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-a-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

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 neuropathologic 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; Strassberger-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., 2010; pycnogenol: 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).

**Tanshinone IIA**

Tanshinone IIA given immediately after 6-OHDA treatment reduced apomorphine-induced contralateral rotations and alleviated 6-OHDA-induced loss of TH+ neurons in the substantia nigra and striatum. Tanshinone IIA also attenuated the reduction of dopamine and its metabolites associated with 6-OHDA lesioning (Zhang et al., 2015). Similar findings with tanshinone 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. Tanshinone 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 dopaminergic 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 Nf2-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 | Mice injected with saline | Mice injected with 6-OHDA had unilateral lesions and aberrant apomorphine-induced rotational behavior | Conclusion |
|-------|--------------------------|-------------------------------------------------------------------------------------------------|------------|
| Fingelton et al. (2017) | Adult C57Bl/6 male mice, 7–8 weeks, anesthetized with ketamine/xylazine and injected with 6-hydroxydopamine (6-OHDA) (10 µg in 0.1 µL of normal saline with 0.02% ascorbic acid) in two separate sites of 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. | Mice injected with saline alone or into two separate sites of the striatum on the right side of the brain served as controls. In 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 open field test. Measurements were repeated daily during the experimental period. In this study, FTY720 alleviated the reduction of extracellular regulated protein kinase (ERK) phosphorylation and Bcl-2 expression in both PD models. The data from young and old mice were consistent and suggested that FTY720 may hold promise as a PD therapeutic agent. | Although S1PR1 expression was significantly increased in mice receiving 6-OHDA or rotenone, FTY720 treatment retained S1PR1 expression. These findings suggest the potential of S1PR2 modulation by FTY720 as a therapy for PD. |
| Ren et al. (2017) | Adult C57Bl/6 male mice, 10 weeks, were injected i.p. with 0.5 mg/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 sacrificed and brains processed. | Mice received an equimolar amount of ethanol (vehicle) twice weekly by voluntary oral dosing with containing tablets incorporated into the correct volume of FTY720. FTY720 was given by gavage to mice (i.e., 1 mg/kg/day) was performed in the model and treatment rats for 42 days. | Administration of FTY720 significantly increased the phosphorylation of protein kinase B (AKT), extracellular signal-regulated kinase (ERK) and phospho-ERK (p-ERK) response and reduced autophagy. The data from young and old mice were consistent and suggested that FTY720 may hold promise as a PD therapeutic agent. |
| Vidal-Martinez et al. (2016) | AS3T α-syn (R6/2; Tg: PgrP5NCA+AS3Tβ3Vescl) mice were used to generate a cohort of non-tremor mice overexpressing α-syn in the substantia nigra and in the transgenic (TG) mice. | Tg mice overexpressing human α-syn developed enteric nervous system (ENS) pathology and neurodegeneration, which produced both behavioral and physiological changes. The data from young and old mice were consistent and suggested that FTY720 may benefit PD patients and others with similar pathology. | |
| Ase et al. (2016) | Adult Sprague–Dawley rats, 200–300 g, were randomly divided into 3 groups: control, normal saline (vehicle), or rotenone (2 mg/kg). The latter two groups received rotenone injections. Rats were injected daily with rotenone (2 mg/kg) for 5 days, or reduced brain sensitivity to environmental stimuli and eating difficulties. | The data from young and old mice were consistent and suggested that FTY720 may hold promise as a PD therapeutic agent. |

**Note:** The table summarizes studies on the use of immunomodulatory agents in animal models of Parkinson's disease (PD) with a focus on FTY720 and related compounds. The studies highlight the potential of these agents in modulating PD-related symptoms and neurodegeneration. The data from young and old mice were consistent and suggested that FTY720 may hold promise as a PD therapeutic agent.
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**Table 1 Continued**

