment, the CD11b-immunopositive cells in the substantia nigra were more numerous and larger than those of the vehicle-treated group. Western blot analysis showed a higher expression level of CD11b in the substantia nigra than that of the vehicle-treated group. Tan IIA treatment significantly reduced the number and size of CD11b-immunopositive cells in the substantia nigra, and downregulated the expression of CD11b compared to the MPTP-treated group. Tan IIA alone had no effect on the number and size of CD11b-immunopositive cells in the substantia nigra, or the expression level of CD11b in the substantia nigra. Anti-p47-phox and anti-inducible nitric oxide synthase (iNOS) antibodies were used to detect NADPH oxidase and iNOS which are the main sources of ROS and RNS. After MPTP treatment, there were significantly more p47-phox-immunopositive cells and iNOS-immunopositive cells in the substantia nigra than for the vehicle-treated group. Western blot analysis showed a higher expression level of p47-phox and iNOS in the substantia nigra than for the vehicle-treated group. Tan IIA treatment significantly reduced the number of p47-phox-immunopositive cells and iNOS- immunopositive cells in the substantia nigra, and there was a lower expression level of p47-phox and iNOS in the substantia nigra, compared to the MPTP-treated group. Tan IIA alone had no effect on the number of p47-phox-immunopositive cells and iNOS- immunopositive cells in the substantia nigra, or the expression level of p47-phox and iNOS in the substantia nigra. Double immunofluorescence staining showed that p47-phox-immunopositive cells and iNOS-immunopositive cells had co-localization with TH-immunopositive cells, showing dopaminergic neurons can express NADPH oxidase and iNOS.	Tan IIA treatment alleviated MPTP-induced loss of TH immunopositive cell bodies in the substantia nigra and fibers in the striatum and alleviated the decrease of the expression level of TH in the substantia nigra. Tan IIA has anti-inflammatory and anti-oxidative properties and may have therapeutic benefit in the treatment of PD.
FK506 Van der Perren et al. (2015)	Adult female Wistar rats (200–250 g) were anesthetized and placed in a stereotactic apparatus. Animals were injected in substantia nigra with 3 μL rAAV2/7A53T α-syn at a rate of 0.25 μL/min. FK506-treated animals received daily injections i.v. (tail vein) of FK506 (1 mg/kg) in saline starting 1 day after rAAV2/7 α-syn injection. Behavioral testing using the cylinder test was performed to quantify forelimb use. Contacts made by each forepaw with the wall of a 20-cm-wide clear glass cylinder were scored. The number of impaired forelimb contacts was expressed as a percentage of total forelimb contacts. To analyze the bioavailability and presence of FK506 in the brain, a separate group of Wistar rats (n = 3–5) was injected i.v. for 3 days (to obtain steady state conditions) with FK506 (0.5, 1.0, 1.5, 2.0, 3.0 mg/kg). At 1 hour after the last i.v. injection, blood (tail vein) and CSF (cistern magna) samples were collected to determine peak values. The animals were injected for 5 more days and 24 hours after the last i.v. injection blood and CSF samples were taken. For immunohistology, rats were euthanized, perfused intracardially with 4% paraformaldehyde in PBS. After postfixation, coronal brain sections were made and immunostained.	Animals injected in substantia nigra with rAAV2/7 eGFP as control. Placebo-treated animals were injected daily i.v. with saline.	Upon administering FK506 (1 mg/kg) daily i.v. for 4 weeks in A53T α-syn rAAV2/7 rats, elevated blood levels of FK506 were found at 4 days, 15 days and 29 days compared with placebo-treated animals. Quantification of the number of TH^+ nigral neurons at 20 days post injection showed a > 2-fold higher survival of dopaminergic neurons in rats treated with FK506 compared with placebo controls. Rats treated with FK506 (1.0 mg/kg) showed a nonsignificant improvement in forelimb use. Quantification of the total number of α-syn positive cells in the substantia nigra revealed a significant increase in the animals treated with FK506 (1 mg/kg) at 29 days. Upon dividing the α-syn positive cells into those with and without aggregates, no difference was observed in the ratio of aggregate-positive to aggregate-negative cells. FK506 did not appear to have an effect *via* α-syn aggregation, The microglial cells in the injected substantia nigra were visualized by Mac1 (CD11b) staining which detects all microglia and macrophages present (proinflammatory, anti-inflammatory, infiltrated macrophages, and proliferated microglia). The number of Mac1-postive microglia and macrophages increased in both groups over time, but FK506-treated rats showed significantly less Mac1-positive cells compared to placebo controls at 15 and 29 days postinjection. Only a few microglia were detected in control animals injected with rAAV2/7 enhanced green fluorescent protein (eGFP) and in the contralateral substantia nigra of all rats. The expression levels of CD68 as a marker of phagocytic microglia and macrophages and MHC II for antigen presentation were analysed. An increase in CD68- and major histocompatibility complex (MHC) II-positive microglia was observed over time in both placebo and FK506-treated rats but with no significant differences between the groups. Control animals injected with rAAV2/7 eGFP showed a similar infiltration of CD68- and MHC II-positive microglia compared to rAAV2/7A53T α-syn-injected rats at day 4, but no increase was observed over time. T-cell infiltration in the substantia nigra was investigated. The number of CD4-positive cells significantly increased in both groups over time, but FK506-treated rats showed significantly less CD4-posutive cells compared to placebo controls at 29 days postinjection. There was a delayed infiltration of CD8-positive T cells in FK506-treated rats, reaching a maximum at 29 days postinjection, compared with placebo-treated animals which showed peak infiltration of CD8-positive cells at 15 days postinjection.	The anti-inflammatory properties of FK506 decrease neuro-degeneration in the α-syn-based PD model.

