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Paroxetine Prevents Loss of Nigrostriatal Dopaminergic Neurons by Inhibiting Brain Inflammation and Oxidative Stress in an Experimental Model of Parkinson’s Disease

Young C. Chung, Sang R. Kim, and Byung K. Jin

The present study examined whether the antidepressant paroxetine promotes the survival of nigrostriatal dopaminergic (DA) neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson’s disease. MPTP induced degeneration of nigrostriatal DA neurons and glial activation as visualized by tyrosine hydroxylase, macrophage Ag complex-1, and/or glial fibrillary acidic protein immunoreactivity. Real-time PCR, Western blotting, and immunohistochemistry showed upregulation of proinflammatory cytokines, activation of microglial NADPH oxidase and astroglial myeloperoxidase, and subsequent reactive oxygen species production and oxidative DNA damage in the MPTP-treated substantia nigra. Treatment with paroxetine prevented degeneration of nigrostriatal DA neurons, increased striatal dopamine levels, and improved motor function. This neuroprotection afforded by paroxetine was associated with the suppression of astroglial myeloperoxidase expression and/or NADPH oxidase-derived reactive oxygen species production and reduced expression of proinflammatory cytokines, including IL-1β, TNF-α, and inducible NO synthase, by activated microglia. The present findings show that paroxetine may possess anti-inflammatory properties and inhibit glial activation-mediated oxidative stress, suggesting that paroxetine and its analogues may have therapeutic value in the treatment of aspects of Parkinson’s disease related to neuroinflammation.


Parkinson’s disease (PD) is a common neurodegenerative disease characterized by abnormal motor behavior, characterized by resting tremor, rigidity, and bradykinesia (1, 2). The neuropathological features of PD are progressive loss of dopaminergic (DA) neurons in the substantia nigra (SN) and depletion of dopamine in the striatum (STR), the site to which these neuronal nerve terminals project (3). Although PD is a sporadic disease of unknown pathogenesis, accumulating evidence suggests that glial activation-derived oxidative stress increases the risk of developing PD (4). In vivo and in vitro 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD show that key enzymes involved in the production of reactive oxygen species (ROS)/reactive nitrogen species (RNS), such as microglial NADPH oxidase and inducible NO synthase (iNOS), and astroglial myeloperoxidase (MPO) are upregulated in damaged areas and contribute to DA neuronal death (5–8). Additionally, proinflammatory cytokines, such as TNF-α and IL-1β, are also increased and contribute to DA neuronal death in the MPTP model of PD (9).

Paroxetine, a common selective serotonin reuptake inhibitor, is widely prescribed as an antidepressant because it has fewer side effects and lower toxicity than earlier-generation selective serotonin reuptake inhibitors (10). The most frequent neuropsychiatric symptom in PD is depression (11), and several clinical reports have shown that paroxetine is safe and effective in treating PD-associated depression (12, 13). Antidepressants such as fluoxetine and imipramine have been shown to possess potent anti-inflammatory effects in the rat cerebral ischemia model of middle cerebral artery occlusion (14) and in a mouse model of Alzheimer’s disease (15). These studies demonstrated that fluoxetine and/or imipramine attenuated expression of TNF-α and IL-1β. Similarly, paroxetine was found to decrease the release of TNF-α in rat splenocytes (16) and inhibit infiltration of peripheral immune cells and production of the proinflammatory molecule substance P in an animal model of atopic dermatitis (17). However, little is known about the effects of paroxetine in the CNS, especially in the nigrostriatal DA system in the context of PD. Thus, the current study sought to determine whether paroxetine could prevent the degeneration of nigrostriatal DA neurons by inhibiting glial activation and, ultimately, reducing oxidative stress in the MPTP model of PD.