| Study | No. of animals, gender, ages, treatment | Comparison | Functional outcomes | Conclusion |
|-------|----------------------------------------|------------|--------------------|------------|
| Amphetamine-regulated transcript peptide (CART) | Adult male Sprague-Dawley rats (230–230 g) were stereotactically cannulated targeting left lateral ventricle and right substantia nigra. At 7 days after i.c.v. or infra-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, 0.1% ascorbic acid in 0.9% saline and administered unilateral (right substantia nigra) to produce lesions in the nigro-striatal tract. While apomorphine (0.3 mg/kg, i.p.) 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 pretreated (PD-like condition) rats. In 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 (infra-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-lesioned rats treated with artificial CSF (aCSF) or CART (n = 5 each group) were isolated and processed for immunohistochemical labeling with TH antibody. | The sham-operated animals were injected with the vehicle (0.1% ascorbic acid in 0.9% saline intrastriatally) and 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 a significant decrease in rotational activity in a dose-dependent manner compared to the aCSF-treated group. CART 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 showed significant decrease in apomorphine-induced rotations, but at lower dose (25 ng/rat) it failed to change any effect. CART antibody (dilutions 1:250 and 1:500) did not have any effect on the levodopa-induced rotation. |  |

**Tanshinone I**

| Study | No. of animals, gender, ages, treatment | Comparison | Functional outcomes | Conclusion |
|-------|----------------------------------------|------------|--------------------|------------|
| 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-hour 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 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. Mice injected with vehicle for 3 days prior to lesioning. On the 3rd day of treatment, 1 hour after final dosing, mice were euthanized and injected stereotactically with 6-OHDA (8 µg) in 2 µL saline containing 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 treatment, 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. | 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. Apgomorphine produced contralateral rotations and the counts were significantly greater than those in the naive rats. Prior treatment with CART (50 or 100 ng/rat, i.c.v.) significantly decreased apomorphine-induced contralateral rotations compared to those of aCSF pretreated 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, cocaine-immune 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 contralateral rotations in a dose-dependent manner compared to that in the aCSF-treated group. CART 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 (30 or 80 ng/rat, i.c.v.) significantly decreased the levodopa (20 mg/kg) induced contralateral rotations, but at lower dose (15 mg/kg) it failed to change any effect. CART antibody (dilutions 1:250 and 1:500) did not have any effect on the levodopa-induced rotation. | While CART-immunoreactivity in the accumbal nucleus, parietal cerebral nucleus, 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. |
| Study | No of animals, gender, ages, treatment | Comparison | Conclusion |
|-------|----------------------------------------|------------|------------|
| Ding et al. (2016) | Colonies of Nrf2–/– mice and Nrf2+/+ littermates were established. Each experimental group comprised 5–8 animals. An adenovirus-based pseudotyped 6 (AAV6) 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 ventral tegmental area (SNc). Dimethyl fumarate (DMF; 200 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 test was held in the vertical axis and a swing was recorded whenever the animal moved in 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. Immunohistochemistry of mouse tissues was also performed. | A control AAV6 expressing GFP was used. Mice received vehicle (VEH) by oral gavage. A control AAV6 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 study showed that DA levels were decreased in the ventral midbrain (VMB) of Nrf2–/– and Nrf2+/+ mice. A control AAV6 expressing GFP did not have any effect on DA levels. The expression of Nrf2 was monitored daily by oral gavage for 1 week, and every other day for 8 weeks after stereotactic injection. One day before sacrifice, mice were anesthetized for 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 injection, Nrf2+/+ mice showed a reduction in TH expression of all animals expressed human α-Syn. In 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 fibres in striatum. Stereological analysis of TH neurons of Nrf2+/+ showed that vehicle (VEH), VEH, and Nrf2+/+ in DMF were partially lost at postinfection week 1 (~50%) and increased throughout 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 Nrf2+/+ 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 microglial and astroglial activation in the basal ganglia. Regarding astroglial, α-Syn toxicity correlated with a significant increase in glial fibrillary acidic protein (GFAP) astrocytes in the ipsilateral VMB side of Nrf2–/– mice, with a maximum at 3 weeks after infection, and an astrocyte scar remained visible in both PD models. DMF reduced astrogliosis in Nrf2+/+ mice treated with DMF, with a shorter effect on Nrf2–/– mice. Similarly, the astrocyte response in the ipsilateral striatum for vehicle-treated Nrf2–/– mice was abolished in the DMF-treated Nrf2–/– without any effect in Nrf2+/+ mice. Regarding microglia, the immunohistochemistry showed that α-Syn expression induced microglia at the ipsilateral side of whole-treated Nrf2–/– mice after 1 week, reaching a maximum after 3 weeks, and returning close to basal levels after 8 weeks. DMF greatly ameliorated the microgliosis of Nrf2+/+ and a minor effect on Nrf2–/– mice. | The dopaminergic neuron loss was greatly attenuated in Nrf2+/+ mice treated with DMF (>75% of neurons preserved). Targeting Nrf2 may be a therapeutic strategy to reinforce endogenous brain defensive mechanisms against α-Syn toxicity. |