Table 1 Continued

Study	No. of animals, gender, ages, treatment	Comparison	Functional outcomes	Conclusion
Lenalidomide Valera et al. (2015)	Mice, 9 months, expressing human α-syn under the control of the murine Thy1 promoter (mThy1-α-syn Tg) were used (n = 38). The mThy1-α-syn Tg mice and their non-Tg littermates were treated for 5 weeks with lenalidomide or thalidomide (100 mg/kg) administered by gavage 5 times each week. Behavioral assessment of the mice was performed using the open field and the round beam tests. Total activity test was conducted for four trials each day for a period of 4 days. Total activity was calculated as total beam breaks in 10 minutes and thigmotaxis was calculated as the percentage of time spent in the periphery. Three consecutive trials of 1 minute each were run in 1 day. The total distance traveled forward and the number of foot slippages was recorded. Mice were euthanized under anesthesia and brains removed. The right hemibrain was fixed in 4% paraformaldehyde in PBS (pH 7.4) and sectioned. The left hemibrain was stored at –80°C for biochemical analysis.	mThy1-α-syn Tg mice and their non-Tg littermates were treated with vehicle (0.5% methylcellulose) by gavage 5 times each week.	The mThy1-α-syn Tg mice showed an increase in activity and motor errors. Lenalidomide, but not thalidomide, reduced total activity, increased speed, and reduced the number of errors in the round beam test. In mThy1-α-syn Tg mice there was a loss of dopaminergic TH immunoreactive fibers in the striatum when compared to non-Tg mice. Both lenalidomide and thalidomide restored TH immunoreactivity in the striatum of mThy1-α-syn Tg mice to levels similar to non-Tg mice. The mThy1-α-syn Tg mice showed α-syn accumulation in neuronal bodies and neuropil of striatum, hippocampus and other brain regions. Lenalidomide and thalidomide had no effect on α-syn accumulation in the mThy1-α-syn Tg mice, indicating that the behavioral improvement observed with lenalidomide was not a consequence of reduced α-syn accumulation. Glial inflammatory responses were analyzed by immunostaining against the glial markers GFAP (astrocytes) and Iba1 (microglia). Although there was an increase in microglial reactivity in the striatum of mThy1-α-syn Tg mice, the difference with non-Tg animals was not statistically significant at this age. Treatment with lenalidomide or thalidomide did not affect GFAP staining. However, treatment with lenalidomide, but not thalidomide, reduced Iba1 immunoreactivity both in striatum and hippocampus. This amelioration of microgliosis was not observed in other brain areas. Immunoblot analysis of α-syn levels in cytosolic and particulate (membrane) fractions revealed that treatment with lenalidomide or thalidomide did not modify α-Syn levels. Measurement of nuclear factor-κB (NF-κB) activation showed that lenalidomide significantly inhibited NF-κB signaling. TNF-α protein lrevels were not significantly elevated in mThy1-α-syn Tg mice compared to non-Tg controls, but both drugs were able to reduce basal TNF-α levels. Chemokine CX3CL1 (fractalkine) levels were reduced in mThy1-α-syn Tg mice and increased by lenalidomide treatment. Fractalkine expression is regulated by NF-κB and is invoved in microglial activity with neuroprotective properties. Levels of the ant-inflammatory cytokine IL-13 were elevated after lenalidomide treatment of mThy1-α-syn Tg mice.	The mThy1-α-syn Tg mice showed a significant increase in TNF-α mRNA levels, and both lenalidomide and thalidomide reduced TNF-α expresion. Lenalidomide and thalidomide also reduced IL-6, IL-1β, and IFN-γ expression. Interestingly, lenalidomide increased the expression of the anti-inflammatory cytokine IL-10, Lenalidomide may have therapeutic potential for reducing neuro-inflammation in PD.
Thalidomide Palencia et al. (2015)	Adult C57Bl/6NHsd male mice, 25–30 g, were divided into groups (n = 10/group). Experiment A: Preventive Group B received 3 oral doses of thalidomide (200 mg/kg) at 2 hours intervals. Group C received an injection s.c. of MPTP (40 mg/kg). Group D received thalidomide as in group B and MPTP was administered immediately after the last dose of thalidomide as in group C. Animals were euthanized 72 hours after the last treatment, and brain striatum and substantia nigra were dissected. Experiment B: Therapeutic Group B received oral doses of thalidomide (200 mg/kg) once daily for 10 days. Group C received an injection s.c. of MPTP (40 mg/kg). Group D received MPTP as in group C and on the next day thalidomide was administered as in group B. Animals were euthanized 72 hours after the last treatment, and brain striatum and substantia nigra were dissected. Contents of dopamine and HVA in striatum and substantia nigra were measured by HPLC. Brain striatal monoamine oxidase-B (MAO-B) activity was measured. The amount of products generated by lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) assay.	Group A was controls in both experiments.	Administration of MPTP significantly decreased content of dopamine in striatum compared to controls in both experiments A and B. Exposure to MPTP significantly increased contents of MAO-B in striatum and substantia nigra compared to controls in both experiments A and B. The striatal content of dopamine after thalidomide alone was similar to that of controls in both experiments A and B. The content of MAO-B in striatum and substantia nigra after thalidomide alone was similar to that of controls in both experiments A and B. Brain contents of HVA were similar in all groups of mice, controls and mice after administration of MPTP or thalidomide, or both. The administration of thalidomide before MPTP exposure (experiment A) or after MPTP exposure (experiment B) significantly increased dopamine content in the striatum compared to MPTP alone. The administration of thalidomide before or after MPTP exposure significantly decreased MAO-B content in striatum and substantia nigra compared to MPTP alone. MPTP treatment induced increased levels of lipid peroxidation (TBARS) products in striatum and substantia nigra, and which were significantly lowered by thalidomide in both experiments A and B. Mice treated with thalidomide alone showed values similar to those of controls.	Thalidomide could be a potential adjuvant therapy for PD.