Materials and Methods

Animals and treatments

All of the experiments were done in accordance with approved animal protocol and guidelines established by Kyung Hee University. All of the experiments were conducted with 8- to 10-wk-old male C57BL/6 mice.
Animals were transcardially perfused with a saline solution containing 0.5% sodium nitrate and heparin (10 U/ml) and then fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were dissected from the skull, postfixed overnight in buffered 4% paraformaldehyde at 4˚C, stored in a 30% sucrose solution for 24–48 h at 4˚C until they sank, frozen, and sectioned on a sliding microtome in 30-μm-thick coronal sections. All of the sections were collected in six separate series and processed for immunohistochemical staining as described previously (19). The primary Abs included those directed against tyrosine hydroxylase (TH; 1:2000; Pel-Freeze, Brown Deer, WI), macrophage Ag complex-1 (MAC-1; 1:200; Serotec, Oxford, U.K.), ionized calcium-binding adaptor molecule 1 (Iba-1; 1:1000; Wako Chemicals, Osaka, Japan), CD68 (ED-1; 1:1000; Serotec), glial fibrillary acidic protein (GFAP; 1:5000; Neuronics, Edina, MN), NADPH-D (1:50; Thermoscientific, Fremont, CA), 8-hydroxy-2′-deoxyguanosine (8-OHdG; 1:200; JaICIA, Shizuoka, Japan), iNOS (1:200, BD Biosciences, San Jose, CA), IL-1β (1:200, R&D Systems, Minneapolis, MN), and p47phox (1:200; Santa Cruz Biotechnology, Santa Cruz, CA). Stained tissues were viewed and analyzed under a bright-field microscope (Nikon, Tokyo, Japan) or viewed with a confocal laser-scanning microscope (Olympus Optical, Tokyo, Japan). For Nissl staining, SN tissues immunostained with TH Ab were mounted on gelatin-coated slides, dried for 1 h at room temperature, and stained with 0.5% cresyl violet (Sigma-Aldrich). To validate immunohistochemical data, we performed immunohistochemistry with each isotype-matched control Ab (Supplemental Fig. 3, Supplemental Table I).

**HPLC analysis**

To measure contents of dopamine in STR, we performed reverse-phase HPLC with electrochemical detector as previously described (24). Dissected striatal tissues were homogenized with 0.1 M perchloric acid and 0.1 mM EDTA buffer and centrifuged at 9000 rpm for 20 min. The supernatant was injected into an autosampler at 4˚C (Waters 717 Plus Autosampler, Waters Division, Milford, MA) and eluted through μBondapak C18 column (3.9 × 300 mm × 10 μm; ESA, Chelmsford, MA) with mobile phase for catecholamine analysis (Chromsystems, Munich, Germany). The peaks of dopamine content were analyzed by ESA Coulombic II electrochemical detector and integrated using a commercially available program (Breeze; Waters). All of the samples were normalized for protein content as spectrophotometrically determined using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA).

**Quantitative real-time-PCR**

Animals treated with or without paroxetine (10 mg/kg, i.p.) were humanely killed 24 h after injection of MPTP, and the bilateral SN regions were immediately isolated. In brief, tissues were homogenized and total RNA was extracting with RNAzol B (Tel-Test, Friendswood, TX). Reverse transcription was performed with SuperScript II Reverse Transcriptase (Life Technologies, Rockville, MD). The primer sequences used in this study were as follows: 5′-CTGCTGGTTGGTGAACACATCTT-3′ (forward) and 5′-ATGTGATGACAAAGGGCGGAAAC-3′ (reverse) for iNOS; 5′-GCCAGTTGGGGAGGCGGCGG-3′ (reverse) for TNF-α; 5′-GCAACTGTTCCTGGAACAG-3′ (forward) and 5′-CTTCTTTGCGCCCTCAACCT-3′ (reverse) for IL-1β; 5′-GCAACTGTTCCTGGAACAG-3′ (forward) and 5′-GCAACTGTTCCTGGAACAG-3′ (reverse) for G3PDH. Real-time PCR was performed in a reaction volume of 10 μl including 2 μl 1:50 diluted reverse transcription product as a template, 5 μl SYBR Premix EX Taq (Takara, Shiga, Japan) and 10 pmol of each primer described above. The PCR amplifications were performed with 50 cycles of denaturation at 95˚C for 5 s, annealing at 60˚C for 10 s, and extension at 72˚C for 20 s using LightCycler (Roche Applied Science, Indianapolis, IN). The ΔCt value was determined by subtracting average Ct values of G3PDH from average Ct values of IL-1β, TNF-α, and iNOS from PCR reactions (Supplemental Table II). To express the relative amounts of IL-1β, TNF-α, and iNOS, ΔΔCt values were calculated by subtracting the ΔCt value of the control group from the ΔCt values of each group. The ratios of expression levels of IL-1β, TNF-α, and iNOS were calculated as 2^-[ΔΔCtΔCt].