| Jing et al. (2013) | Adult CS/B6 male mice, 8 weeks, were treated with DMF (50 mg/kg) by oral gavage for 7 days prior to lesioning. On the 7th day of treatment, 1 hour after final dosing, mice were vehicle by oral gavage. Control mice received saline. Apomorphine (0.1 mg/kg) was injected i.p., mice placed individually in plastic boxes and videotaped from above for 30 min. Mice were euthanized at different time points following-DMF for biochemical or histological assessment. | The levels of ROS and GSH at 7 days after 6-OHDA lesioning were measured to test if the time course of ROS/APE activity of 6-OHDA-induced oxidative stress. 6-OHDA treatment stimulated a significant increase in striatal ROS formation at 7 days after injection. DMF pretreatment reduced the 6-OHDA-induced striatal ROS formation. DMF treatment also protected against 6-OHDA-associated oxidative stress by increasing GSH striatal levels at 7 days after injection. 6-OHDA administration markedly increased microglial and astroglial infiltration such as reactive astrocytes and microglia, whereas in vehicle-treated animals, only a few immuno-reactive astrocytes and microglial cells were observed in the striatum. DMF administration significantly decreased microglial and astroglial activation. In brains collected at 13 days after 6-OHDA lesioning, IHC staining showed that the 6-OHDA-induced loss of TH neurons in the substantia nigra was markedly attenuated by DMF treatment. Immunoreactivity of striatal astrocytes was evaluated using an anti-TH antibody. Mice treated with DMF exhibited a significant increase in TH protein levels in the 6-OHDA-lesioned mice. Here, a 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, HVA at 2 weeks and returned to basal levels after 8 weeks. DMF greatly ameliorated the microgliosis of Nrf2+/+ and a minor effect on Nrf2–/– mice. | DMF may be beneficial for the treatment of PD. |

| Gasser et al. (2015) | Adult CS/B6 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-tetrahydroxyindole (MPTP) 50 mg/kg group, MPTP 50 mg/kg + Rg1 20 mg/kg group. Mice were treated i.p. with MPTP hydrochloride, 50 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 injection. Group with Rg1 only was treated with Rg1 for 15 consecutive days. All of the mice were anesthetized and 0.5–0.6 mL of peripheral blood was drawn through the jugular vein. Serum was separated from the whole blood by centrifugation and stored at −80°C until ELISA analysis. Peripheral blood mononuclear cells (PBMC) were isolated with leucocytes and incubated with fluoroisothiocyanate-labeled antibodies against CD3, CD4, CD8 T cells and CD45, CD62, 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 31. PBS for immunohistochemistry to examine the number of TH neurons. | Control group injected ip. with 0.5 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 trend of higher 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 and compared with the MPTP group, the ratio of CD3+ CD4+ T cells was significantly lower in the MPTP + Rg1 groups than in the MPTP group. The percentage of CD3+ CD8+ T cells in the MPTP + Rg1 groups was significantly lower in the MPTP + Rg1 groups than in the MPTP group. The percentage of CD4−FoxP3− regulatory T cells was significantly lower in the MPTP + Rg1 groups than in the MPTP group. The percentage of CD4−FoxP3+ regulatory T cells was significantly higher in the MPTP + Rg1 groups than in the MPTP group. The ratio of CD4+CD8+ T cells was significantly lower in the MPTP + Rg1 groups than in the MPTP group. | Rg1 may be a promising drug for the treatment of PD at the regulation of peripheral and central inflammation. |
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Study | No. of animals, gender, age, treatments | Comparison | Functional outcomes | Conclusion
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Tan shine (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-OHDA (in 2 µL saline containing 0.1% 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 containing 1% DMSO) up to 6-OHDA lesion. + Tan IIA group immediately after 6-OHDA lesion. Mice were euthanized at a time point between 1 and 21 days following 6-OHDA injection and tissue collected for biochemical and histological assay. 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 boxes and videotaped from above for 30 minutes. Analysis of complex 3 (60°) rotation was made. Animals were euthanized with sodium pentobarbital at 3 weeks after 6-OHDA administration, then perfused with saline, followed by 4% paraformaldehyde in 1:1 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 stratum 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 (n = 10) | Apomorphine-induced contralateral rotation was significantly reduced with Tan IIA treatment compared to controls treated with 6-OHDA + vehicle. | Tan IIA may be beneficial for the treatment of PD. Using 8-OH-SY5Y cells treated with 6-OHDA, Tan IIA abolished the enhanced expression of mTOR.