Cyclosporin Tamburino et al. (2015)	Adult female Sprague-Dawley rats, 3 months (225–250 g) were injected with either 3 µL of AAV-α-syn vector into the substantia nigra or 3 µL of 6-OHDA (3.5 µg/µL in 0.9% saline containing 0.2 mg/mL L-ascorbic acid) into the medial forebrain bundle (MFB) at a rate of 0.2 µL/min. Injections were performed unilaterally on the right side using stereotactic apparatus. After 8 weeks, when marked behavioral impairments were observed in both models using the cylinder and amphetamine-induced rotation tests, rats were assigned to receive an intra-striatal graft of mesencephalic progenitors and motor performance was monitored up to 12 weeks post-transplantation. Ventral mesencephalon (VM) from E14 rat embryos were dissected. Under anesthesia, about 1.5 × 10^5 cells were injected stereotactically as two 0.75 µL deposits in the lesioned striatum. A second set of experiments was performed in which rats received daily injections i.p. of cyclosporin A (CsA; 10 mg/kg) at 8 weeks after intra-nigral injection of AAV-α-syn and 3 days before receiving a neural graft. Animals were tested for motor behavior at 6 and 12 weeks post-transplantation, while maintained under CsA treatment. Thy1-α-syn Tg mice, 10 months, received daily injections i.p. of CsA (20 mg/kg) for 6 weeks and examined for behavioral deficits. Mice receiving increasing doses of MPTP over 4 weeks were treated with CsA for 4 weeks starting 3 days after the last MPTP injection, and examined for behavioral deficits.	Sham control rats were injected with vehicle only. Also subjected to sham surgery.	Neural grafting provided an efficient and full recovery of motor function in both animal models and in both tests 6-OHDA lesioned rats showed no sign of a motor deficit on the cylinder test at 12 weeks after transplantation and elicited an overcompensation on the rotation test. Likewise, in the AAV-α-syn model, transplantation of fetal mesencephalic cells fully restored behavioral deficit in the cylinder test and in the rotation test, albeit with no overcompensation. Postmortem analysis revealed that survival of the grafts was reduced in the AAV-α-syn model as compared to the 6-OHDA group. The transplants were significantly smaller in volume in the AAV model as compared to the neurotoxin model. Stereological quantification showed that grafts in the AAV-α-syn group contained fewer TH^+ neurons compared to the 6-OHDA group. Staining of $Iba\text{-}1^+$ microglia revealed sustained inflammation in the striatum of rats treated with the AAV-α-syn vector, while rats lesioned with 6-OHDA did not show such a response at 8 weeks postlesion. The pro-inflammatory cytokines IL-1β, IFN-γ, and TNF-α were significantly elevated in the striatum of rats overexpressing human α-syn. Immunosuppression with CsA had no effect on the speed of functional recovery and did not affect the magnitude of behavioral restoration although overcompensation now occurred in the rotational test. Thy1-α-Syn transgenic mice treated with CsA showed enhanced motor and cognitive function, and CsA lowered the striatal level of human α-syn and partially restored the level of TH protein. Glial response was reduced in the hippocampus and cortical levels of synaptic markers MAP-2 and synaptophysin were partially restored by CsA treatment. CsA treatment enhanced motor function of MPTP-treated mice. However, counting of nigral dopamine neurons, measurement of striatal TH^+ fibers, and analysis of striatal level of TH protein did not support a direct involvement of the dopaminergic system in this therapeutic effect. The increased expression of the axonal sprouting marker SCG10 suggested that CsA may have had an effect on axonal regeneration. CsA treatment lowered the expression level of NFATc3, a direct target of calcineurin regulating the level of inflammatory factors, in the midbrain of MPTP-treated animals. MPTP-induced mitochondrial stress in the midbrain was also alleviated by CsA as shown by the reduced expression of cytochrome c, which is a marker of ROS production. CsA treatment reduced the expression level of GFAP and GLT-1 (glutamate transporter-1 expressed by astrocytes) in the striatum of MPTP-treated animals. GFAP is a marker of astrogliosis. MPTP lesions can provoke an elevation of striatal GLT-1, suggesting Increased levels of glutamate, which can modulate the activity of striatal medium spiny neurons and possibly also induce excitotoxicity.	Consistent with these observations, morphological analysis showed that CsA treatment resulted in larger-sized grafts with an increased number of graft-derived dopamine neurons. CsA treatment also induced partial alleviation of the motor deficit in sham-operated animals compared to vehicle-treated animals. CsA treatment may act as a disease modifying therapy in PD.