**Measurement of TNF-α and IL-1β**

Animals treated with or without paroxetine (10 mg/kg, i.p.) were humanely killed 72 h after injection of MPTP, and the bilateral SN regions were immediately isolated. The production of TNF-α and IL-1β from mice SN tissues was determined by sandwich ELISA techniques. Tissues were homogenized in 200 μl ice-cold radioimmunooprecipitation buffer (60 mM NaCl, 0.1% SDS, 0.1% Nonidet P-40, 0.5% sodium deoxycholic acid, and 50 mM Tris [pH 8]) and centrifuged at 14,000 × g at 4˚C for 20 min. Equal amounts of samples (100 μg) were placed in ELISA kit
strips coated with the appropriate Ab. Sandwich ELISA was then performed according to the manufacturer’s instructions (BioSource International, Camarillo, CA). The detection limits of TNF-α and IL-1β were 5 and 25 pg/ml, respectively. We diluted TNF-α samples and IL-1β samples as 1:10, respectively.

Western blotting

For separating the cytosolic and membrane fraction, we dissected SN tissues from the animals at 3 d after injection of MPTP. As previously described (25, 26), SN samples were homogenized with using a glass homogenizer in ice-cold buffer consisting of the following: 20 mM HEPES, 250 mM sucrose, 10 mM KCl, 1.5 mM MgCl₂, 2 mM EDTA, and protease inhibitor mixture (Sigma-Aldrich). Homogenates were centrifuged for 5 min at 500 × g at 4°C, and supernatants were collected and centrifuged for 20 min at 13,000 × g at 4°C. The pellets were further centrifuged for 1 h at 100,000 × g at 4°C, and the resulting supernatants and pellets were designated as the cytosolic and membrane fractions, respectively. Equal amounts of proteins (30 μg) were separated by 12% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA) using an electrophoretic transfer system (Bio-Rad). The membranes were immunoblotted with goat anti-actin (1:1000; Santa Cruz Biotechnology), rabbit anti-p47phox (1:1000; BD Biosciences), and mouse anti-Rac1 (1:500; Santa Cruz Biotechnology). To determine the relative degree of membrane purification, the membrane fraction was subjected to immunoblotting for calnexin, a membrane marker, using a rabbit anti-calnexin (1:1000; Stressgen, Victoria, BC, Canada). For semiquantitative analyses, the densities of bands on immunoblots were measured with a computer imaging device and accompanying software (Fujifilm, Tokyo, Japan).

Statistical analysis

All of the values are expressed as mean ± SEM. Statistical significance (p < 0.05 for all analyses) was assessed by ANOVA using Instat 3.05 (GraphPad, San Diego, CA), followed by Student–Newman–Keuls analyses.

Results

Paroxetine protects nigrostriatal DA neurons from MPTP-induced neurotoxicity in vivo

In the brain, MPTP is converted to 1-methyl-4-phenyl-pyridinium (MPP⁺), an active toxic metabolite that is primarily responsible for MPTP neurotoxicity (27). Consistent with this, striatal MPP⁺ content correlates linearly with MPTP toxicity (28). Striatal levels of MPTP and MPP⁺ at specific time points after the last MPTP injection were measured and quantified by liquid chromatography with electrospray ionization mass spectrometry. MPP⁺ levels peaked within 30 min at 14.9 ± 2.4 μg/mg protein and then gradually declined, becoming almost negligible (0.2 ± 0.1 μg/mg protein) after 12 h. These data indicate that MPTP is completely converted to MPP⁺ and largely cleared within 12 h after injection. Because paroxetine was administered 12 h after the final injection of MPTP for all in vivo experiments, its effects could not be attributed to reduced metabolism of MPTP to MPP⁺ or uptake of MPP⁺ into DA neurons. At this time point, the extent of damage of the nigrostriatal DA system was assessed by TH immunostaining and the results are shown in Supplemental Fig. 1.