Ren et al. (2015) | Adult C57Bl/6 male mice, 3–4 months, were randomly divided into groups. Mice in group A (n = 6) were injected with MPTP hydrochloride (20 mg/kg) in PBS 4 times at 2-hour intervals, and then with vehicle including 1% DMSO at 12 hours after the last injection of MPTP and once a day for the 6 days. Mice in group B (n = 6) were injected with MPTP hydrochloride as in group A 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 6 days. Mice in group C (n = 6) were injected with MPTP hydrochloride as in group B mice and also received Tan IIA as in group C mice. Mice were euthanized at the chosen time points and the ventral midbrain dissected out. Rotated test was used to measure the dyskinesia of mice. Before MPTP administration, mice were treated on the rotated at 20 r/min for 5 minutes, 4 times per day for 7 consecutive days. Mice were then chosen that did not fall off during the training for the next step. At 7 days after the last MPTP administration, mice receiving 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 rotated test, mice were euthanized and the content of dopamine in the stratum was measured by HPLC. Mice of each group were euthanized at 3 and 7 days after the final injection of MPTP. | Mice in group A (n = 6) were injected with PBS 4 times at 2-hour intervals, and then with PBS including 1% DMSO at 12 hours after the last injection of PBS and once a day for the 6 days. | MPTP is completely converted to MPP+ and largely cleared within 12 hours after injection. MPTP reduced the number of TH immunopositive cells/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 on 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. The content of dopamine in the stratum was much less than that of the vehicle-treated group. Tan IIA treatment recovered the behavioral dysfunctions of mice as well as the content of dopamine in the stratum. 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 vehicle-treated group. Tan IIA alone had no effect on the number and size of CD11b-immunopositive cells 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 sources of ROS and RNS. After MPTP treatment, there were significant increases in microglia, pro-inflammatory and anti-inflammatory 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. Double immunofluorescence staining showed that p47-phox-immunopositive 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.

FSK06 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 1 µL rAAV2/7 eGFP as a control at 1 x 10^12 viral particles. FSK06-treated animals received daily injections of FK06 (1 µg/kg) in saline starting 1 day after rAAV2/7 eGFP injection. Behavioral testing started the cylinder test was used to test for any locomotor contacts. Contact made by each forepaw with the wall of a 20-cm-wide clear glass cylinder were scored. The number of impaired or limb contacts was assessed in a total of 4 different forepaw contacts. To analyze the bioavailability and presence of FK06 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 FK06 (50 mg/kg in saline). At 1 hour after the last i.v. injection, blood (tail vein) and CSF (lumbar puncture) samples were collected to determine peak values. The animals were anesthetized and the brain removed. Tissues were homogenized in saline and total FK06 levels were determined by an ELISA assay. | Animals injected in substantia nigra with rAAV2/7 eGFP as control. Placebo-treated animals were injected daily i.v. with saline. | Upon administering FK06 (1 mg/kg) daily i.v. for 4 weeks in A53T α-syn rAAV2/7 rats, detailed blood levels of FK06 were found at 4 days, 13 days and 29 days compared with placebo-treated animals. Quantification of the number of TH+ nigral neurons 20 days post injection showed a 2-2 fold higher survival of dopamine neurons in rats treated with FK06 compared with placebo rats. Controls treated with FK06 (1 µg/kg) showed significant improvements in a rotarod test performance. Analysis of the total number of α-syn positive cells in the substantia nigra revealed a significant increase in the animals treated with FK06 (1 µg/kg) at 29 days. Upon analyzing the α-syn positive cells in a population of dopamine neurons, no significant differences were observed in the duration of α-syn aggregates. No differences were observed in the duration of α-syn aggregates in the substantia nigra and striatum in both groups over time, but FK06-treated rats showed significantly less CD4-positive cells compared to placebo controls at 13 and 29 days post-injection. One significant difference was observed in the number of α-syn and TH+ animals injected with rAAV2/7 enhanced green fluorescent protein (eGFP) in the contralateral substantia nigra of all rats. The expression levels of CD68 as a marker of phagocytic and microglia/macrophages and MHC II for antigen presentation were analyzed. An increase in CD68-positive cells and microglia/macrophages in the substantia nigra at 20 days post injection showed a > 2-fold increase in the number of α-syn and TH+ animals injected with rAAV2/7 enhanced green fluorescent protein (eGFP) in the contralateral substantia nigra of all rats. The expression levels of CD68 as a marker of phagocytic and microglia/macrophages and MHC II for antigen presentation were analyzed. | The anti-inflammatory properties of FSK06 decrease neurodegeneration in the α-syn PD model.
### Table 1 Continued