Table 1 Continued

Study	No. of animals, gender, ages, treatment	Comparison	Functional outcomes	Conclusion
SA00025 Smith et al. (2015)	Adult female Sprague-Dawley rats (~250 g). In the first experiment, naïve rats were gavaged daily for 7 consecutive days with the Nurr1 agonist (SA00025, 30 mg/kg) in 0.6% methylcellulose and 0.5% Tween-80 in distilled water. Rats were euthanized at 1, 4, 12 or 24 hours after the last gavage (n = 3–4/group). In the second experiment, treatment with SA00025 was started 1 day after intra-nigral polyinosinic-polycytidylic acid (polyI:C) injection (day 1) and was administered daily for the duration of the experiment (32 days), including during the day rats received intra-striatal 6-OHDA (day 12). Rats were euthanized at 24 hours after the final administration of SA00025 (day 33) (n = 8/group). For stereotactic delivery of polyI:C and 6-OHDA, rats were anesthetized and 20 µg polyI:C in 2 µL was delivered to the substantia nigra at a rate of 0.5µl/min. Afterwards, rats were rested for 11 days before being re-anesthetized for intra-striatal delivery of 5µg 6-OHDA in 3.5 µL at a rate of 0.5 µL/min.	Control rats were gavaged daily for 7 days with 0.6% methylcellulose and 0.5% Tween-80 in distilled water.	Following 7 days of daily gavage, pharmacokinetic analysis and postmortem showed that SA00025 entered the brain and confirmed elevated brain exposure at 1, 4, and 24 hours after the last administration. SA00025 treatment significantly modified the expression of Nurr1 and dopaminergic target genes from 1–48 hours after daily gavage for 7 days. A normalization of Nurr1, TH and VMAT mRNA expression was observed at 4 hours post gavage and an increase in c-Ret at 24 hours. Concomitantly, protein levels of TH were significantly elevated at 4 hours following SA00025 treatment compared to vehicle treatment. In the 6-OHDA lesion model, SA00025 was neuroprotective on TH^+ and $NeuN^+$ neurons within the substantia nigra and also preserved TH^+ fibers in the striatum. The intensity of TH immunostaining within individual dopaminergic cell bodies of SA00025 treated rats was significantly higher compared to vehicle treated rats on the ipsilateral side and the contralateral side. Dopamine neuron fibers in the rostral striatum were also significantly spared by SA00025 treatment compared to vehicle treatment. At the end of SA00025 administration to rats that received an intra-nigral polyI:C injection followed by an intra-striatal 6-OHDA injection, there was a significant morphological change of Iba-1^+ microglia in the substantia nigra. SA00025 treatment caused significantly more microglia residing in a resting state and a significant decrease in reactive microglia compared to vehicle treatment. SA00025 treatment decreased immunofluorescence intensity of both Iba-1^+ microglia and $GFAP^+$ astrocytes in the substantia nigra compared to vehicle treatment. A significant reduction in protein level of IL-6 was observed with SA00025 treatment, but IL-10, IL-1α, monocyte chemotactic protein 1 (MCP1), macrophage inflammatory protein (MIP)1a, MIP2, MIP3a, regulated on activation, normal T cell expressed and secreted factor (RANTES), Fractalkine, TNF-α, IL-1β, IL-2, macrophage-derived chemokine (MDC), transforming growth factor-β1 (TGF-β1) were unchanged.	Rats had a significant sparing of dopaminergic neurons in the substantia nigra after 32 days of SA00025 treatment compared to vehicle treatment. Nurr1 agonist SA00025 causes neuroprotection and anti-inflammatory effects in an inflammation exacerbated 6-OHDA lesion model of PD.
IFN-β Ejerskov et al. (2015)	*Ifnb*$^{-/-}$ mice and *Ifnb*$^{+/+}$ littermates. Behavioral measurements included evaluating motor-coordination and -learning with an accelerating RotaRod automatically recording time before falling. Neuromuscular strength was recorded by forelimb hanging time on a bar. Heat and cold tail-pain sensitivity was measured by tail-flick latency time after exposure. Spatial learning and reference memory were assessed by Morris water maze. For IHC and immunofluorescence, either mice were perfused and brains fixed in 4% paraformaldehyde and sections cut or brains were dissected and snap-frozen before sectioning. Primary cerebellar granular neurons (CGNs) were from 6- or 7-day-old cerebella and cortical neuron (CN) cultures from the cortex of 1-day-old mice. Lentivirus was used to overexpress IFN-β in a familial PD model induced with human α-syn (hSCNA) in substantia nigra of rats.		*Ifnb*$^{-/-}$ mice were significantly impaired in motor coordination and learning from 3 months compared to age- and weight-matched wild-type (WT) *Ifnb*$^{+/+}$ littermates and in latency-to-fall time in a wire-suspension test. Latency to tail flick was considerably shorter in *Ifnb*$^{-/-}$ mice than *Ifnb*$^{+/+}$ mice, indicating hyperalgesia and defective nociception towards temperature-induced pain. Forced swimming tests found no differences between *Ifnb*$^{-/-}$ and *Ifnb*$^{+/+}$ mice in swimming pattern, climbing effort, or immobility, so *Ifnb*$^{-/-}$ mice were not defective in water in contrast to land. In water maze tests, *Ifnb*$^{-/-}$ mice had significant spatial- and memory-learning deficits that increased with age. Apoptotic cells were detected in 1.5-month-old *Ifnb*$^{-/-}$ granular layers of olfactory bulbs, the granular dentate gyrus of hippocampus and the subventricular zone, and the striatum caudate putamen including the ependymal cell layer in 12 month-old-*Ifnb*$^{-/-}$ mice, but not detected in *Ifnb*$^{+/+}$ sagittal brain sections at similar ages. Neurons were significantly reduced in the hippocampal CA1 region in 3- to 6-month-old *Ifnb*$^{-/-}$ mice and decreased in Purkinje cells of cerebellum. TH^+ fiber density and TH^+ (dopamine producing) neurons were significantly reduced in the striatum and substantia nigra in *Ifnb*$^{-/-}$ mice *versus* WT. $NeuN^+$ and $NeuN^+TH^+$ cells were reduced in the ventral midbrain, which was correlated with reduced TH protein in basal ganglia in *Ifnb*$^{-/-}$ mice while total cells were unaffected. Histological examination showed that *Ifnb*$^{-/-}$ neuron degeneration was associated with age-dependent α-synucleinpathy. Staining for α-syn was normal in 1.5-month-old *Ifnb*$^{-/-}$ brains; by 3 months, α-syn was found in Lewy body-like structures in substantia nigra; however, α-syn intensity was reduced in 12-month-old *Ifnb*$^{-/-}$ mice, likely reflecting degeneration of TH^+ neurons. α-Syn and large aggregates of phosphorylated α-syn were found in TH^+ neuons of substantia nigra. At 3 months, α-syn aggregates were widespread in the striatum, frontal cortex, hipocampus and cerebellum. α-Syn aggregates and neurites were found sporadically in thalamus, brainstem, and subthalamic regions of 3-month-old *Ifnb*$^{-/-}$ mice. Neurons with α-syn^+ Lewy body-like structures increased with age (6- and 12-month-old) in *Ifnb*$^{-/-}$ mouse thalamus. Gene set enrichment analysis (GSEA) was used to identify cellular pathways involved in *Ifnb*$^{-/-}$ neuron pathology. In the top 20 deregulated pathways, 3 were associated with autophagy, which were restored with recombinant IFN-β (rIFN-β). Microtubule-associated protein 1 light chain 3B-II (LC3B-II) increased in basal ganglia of 1.5-month-old old *Ifnb*$^{-/-}$ mice, correlating with increased p62, NBR1 and Rab7, supporting defects in autophagy before α-syn, ubiquitin, pTau, and Lewy body aggregation. LC3B-II and p62 were higher in untreated *Ifnb*$^{-/-}$ cortical neurons, and while overnight IFN-β treatment promoted LC3B-II conversion and reduced p62 in *Ifnb*$^{+/+}$ cortical neurons, indicating increased autophagy flux, rIFN-β reduced p62 but only slightly increased LC3B-II in *Ifnb*$^{-/-}$ cortical neurons. By promoting autophagy, rIFN-β reduced α-syn in both *Ifnb*$^{+/+}$ and *Ifnb*$^{-/-}$ cortical neurons. Injection of hSCNA and control lentiviruses blocked autophagy in rat basal ganglia 10 days after substantia nigra injection. *Ifnb* overexpression prevented hSCNA and pSer129-α-syn accumulation and restored TH loss. The rats showed improved left paw use compared to right paw use; contralateral to injection side of hSCNA/*Ifnb* and hSCNA/control viruses, respectively, 21 days post injection which was associated with preservation of TH^+ fibers in substantia nigra. *Ifnb* gene therapy significantly protected TH^+ dopaminergic neurons from hSCNA-induced substantia nigra damage.	*Ifnb*$^{-/-}$ mice developed spontaneous pathologies mimicking major aspects of human neurodegeneration such as PD. Deleting neural autophagy-regulating genes leads to neurodegeneration. A central role is indicated for IFN-β in neuronal homeostasis and a regulator of autophagy-mediated protein degeneration, and accentuates *Ifnb*$^{-/-}$ mice as a model for PD with α-synucleinopathy such as PD. IFN-β prevented pathology in a rat familial model of PD by inducing autophagy and α-syn clearance.