The mice in each group received four i.p. injections of MPTP (20 mg/kg) or PBS as a control at 2 h intervals. Seven days later, brains were removed and sections were immunostained for TH to specifically detect DA neurons, and then SN tissues were processed for Nissl staining. Similar to a recent report (18), there was a considerable loss of TH-immunopositive (ip) cells bodies in the SN (Fig. 1E, I/F) and fibers in the STR (Fig. 2C) at 7 d in the MPTP-injected mice compared with those of the PBS-treated control mice (Figs. 1A, 1B, 2A). TH-ip cells in the SN and their nerve terminals in the STR were quantified by stereological counts and densitometric analyses, respectively. MPTP treatment decreased the number of TH-ip neurons by 63% in the SN (Fig. 1H) and reduced the OD of TH-ip fibers by 62% in the STR (Fig. 2E) compared with those of the PBS-treated mice. Moreover, PBS-treated SN had prominent Nissl substances (Fig. 1B) when compared with MPTP-treated SN, showing marked loss of Nissl substances with gliosis (Fig. 1F). The stereological counts of the substances revealed that MPTP decreased the number of Nissl-positive neurons by 61% in the SN compared with those of the PBS-treated SN (Fig. 1A).

To investigate whether paroxetine altered MPTP-induced neurotoxicity of nigrostriatal DA neurons, we administered paroxetine (10 mg/kg body weight) for 6 d, starting 12 h after the last MPTP injection. The results of TH immunohistochemistry showed that paroxetine treatment effectively reduced MPTP-induced loss of DA neurons in the SN (Fig. 1G, 1H) and their nerve terminals in the STR (Fig. 2D). When quantified and expressed as a percentage of TH-ip neurons or fibers in the control SN and STR, respectively, paroxetine was found to increase the number of TH-ip neurons by 26% (Fig. 1I; p < 0.01) and the OD of TH-ip fibers by 21% (Fig. 2E; p < 0.001). Consistent with these results, paroxetine was found to increase the number of Nissl-stained neurons by 25% in the SN (Fig. 1J; p < 0.01). Paroxetine alone had no effects on the number of neurons in the SN (Fig. 1C, 1D, 1I) or DA fibers in the STR (Fig. 2B, 2E).

Paroxetine increases striatal dopamine levels and improves motor behavior in MPTP mice

We next determined whether paroxetine improves MPTP-induced motor deficits by testing performance on a rotarod apparatus with some modifications (9). Animals receiving various treatment
Paroxetine inhibits MPTP-induced expression of IL-1β, TNF-α, and iNOS

It has been shown that mice expressing a dominant-negative inhibitor of IL-1β-converting enzyme or those deficient in TNF-α or iNOS are resistant to MPTP-induced neurotoxicity (6, 32, 33). Thus, we examined whether paroxetine might modulate DA neuronal survival by affecting MPTP-induced expression of IL-1β, TNF-α, and/or iNOS in the SN. For this purpose, paroxetine was administered 12 h after the last MPTP injection and animals were sacrificed 12 h later. An analysis of dissected brain tissues by real-time PCR revealed that paroxetine administered after MPTP significantly inhibited MPTP-induced expression of proinflammatory cytokines, reducing IL-1β, TNF-α, and iNOS expression by 57, 60, and 76%, respectively (Fig. 4A), but had no effect when administered alone. Consistent with real-time PCR analyses, ELISA results showed that the levels of IL-1β and TNF-α protein were significantly increased at 3 d in the MPTP-treated SN compared with those of the PBS-treated SN (Fig. 4B). These MPTP-induced increases were significantly attenuated by treatment with paroxetine, which had no effect alone. To identify the cell types expressing IL-1β and iNOS protein, we performed
double-immunofluorescent staining in SN sections obtained 3 d after MPTP injection using a combination of Abs against MAC-1 and IL-1β or iNOS. Simultaneous imaging of immunofluorescence in the same tissue sections revealed that IL-1β (Fig. 4C) and iNOS (Fig. 4D) immunoreactivity was localized within MAC-1- and iNOS-expressing microglia.