| Study | No. of animals, gender, ages, treatment | Comparison | Functional outcomes | Conclusion |
|-------|----------------------------------------|------------|--------------------|------------|
| Lenalidomide (Bartolini et al. 2015) | Mice, 9 months, expressing human α-syn under the control of the murine Thy1 promoter (mThy1-α-syn Tg) mice were used, n = 38. | mThy1-α-syn Tg mice and their non-Tg littermates were treated for 5 weeks with lenalidomide or thalidomide (300 mg/kg) administered by gavage 5 times each week. Behavioral assessment of the mice was performed using the open field and the rotarod tests. Total activity was calculated as total beam breaks in 10 minutes and bruxism was calculated as the percentage of time spent in the periphery. The total distance traveled forward and the number of foot slippages were recorded. Mice were euthanized under anesthesia and brains removed. The right hemibrain was fixed in 4% formaldehyde in PBS (pH 7.4) and sectioned. The left hemibrain was stored at −4°C for biochemical analysis. | The mThy1-α-syn Tg mice showed an increase in activity and motor errors. Lenalidomide, but not thalidomide, led to a significant and consistent increase in total errors in the rotarod test. In mThy1-α-syn Tg mice, there was a loss of dopaminergic TH immunoreactive fibers in the striatum compared to non-Tg mice. Both lenalidomide and thalidomide restored TH immunoreactivity to levels similar to non-Tg mice. The mThy1-α-syn Tg mice showed a significant reduction in the expression of the anti-inflammatory cytokine IL-10, which indicated that the behavioral improvement observed with lenalidomide was not a consequence of changes in the levels of pro-inflammatory cytokines. 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 compared to 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 levels 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 CCL3L1 (fractalkine) levels were reduced in mThy1-α-syn Tg mice and increased by lenalidomide treatment. Fractalkine expression is regulated by NF-κB and is involved in neuroprotective properties. In lesioned brain areas of α-syn Tg mice, CSF-1R expression was reduced by lenalidomide treatment. | Lenalidomide increased the expression of the anti-inflammatory cytokine IL-10. Lenalidomide may have neuroprotective potential for reducing neuroinflammation in PD. |
| Thalidomide (Patel et al. 2015) | Adult C57BL/6N mice, male 25–30 g, were divided into groups (n = 10/group). Group A: control group (vehicle). Group B: rats received daily oral doses of thalidomide (200 mg/kg) at 3 hours intervals. Group C received an injection sc. 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. Group E: MPTP exposure was performed in lesioned rats to receive an intra-striatal graft of mesencephalic progenitors and motor performance was monitored up to 13 weeks. Stereotaxic injections were performed at 13 weeks. | 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. | The 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 administration was similar to that of controls in both experiments A and B. The content of MAO-B in striatum and substantia nigra after thalidomide administration 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 treatment with MPTP or thalidomide, or both. The administration of thalidomide before MPTP exposure significantly decreased MAO-B content in striatum and substantia nigra compared to MPTP alone. Thalidomide-induced reduction of MAO-B correlated with reduced production of reactive oxygen species (ROS) and reduced neuroinflammation