Table 1 Continued

Study	No. of animals, gender, ages, treatment	Comparison	Functional outcomes	Conclusion
CNI-1493 Noelker et al. (2013)	Adult C57Bl/6 male mice, 9 weeks, were divided into groups (n = 8–10/group). Animals investigated at 2 days and 7 days after MPTP intoxication: CNI-1493/saline treated mice, saline/MPTP-treated mice, CNI-1493/MPTP-treated animals. Mice were injected i.p. 4 times at 2 hours intervals with either MPTP hydrochloride (20 mg/kg) or a corresponding volume of saline with and without CNI-1493 treatment. CNI administration (8 mg/kg, i.p.) started 1 day before MPTP intoxication and was repeated daily until being euthanized. At day 2 (for Iba-1 staining and neurochemical analysis) or day 7 (for TH staining and neurochemical analysis) after MPTP intoxication, animals were euthanized and brains processed for further analysis.	Controls were saline/saline-treated mice.	MPTP induced a significant loss of dopaminergic neurons in the substantia nigra of saline-treated mice. Additional administration of CNI-1493 attenuated dopaminergic cell loss in the substantia nigra demonstrating that CNI-1493-treated mice were partially protected against MPTP toxicity. MPTP induced a 90% reduction of striatal dopamine content of saline-treated mice compared to controls, whereas an increase in striatal dopamine was observed in CNI-1493-treated mice compared to MPTP-treated mice at 2 days and 7 days after MPTP intoxication. The increase at 7 days was significant. CNI-treated mice showed a significantly lower number of activated Iba-1^{+} microglial cells in the substantia nigra 2 days after MPTP intoxication compared to MPTP-treated mice.	The neuroprotective effect of CNI-1493 in mediating microglial cell activation and dopamine degeneration in MPTP mouse model of PD suggests it might be a valuable candidate in the future treatment of PD.
Pycnogenol Khan et al. (2013)	C57Bl/6 mice were randomly divided into groups (n = 6/group). Group 2 was MPTP-injected, 4 injections i.p. of MPTP (18 mg/kg in saline at 2 hours intervals) for 1 day only. Group 3 received pretreatment with pycnogenol (PYC, 20 mg/kg) 30 minutes before each MPTP injection and one additional injection on the next day and then euthanized. Group 5 was only MPTP injected. Group 6 received PYC 30 minutes before each MPTP injection and continued once daily for 7 days. Behavioral tests were performed using rotarod, grip test, footprint analysis, drag test. Animals were euthanized and brains removed to dissect out the striatum and then homogenized in PBS with protease inhibitor. Tissue homogenates were centrifuged to obtain post mitochondrial supernatant for biochemical studies.	Group 1 was saline-treated and served as control for day 1. Group 4 was saline-treated control for 7 days and served as control for day 7. Total number of saline injections in control mice was 4 for both day 1 and day 7 controls.	In the rotarod test, a significant decrease in motor coordination skill in MPTP injected groups compared to control groups was observed after day 1 and day 7. PYC (20 mg/kg) was effective in partial recovery of motor coordination in 7 day PYC+MPTP injected group. A significant decrease in motor strength as measured by grip strength test was found in MPTP injected groups compared to controls. PYC treatment (7 days) significantly protected mice from MPTP-induced decline in motor activity. The forepaw step distance was significantly decreased in MPTP injected groups compared to controls. The forepaw step distance was improved by 7 days PYC treatment compared to MPTP injected group. Number of steps in the drag test was significantly decreased in MPTP groups compared to controls. PYC treatment followed by MPTP for 7 days significantly improved the number of footsteps compared to MPTP group. PYC treatment for 1 day did not significantly improve motor coordination skill, grip strength, forepaw step distance and step changes as measured by the drag test compared to MPTP injected group. There was a significant increase in TBARS contents on day 1 and day 7 following MPTP administration compared to control. PYC treatment followed by MPTP administration significantly prevented the increase in TBARS content compared to MPTP injected group. GSH content was significantly reduced in MPTP groups at day 1 and day 7 compared to control group. The GSH content was significantly protected in the PYC treated group compared to MPTP injected groups. The activities of antioxidant enzymes GPx, GR, and SOD in MPTP groups were significantly decreased compared to control groups. PYC treatment for 7 days followed by MPTP injection significantly preserved the activities of these enzymes compared to MPTP injected groups. PYC treatment for 1 day only was unable to prevent the loss of antioxidant enzyme activities. The MPTP-induced dopamine depletion was attenuated in mice treated with PYC for 7 days compared to MPTP-injected mice. Increased expression of Iba-1 indicating increased numbers and activation of microglia were observed as an index of inflammatory response in MPTP injected mice. PYC treatment for 7 days followed by MPTP administration significantly prevented the MPTP-induced increase in the number of microglia and their activation. Higher expressions of GFAP indicating increased numbers of astrocytes with astrocyte hypertrophy inflammatory response characteristics were seen in MPTP injected groups after day 1 and day 7 compared to control groups. PYC treatment attenuated higher expressions of GFAP in PYC + MPTP group compared to MPTP groups. MPTP injected mice demonstrated significantly higher levels of NF-κB p65 protein in nuclear extracts after 1 day and 7 days compared with control mice. PYC treatment prior to MPTP significantly attenuated the activation of NF-κB. Significantly higher striata protein levels of cyclooxygenase-2 (COX-2) and iNOS were found in MPTP-injected mice compared to controls. The MPTP-induced expression of COX-2 and iNOS protein was almost completely blocked by PYC treatment for 7 days. Secretion of inflammatory cytokines IL-1β and TNF-α in striatum was significantly increased at day 1 and day 7 post MPTP injection compared with controls, The MPTP-induced increase in the secretion of IL-1β and TNF-α was significntly blocked in PYC-treated mice at day 7 post-injection.	PYC-treated mice show significantly reduced nigrostriatal dopaminergic neuron loss following MPTP injection. PYC-induced adaptation to oxidative stress and inflammation could suggest a novel approach for clinical intervention in neuro-degenerative diseases including PD.