**Paroxetine attenuates MPTP-induced oxidant production and oxidative stress via microglial NADPH oxidase**

The production of ROS, such as hydrogen peroxide and superoxide, are increased in the midbrain of MPTP-treated mice, and oxidant originating from microglia is thought to mediate the loss of DA neurons in the SN (34). Thus, we examined whether paroxetine rescued nigral DA neurons by inhibiting MPTP-induced oxidant production. The fluorescent product of oxidized hydroethidine (i.e., ethidium) was significantly increased in the MPTP-injected SN 72 h after administration (Fig. 5B) compared with that of the PBS-injected SN (Fig. 5A). Paroxetine dramatically decreased MPTP-induced oxidant production in the SN in vivo (Fig. 5C) but had no effect alone (data not shown).

NADPH oxidase is composed of the cytosolic components p47phox, p67phox, and Rac1 and the membrane components gp91phox and p22phox (35, 36). Translocation of NADPH oxidase subunits from the cytosol to the plasma membrane in activated microglia produces ROS, which ultimately contribute to DA neuronal death in the MPTP mouse model of PD (7). To investigate whether paroxetine modulates the activity of NADPH oxidase, we performed Western blotting after separating cell lysates into membrane and cytosolic components. Western blot analyses showed that the levels of p47phox and Rac1 in the MPTP-treated SN were significantly increased in membrane fractions 3 d after MPTP injection compared with those of the PBS-treated SN controls (Fig. 5D, 5E), indicating increased translocation of these subunits. Administration of paroxetine after MPTP injection significantly decreased translocation of p47phox and Rac1 from the cytosol to the membrane in the SN (Fig. 5D, 5E). Paroxetine alone had no effects on the level of p47phox or Rac1 in either cytosolic or membrane fractions. These results confirm that MPTP-induced activation of NADPH oxidase is inhibited by paroxetine. Moreover, the p47phox-ip cells present in the SN 3 d after MPTP injection corresponded to activated microglia (Fig. 5F) but not astrocytes or neurons (data not shown).
In MPTP mice, DA neuronal cell death is accompanied by increased levels of 8-OHdG, a marker of oxidative nucleic acid damage (37). To determine whether paroxetine prevented MPTP-induced oxidative damage to DNA in the SN, we immunostained for 8-OHdG. Our results revealed that the levels of 8-OHdG were dramatically increased in the SN 3 d after MPTP injection (Fig. 5H) compared with those of the PBS-treated SN (Fig. 5G). This MPTP-induced oxidative DNA damage was dramatically inhibited by paroxetine (Fig. 5F), which had no effect alone (data not shown).

**Paroxetine inhibits MPTP-induced astroglial activation and MPO expression in the SN in vivo**

It has been shown that MPO is upregulated in astrocytes in the SN of PD patients and MPTP-treated mice; furthermore, mice deficient in MPO are resistant to MPTP neurotoxicity (5). Thus, we next examined whether paroxetine inhibited MPTP-induced astroglial activation and expression of MPO in the SN, which would be predicted to enhance neuronal survival. To ascertain this, we immunostained sections adjacent to those used for MAC-1 immunostaining for GFAP and MPO 3 d after MPTP injection. In agreement with previous studies (6, 30), resting astrocytes (Fig. 6A; small somas and thin dendrites) were transformed into cells with enlarged bodies and thick dendrites in MPTP-treated mice (Fig. 6B). Treatment with paroxetine (10 mg/kg, i.p.) after MPTP injection dramatically decreased the number of activated astrocytes in the MPTP-treated mice (Fig. 6C). There was also an increase in immunostaining for MPO-ip cells in the MPTP-treated SN (Fig. 6E) compared with that of the control SN (Fig. 6D). An increase that was significantly attenuated by paroxetine (Fig. 6F).