observed in the lesioned striatum of MPTP-treated rats. Thalidomide and melatonin-induced reduction of MAO-B correlated with reduced production of reactive oxygen species (ROS) and reduced neuroinflammation observed in the lesioned striatum of MPTP-treated rats. Thalidomide treatment resulted in a significant increase in MAO-B mRNA levels, and both lenalidomide and thalidomide induced TNF-α expression. Lenalidomide and thalidomide also resulted in IL-6, IL-8, and TNF-α expression. Interestingly, lenalidomide increased the expression of the anti-inflammatory cytokine IL-10. | MPTP treatment induced increased contents of MAO-B in striatum and substantia nigra compared to controls. Thalidomide-induced reductions of MAO-B correlated with reduced production of reactive oxygen species (ROS) and reduced neuroinflammation observed in the lesioned striatum of MPTP-treated rats. Thalidomide treatment resulted in a significant increase in MAO-B mRNA levels, and both lenalidomide and thalidomide induced TNF-α expression. Lenalidomide and thalidomide also resulted in IL-6, IL-8, and TNF-α expression. Interestingly, lenalidomide increased the expression of the anti-inflammatory cytokine IL-10. Thalidomide could be a potential adjuvant therapy for PD. |
| Cyclosporin A (Tamburino et al. 2015) | Adult female Sprague-Dawley rats, 3 months (225–270 g) were injected with 3.5 ml of a 0.1% AAV-α-syn vector into the substantia nigra or 3 ml of 6-OHDA (0.5 µg) in 0.9% saline containing 0.2 mg/ml L-aspartic acid into the medial forebrain bundle (MFB). at a rate of 2 µl/min. The animal was unanesthetized and the injection site was stimulated for 10 min with a hand-held photodetector. | Group A was controls in both experiments. Group B was injected with vehicle, n = 10. Group C was injected with vehicle, n = 8. Group D was injected with vehicle, n = 9. Group E was injected with vehicle, n = 10. Group F was injected with vehicle, n = 10. Group G was injected with vehicle, n = 10. Group H was injected with vehicle, n = 10. Group I was injected with vehicle, n = 10. Group J was injected with vehicle, n = 10. Group K was injected with vehicle, n = 10. Group L was injected with vehicle, n = 10. Group M was injected with vehicle, n = 10. Group N was injected with vehicle, n = 10. Group O was injected with vehicle, n = 10. Group P was injected with vehicle, n = 10. Group Q was injected with vehicle, n = 10. Group R was injected with vehicle, n = 10. Group S was injected with vehicle, n = 10. Group T was injected with vehicle, n = 10. Group U was injected with vehicle, n = 10. Group V was injected with vehicle, n = 10. Group W was injected with vehicle, n = 10. Group X was injected with vehicle, n = 10. Group Y was injected with vehicle, n = 10. Group Z was injected with vehicle, n = 10. | The 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 administration was similar to that of controls in both experiments A and B. The content of MAO-B in striatum and substantia nigra after thalidomide administration 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 treatment with MPTP or thalidomide, or both. The administration of thalidomide before MPTP exposure significantly decreased MAO-B content in striatum and substantia nigra compared to MPTP alone. Thalidomide-induced reduction of MAO-B correlated with reduced production of reactive oxygen species (ROS) and reduced neuroinflammation observed in the lesioned striatum of MPTP-treated rats. | Thalidomide could be a potential adjuvant therapy for 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