Martinez B, Peplow PV (2018) Neuroprotection by immunomodulatory agents in animal models of Parkinson's disease. Neural Regen Res 13(9):1493-1506. doi:10.4103/1673-5374.237108

idative stress by reducing ROS and increasing glutathione in the striatum. It protected against 6-OHDA-induced loss of TH^+ neurons in the substantia nigra and striatum, decreased microgliosis and astrogliosis, and attenuated the reduction in striatal dopamine and its metabolites in 6-OHDA lesioned animals. Dimethyl fumarate also decreased apomorphine-induced asymmetrical rotations contralateral to the 6-OHDA intrastriatal injection site (Jing et al., 2015).

Lenalidomide

Lenalidomide reduced motor deficits and ameliorated dopamine fiber loss in the striatum, together with a decrease in microgliosis in the striatum and hippocampus, in mThy1-α-syn transgenic animals. Lenalidomide reduced the expression of the proinflammatory cytokines TNF-α, IL-6, IL-1β, and IFN-γ and increased the expression of the anti-inflammatory cytokines IL-10 and IL-13, as well as inhibiting NF-κB signaling in mThy1-α-syn transgenic animals. CX3CL1 (Fractalkine) level in transgenic animals was increased by lenalidomide treatment (Valera et al., 2015).

Thalidomide

Thalidomide, like lenalidomide, restored dopamine fiber loss in the striatum, and reduced TNF-α, IL-6, IL-1β, and IFN-γ expression in mThy1-α-syn transgenic animals. However, it did not affect microgliosis in the striatum and hippocampus, or the expression of IL-10 in transgenic animals (Valera et al., 2015). Thalidomide treatment before or after MPTP exposure increased dopamine content in the striatum and decreased monoamine oxidase B in the substantia nigra and striatum. In addition, thalidomide given before or after MPTP exposure lowered lipid peroxidation products in the substantia nigra and striatum (Palencia et al., 2015).

Ginsenoside Rg1

Ginsenoside Rg1 decreased MPTP-induced dopaminergic neuronal loss in the substantia nigra. The ratio of $CD3^+CD4^+$ to $CD3^+CD8^+$ T cells and $CD4^+CD25^+Foxp3^+$ regulatory T cells in the blood were increased in MPTP-induced animals following Rg1 treatment. The serum levels of TNF-α, IFN-γ, IL-1β and IL-6 in MPTP-induced animals were reduced by Rg1. Microgliosis was inhibited and infiltration of $CD3^+$ T cells into the substantia nigra of MPTP-lesioned animals was reduced by Rg1 treatment (Zhou et al., 2015).

IFN-β

Age-associated motor learning defects, neuromuscular deficiencies, and cognitive impairment were caused by deletion of *Ifnb* gene, which encodes IFN-β. $Ifnb^{-/-}$ pathology was associated with LBs resulting from defective neuronal autophagy (Ejerskov et al., 2015).

CNI-1493

CNI-1493 attenuated dopaminergic cell loss in the substantia nigra and alleviated striatal loss of dopamine content in MPTP-injected animals. CNI-1493 reduced microgliosis in the substantia nigra of MPTP-treated animals (Noelker et al., 2013).

Pycnogenol

Pycnogenol improved behavioral motor deficits of MPTP-injected animals. Lipid peroxidation products were reduced by pycnogenol and the activities of antioxidant enzymes and glutathione were increased by pycnogenol in MPTP-lesioned animals. Also pycnogenol attenuated dopamine depletion in the striata and reduced the nigrostriatal dopaminergic neuron loss following MPTP injection. Pycnogenol treatment reduced microgliosis and astrogliosis in MPTP-injected animals. In addition, pycnogenol pretreatment attenuated the activation of NF-κB in nuclear extracts. The MPTP-induced expression of cyclooxygenase-2 (COX-2) and iNOS protein and the secretion of TNF-α and IL-1β in the striatum were inhibited by pycnogenol (Khan et al., 2013).

Cyclosporin

Cyclosporin enhanced motor and cognitive function in Thy1-α-syn transgenic animals, decreased the striatal level of human α-syn and partially restored the level of TH protein. Cyclosporin enhanced motor function, exhibited an anti-inflammatory effect by lowering the expression level of NFATc3 and alleviated mitochondrial stress in the midbrain of MPTP-lesioned animals. The expression levels of GFAP and GLT-1 in the striatum of MPTP-treated animals were also reduced by cyclosporine, suggesting that it reduced astrogliosis and glutamate levels, the latter being associated with a lowering of excitotoxicity (Tamburino et al., 2015).