Rats had a significant sparing of dopaminergic neurons in the substantia nigra after 32 days of SAA00025 treatment compared to vehicle treatment. Nurr1 agonist SAA00025 causes neuroprotection and anti-inflammatory effects in an inflammation-exacerbated 6-OHDA lesion model of PD.

**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 SAA00025 entered the brain and confirmed elevated brain exposure at 1, 4, and 24 hours after the last administration. SAA00025 treatment significantly modified the expression of Nurr1 and dopaminergic target genes from 1–4 hours after daily gavage for 7 days. A normalization of Nurr1, TH and VMA1 mRNA expression was observed at 4 hours post-gavage and an increase in c-RET at 24 hours. Consistently, protein levels of TH were significantly elevated at 4 hours following SAA00025 treatment compared to vehicle treatment.

In the 6-OHDA lesion model, SAA00025 was neuroprotective on TH and NeuN neurons within the substantia nigra and also preserved TH fibers in the striatum. The intensity of TH immunostaining, with in individual dopaminergic cell bodies of SAA00025 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 SAA00025 treatment compared to vehicle treatment. At the end of SAA00025 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. SAA00025 treatment caused significantly more microglia residing in a resting state and a significant decrease in reactive microglia compared to vehicle treatment. SAA00025 treatment decreased immunofluorescence intensity of both Iba-1+ and GFAP+ astrocytes in the substantia nigra compared to vehicle treatment. A significant reduction in protein level of IL-6 was observed with SAA00025 treatment. IL-6, IL-1, IL-10, monocyte chemotactic protein (MCP)1, macrophage inflammatory protein (MIP)1a, MIP2, MIP3a, regulated on activation, normal T cell expressed and secreted factor (RANTES), Fractalkine, TNF-α, IL-3, IL-2, macrophage-derived chemokine (MDC), transforming growth factors β1 (TGF-β1) were unchanged.

**Conclusion**

Following 7 days of daily gavage, pharmacokinetic analysis and postmortem showed that SAA00025 entered the brain and confirmed elevated brain exposure at 1, 4, and 24 hours after the last administration. SAA00025 treatment significantly modified the expression of Nurr1 and dopaminergic target genes from 1–4 hours after daily gavage for 7 days. A normalization of Nurr1, TH and VMA1 mRNA expression was observed at 4 hours post-gavage and an increase in c-RET at 24 hours. Consistently, protein levels of TH were significantly elevated at 4 hours following SAA00025 treatment compared to vehicle treatment. In the 6-OHDA lesion model, SAA00025 was neuroprotective on TH and NeuN neurons within the substantia nigra and also preserved TH fibers in the striatum. The intensity of TH immunostaining, with in individual dopaminergic cell bodies of SAA00025 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 SAA00025 treatment compared to vehicle treatment. At the end of SAA00025 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. SAA00025 treatment caused significantly more microglia residing in a resting state and a significant decrease in reactive microglia compared to vehicle treatment. SAA00025 treatment decreased immunofluorescence intensity of both Iba-1+ and GFAP+ astrocytes in the substantia nigra compared to vehicle treatment. A significant reduction in protein level of IL-6 was observed with SAA00025 treatment. IL-6, IL-1, IL-10, monocyte chemotactic protein (MCP)1, macrophage inflammatory protein (MIP)1a, MIP2, MIP3a, regulated on activation, normal T cell expressed and secreted factor (RANTES), Fractalkine, TNF-α, IL-3, IL-2, macrophage-derived chemokine (MDC), transforming growth factors β1 (TGF-β1) were unchanged.

**INF-β**

Ejerskov et al. (2015)

**INF-β**

INF-β mice and INF-β−/− littermates. Behavioral measurements included evaluating motor coordination and learning with an accelerating RotaRod automatically recording time before falling. Neuronal muscular 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 granule neurons (CGNs) were from 7-day-old old cerebellum and cortical neuron (CN) cultures from the cortex of 1-day-old mice. Lentivirus was used to overexpress INF-β in a familial PD model induced with human α-syn (hSCNA) in substantia nigra of rats.

**INF-β**

INF-β−/− mice were significantly impaired in motor coordination and learning from 3 months compared to age- and weight-matched wild-type (WT) INF-β+/+ littermates and in latency-to-fall time in a wire-suspension test. Latency to fall was considerably shorter in INF-β−/− than INF-β+/+, indicating hyperalgiesia and defective nociception towards temperature-induced pain. Forced swimming tests found no differences between INF-β−/− and INF-β+/+ mice in swimming pattern, climbing effort, or immobility, so INF-β−/− mice were not defective in water intake compared to land. In water maze tests, INF-β−/− mice had significant spatial- and memory learning deficits that increased with age. Apoptotic cells were detected in 1.5-month-old INF-β−/− granular layers of olfactory bulbs, the granule dense gyrus of hippocampus and the subventricular zimula, and the striatum caudate putamina including the ependymal cell layer in 12 month-old-INF-β−/− mice, but not detected in INF-β+/+ sagittal brain sections at similar ages. Neurons were significantly reduced in the hippocampal CA1 region in 3- to 6-month-old-INF-β−/− mice and decreased in Purkinje cells of cerebellum. TH fiber density and TH (dopamine producing) neurons were significantly reduced in the stratum and substantia nigra in INF-β−/− mice versus WT. NeuN+ and NeuNTH+ cells were reduced in the ventral midbrain, which was correlated with reduced TH protein in basal ganglia in INF-β−/− mice while total cells were unaffected. Histological examination showed that INF-β−/− neuron degeneration was associated with age-dependent α-synucleinopathy. Staining for α-syn was normal in 1.5-month-old INF-β−/− brains; by 3 months, α-syn was significantly increased in substantia nigra, however, α-syn intensity was reduced in 12-month-old-INF-β−/− mice, likely reflecting degeneration of TH neurons. A α-syn and large aggregates of phosphoylated α-syn were found in TH neurons of substantia nigra. At 3 months, α-syn aggregates were widespread in the striatum, frontal cortex, hippocampus and cerebellum. α-syn aggregates and neurons were found sporadically in thalamus, brainstem, and subthalamic regions of 3-month-old-INF-β−/− mice. Neurons with α-syn Lewy body-like structures increased with age (6- and 12-month-old) in INF-β−/− mouse thalami. Gene set enrichment analysis (GSEA) was used to identify cellular pathways involved in INF-β−/− neuron pathology. In the top 20 deregulated pathways, 3 were associated with autophagy, which were restored with recombinant INF-β (hIFN-β). Microtubule-associated protein 1 light chain 3B (HC3B) increased in basal ganglia of 1.5-month-old-INF-β−/− mice, correlating with increased p62, NR2B and Rab7, supporting defects in autophagosome before α-syn, ubiquitin, Tau, and Lewy body-aggregation. HC3B, NIB1, and p62 were higher in untreated INF-β−/− cortical neurons, and while overnight INF-β treatment promoted LCB3-11 conversion and reduced p62 in INF-β−/− cortical neurons, indicating increased autophagy flux. INF-β−/− reduced p62 but only slightly increased LCB3-11. By promoting autophagy, INF-β−/− reduced α-syn in both INF-β−/− and INF-β+/+ cortical neurons. Injection of hSCNA and control lentiviruses blocked autophagy in rat basal ganglia 10 days after substantia nigra injection. INF-β overexpression prevented hSCNA and p62.29-a-syn aggregation. The results showed improved left paw use compared to right paw use, contralateral to injection side of hSCNA/hSCNA and hSCNA-control viruses, respectively. 21 days post injection which was associated with preservation of TH fibers in substantia nigra and nigrostriatal dopaminergic neurons from hSCNA-induced substantia nigra damage.
Table 1 Continued

| Study | No. of animals, gender, age, treatment | Comparison | Functional outcomes | Conclusion |
|-------|---------------------------------------|------------|--------------------|------------|
| CNI-1493 | Adult C57Bl6 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 | C57Bl6 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 was compared to control groups was observed after day 1 and day 7. PWC (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. PWC 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 PWC treatment compared to MPTP injected group. Number of steps in the drag test was significantly decreased in MPTP groups compared to controls. PWC treatment followed by MPTP for 7 days significantly improved the number of footprints compared to MPTP group. PWC treatment for 1 day did not significantly improve motor coordination skill, grip strength, forepaw step distance and step change 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. PWC 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. PWC treatment for 7 days followed by MPTP injection significantly preserved the activities of these enzymes compared to MPTP injected groups. PWC 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 PWC 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. PWC 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 group. PWC treatment attenuated higher expressions of GFAP in PYC + MPTP group compared to MPTP groups. MPTP injected mice demonstrated significantly higher levels of NF-kB p50 protein in nuclear extracts after 1 day and 7 days compared with control mice. PWC treatment prior to MPTP significantly attenuated the activation of NF-kB. Significantly higher striatal 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 PWC 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 significantly blocked in PYC-treated mice at day 7 post-injection. | The neuroprotective effects of pycnogenol show significantly reduced nigrostriatal dopaminergic neuron loss following MPTP injection. PWC-induced adaptation to oxidative stress and inflammation could suggest a novel approach for clinical intervention in neurodegenerative diseases including PD. |
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 intrastratal injection site (Jing et al., 2015).

**Lenalidomide**

Lenalidomide reduced motor deficits and ameliorated dopaminergic 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 dopaminergic cell loss 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).

Pharmacological 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), tanshinone 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 alleviated 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, anti-diabetic, anti-hypertensive, and anti-hyperlipidemic 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).

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