Rat PD studies

IFN-β

Lentiviral IFN-β overexpression arrested dopaminergic neuron loss in a familial PD model induced by injecting human α-syn (hSCNA) in the substantia nigra of animals (Ejerskov et al., 2015).

Acetoside

Parkinsonism symptoms were attenuated by administration of acetoside in rotenone-injected animals. Acetoside suppressed rotenone-induced α-syn, caspase-3 upregulation and microtubule-associated protein 2 (MAP2) downregulation (Yuan et al., 2016).

CART

Pretreatment with CART restored TH^+ content in the substantia nigra and decreased apomorphine-induced contralateral rotations in 6-OHDA lesioned animals (Upadhya et al., 2016).

FK506

FK506 increased the survival of dopaminergic neurons in a rAAV2/7 α-syn overexpression model. α-Syn aggregation

was not decreased, but the infiltration of both T helper and cytotoxic T cells and the number and subtype of microglia and macrophages were lowered by FK506. At 15 days the percentage of 'isolated activated microglia' in substantia nigra increased but was less prominent in FK506- than placebo-treated animals. At 29 days microglial cells with 'tendency to form clusters' were mainly present in placebo group and more abundant than in FK506-treated animals (Van der Perren et al., 2015).

Cyclosporin

Cyclosporin treatment following AAV α-syn vector injection into substantia nigra and receiving mesencephalic neural cell graft resulted in larger-sized grafts with an increased number of dopamine neurons formed from the graft than in the vehicle-treated group (Tamburino et al., 2015).

Nurr1 agonist SA00025

SA00025 was partially neuroprotective of dopaminergic neurons and fibers in animals receiving a priming injection of polyinosinic-polycytidylic acid (to exacerbate inflammation) and subsequent injection of 6-OHDA. SA00025 brought about changes in microglial morphology indicative of a resting state and a decrease in reactive microglia, together with a decrease in microglial Iba-1 staining intensity in the substantia nigra. SA00025 also decreased astrocyte GFAP staining intensity in the substantia nigra and IL-6 levels (Smith et al., 2015).

Neuroprotective Effects of Immunomodulatory Agents in PD

Neuroprotective therapy for PD aims to protect the at-risk dopaminergic neurons in the substantia nigra from degeneration that results in premature cell death and depletion of dopamine. It is envisaged that neuroprotective drugs could be used to treat patients with early clinical signs of the disease or potentially even prior to disease onset in those identified as having pre-disposing risk, including genetic factors (Tarsy, 2017).

Pharmaceutical therapies that target inflammation and the immune response are promising approaches for the treatment of PD. The pharmaceutical studies described in this review have identified several agents with immunomodulatory properties that protected dopaminergic neurons from degeneration and death in animal models of PD. All of the agents were effective in reducing the motor deficit and alleviating dopaminergic neurotoxicity and, when measured, prevented the decrease of dopamine upon being administered therapeutically after MPTP-, 6-OHDA-, rotenone-lesioning or delivery of AAV-α-syn to the ventral midbrain of animals. Interestingly, pretreatment with FTY720 (Zhao et al., 2017), tanshinoine I (Jing et al., 2016), dimethyl fumarate (Jing et al., 2015), thalidomide (Palencia et al., 2015), or CART (Upadhya et al., 2016) as a preventive strategy ameliorated motor deficits and nigral dopaminergic neurotoxicity in brain-lesioned animals. When tested for in animal models of PD, agents such as tanshinone I (Jing et al., 2016), tanshinone IIA (Ren et al., 2015), dimethyl fumarate (Jing et al., 2015), and pycnogenol (Khan et al., 2013) decreased oxidative stress. Also lipid peroxidation products were lowered by thalidomide (Palencia et al., 2015) and pycnogenol (Khan et al., 2013). Tanshinone I (Wang et al., 2015), lenalidomide (Valera et al., 2015), thalidomide (Valera et al., 2015), Rg1 (Zhou et al., 2015), pycnogenol (Khan et al., 2013) and SA00025 (Smith et al., 2015) decreased the levels of proinflammatory cytokines TNF-α, IL-6, IL-1β, and IFN-γ, while the levels of anti-inflammatory cytokines IL-10 and IL-13 were maintained or increased by tanshinone I (Wang et al., 2015) and lenalidomide (Valera et al., 2015). Cyclosporin exhibited an anti-inflammatory effect by lowering the expression level of NFATc3 in the midbrain of MPTP-lesioned animals (Tamburino et al., 2015). Microgliosis was decreased by dimethyl fumarate (Jing et al., 2015; Lestes-Becker et al., 2016), lenalidomide (Valera et al., 2015), Rg1 (Zhou et al., 2015), CNI-1493 (Noelker et al., 2013), pycnogenol (Khan et al., 2013), and SA00025 (Smith et al., 2015), while astrogliosis was reduced by dimethyl fumarate (Jing et al., 2015; Lestes-Becker et al., 2016), pycnogenol (Khan et al., 2013), cyclosporine (Tamburino et al., 2015), and SA00025 (Smith et al., 2015) in animal PD models. FK506 inhibited the infiltration of both T helper and cytotoxic T cells and decreased the number and subtype of microglia and macrophages (Van der Perren et al., 2015), whereas tanshinone IIA reduced the number and size of $CD11b^+$ cells in the striatum (Ren et al., 2015). Rg1 inhibited the infiltration of $CD3^+$ T cells into the substantia nigra and increased the ratio of $CD3^+CD4^+$ to $CD3^+CD8^+$ T cells and $CD4^+CD25^+Foxp3^+$ regulatory T cells in the blood (Zhou et al., 2015). The actions of the immunomodulatory agents in inhibiting microgliosis and astrogliosis and lowering the levels of pro-inflammatory cytokines and NFATc3, as well as modifying the infiltration of immune cells, are consistent with decreasing the neuroinflammation associated with aggregation of α-syn and thereby reducing neuronal degeneration and death (Rai et al., 2017; von Euler Chelpin and Vorup-Jensen, 2017).

Future Perspectives

Persistent inflammatory responses, involving T cell infiltration and microglial cell activation, are common characteristics of human patients with PD and involved in the degeneration of dopaminergic neurons. There is a need to develop therapeutic strategies that can impede or halt the disease through the modulation of the peripheral immune system by controlling the existing neuroinflammation (von Euler Chelpin and Vorup-Jensen, 2017). Several potential neuroprotective agents for PD had shown some promise in animals and/or humans, including selegiline and rasagiline (both monoamine oxidase inhibitors), and the natural sub-

stance coenzyme Q10. However, no treatment had proven to be effective for neuroprotection in human PD patients (Tarsy, 2017).

6-OHDA administration induces an intense IgG deposition in the substantia nigra as well as increased infiltration of both T- and B- lymphocytes into the injected side of the midbrain. The adaptive immune response was associated with extensive degeneration of dopamine neurons and microglial activation (Theodore and Maragos, 2015). Classically activated neuroinflammatory microglia by secreting IL-1α, TNF-α and C1q induce a subtype of reactive astrocytes termed A1, and are strongly induced by CNS injury and disease. A1 astrocytes lose the ability to promote neuronal survival, outgrowth, synaptogenesis and phagocytosis, and induce neuron and oligodendrocyte death (Liddelow and Barres, 2017; Liddelow et al., 2017). Normal aging was shown to induce neuroinflammatory A1-like astrocyte reactivity (Clarke et al., 2018) and would suggest that it is involved in the onset of PD. Some of the pharmaceutical agents reviewed herein could have potential by reducing the number and functional state of activated microglia and astrocytes (**Figure 1**) and inhibiting T cell infiltration into the PD brain.

Most of the mouse and rat studies reviewed had been performed with relatively young adult animals. Future studies need to be conducted with aged animals. This is particularly relevant with regard to the role of neuromelanin in neuroinflammation. Neuromelanin is formed by the oxidation of dopamine (Segura-Aguilar et al. 2014) and is present in the neurons of the substantia nigra with increasing amounts in cats, dogs, primates and humans (DeMattei et al., 1986). It was previously concluded that rodents did not possess neuromelanin (Marsden, 1983) but this seems to be an artifact of the young age of the animals studied as it has now been established that they can and do accumulate neuromelanin with its concentration being dependent on age (Zecca et al., 2001). In very old rats (23 months), but not in younger animals, neuromelanin granules were detected by electron microscopy (DeMattei et al., 1986). Accumulated neuromelanin is known to trap and bind PD-inducing toxins, making neurons containing these granules more susceptible to toxic insult. The presence of extraneuronal neuromelanin has been investigated in human subjects with idiopathic PD and MPTP exposure (McGeer et al., 1988; Langston et al., 1999). Most of the extraneuronal neuromelanin is phagocytosed by microglia resulting in microglia and astrocyte activation. It suggests that neuromelanin could be the effector of the chronic inflammation in the substantia nigra and degeneration of dopaminergic neurons in PD.

It is likely that many of the human PD patients are taking medication, and an observational study concluded that diabetes prevalence was closely similar between patients with PD and subjects without the disease (Becker et al., 2008). Animal models of PD should also incorporate possible medications that could be used by human PD patients such as L-dopa, antidiabetic, antihypertensive, and antihyperlipidemic drugs. Also both male and female animals should be used. Where gender was specified, the mouse studies had used males, whereas the rat studies had used females. It was surprising that no *in vivo* studies were found in the PubMed search of the effects of immunomodulatory agents in human PD patients. It would seem that some of the pharmaceutical therapies described in the recent animal PD studies and reviewed here would warrant being trialed in human patients. In addition, cell-based therapies with immunomodulatory properties such as mesenchymal stem cells (MSCs), human umbilical cord blood cells, and endothelial progenitor cells could also be investigated for possible benefit in PD patients. MSCs were reported to stabilize axonal transports for autophagic clearance of α-syn in Parkinsonian models (Oh et al, 2017). MSC therapy was found to improve clinical outcome in patients with stable chronic stroke (Steinberg et al., 2016).

A future translational task will be to exploit endogenous mechanisms of neuroprotection for therapeutic purposes by combining behavioral and pharmacological interventions. This type of approach is likely to benefit many PD patients, despite the clinical, etiological, and genetic heterogeneity of the disease (Francardo et al., 2017).

Author contributions: *Both authors contributed to the study equally.*
Conflicts of interest: *None declared.*
Financial support: *None.*

Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

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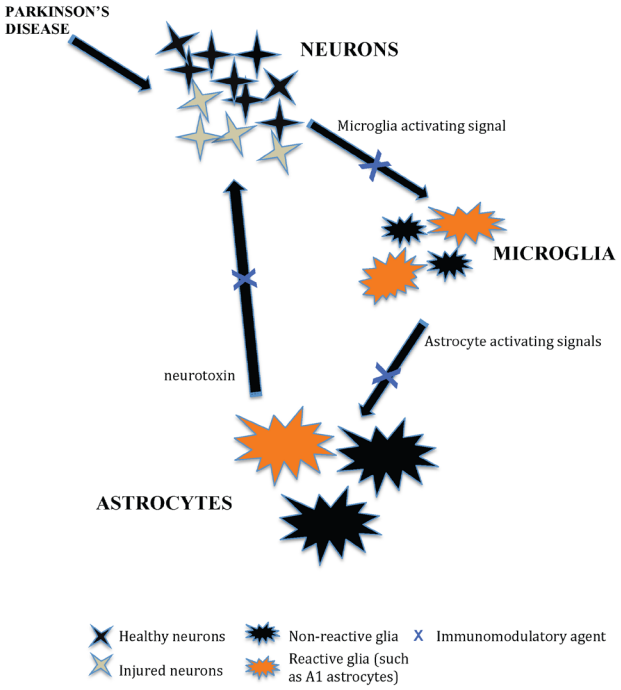

Figure 1 A schematic to illustrate the effect of immunomodulatory agents in enhancing the survival of dopaminergic neurons in animal models of Parkinson's disease by reducing microgliosis and astrogliosis (modified from Liddelow and Barres, 2017).

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