Quantification of MPO-ip cells in the SN by stereological cell counts confirmed these results, showing that the number MPO-ip cells was 263-fold higher in the MPTP-treated SN than that in the SN of PBS-treated controls (Fig. 6G). Paroxetine reduced the number of MPTP-induced MPO-ip cells by 75% (Fig. 6G). As controls, vehicle (PBS) and paroxetine alone had no effects. Additional immunofluorescence staining revealed that MPO immunoreactivity was localized within astrocytes 3 d after MPTP treatment (Fig. 6H).

**Discussion**

In this study, we demonstrated that paroxetine protects nigrostriatal DA neurons from MPTP neurotoxicity in vivo by inhibiting brain inflammation and the resultant oxidative stress. We showed that paroxetine suppressed MPTP-induced ROS generation and reduced oxidative damage to nucleic acids by inhibiting microglia-derived NADPH oxidase and astrocyte-derived MPO, leading to survival of nigrostriatal DA neurons, recovery of striatal dopamine depletion in vivo, and reversal of motor dysfunction. Additionally, paroxetine attenuated the expression of proinflammatory cytokines and iNOS within activated microglia. To our knowledge, this is the first study to show that paroxetine prevents nigrostriatal DA neuronal death through blockade of glial activation in the MPTP model of PD.

Glial cells play an important role in supporting neurons in the CNS. However, in the presence of adverse stimuli, they may contribute to chronic, damaging inflammation and, ultimately, to neuronal cell death. Microglia are intrinsic immune effector cells that are dramatically activated in response to neuronal damage (38). Activated microglia produce several potentially neurotoxic substances, including ROS and/or proinflammatory cytokines (29). ROS, such as O$_2^-$ and O$_2^-$-derived oxidant molecules, may impose an oxidative stress on DA neurons and induce and/or exacerbate neurotoxicity (39). Several studies have demonstrated evidence of oxidative stress in PD patients and in the MPTP model of PD, including oxidative modifications to nucleic acids (40, 41). Importantly, these ROS can be produced by microglia-derived NADPH oxidase and are capable of causing oxidative stress in the MPTP model of PD (29). Several studies, including ours, have demonstrated that activation of microglial NADPH oxidase participates in DA neuronal death in vivo and in vitro (7, 8, 25). The results of the current study show that MPTP activated NADPH oxidase, as demonstrated by the translocation of cytosolic components of NADPH oxidase, p47$^{phox}$, and Rac1. NADPH oxidase activation resulted in increased O$_2^-$ and O$_2^-$-derived oxidants and DNA damage, as visualized by hydroethidium staining and 8-OHdG immunostaining in the SN, respectively. Treatment with paroxetine not only inhibited microglial NADPH oxidase activation but also mitigated ROS production and nucleic acid oxidation. These results verify that paroxetine inhibited MPTP-induced activation of NADPH oxidase and oxidative damage, thereby resulting in neuroprotection in the MPTP model.

Accompanying oxidative stress, other microglial-derived proinflammatory cytokines or cytotoxic factors may be involved in nigrostriatal DA neuronal death. It has been shown that iNOS (42) and proinflammatory cytokines, such as TNF-α and IL-1β (43), are increased in the brains of PD patients. Moreover, NO, generated by iNOS, may also be involved in the pathogenesis of PD (27), although the presence of iNOS in human microglia remains controversial (44). The neurotoxic effects of NO are attributed to its reaction with O$_2^-$ to form peroxynitrite, which can cause oxidative damage to proteins in the MPTP model (6). TNF-α and IL-1β, originating from activated glia, may trigger intracellular death-related signaling pathways or participate in...
the induction of iNOS expression in the MPTP model (45). The present data showed that MPTP increased the expression of iNOS, TNF-α, and IL-1β mRNA in the SN. Treatment with paroxetine inhibited the expression of these three molecules within activated microglia after MPTP injection, an effect that likely accounts, at least in part, for the observed paroxetine-induced neuroprotection. These results collectively suggest that paroxetine has anti-inflammatory properties that contribute to its neuroprotective effects. Similar to our results, a recent report showed that minocycline prevents MPTP neurotoxicity through relatively small changes in protein levels of IL-1β and TNF-α (46). Although genetic ablation of TNF-α decreased microglial activation and prevented disruption of blood–brain barriers, survival of DA neurons was not increased in the SN. Thus, biological relevance of these molecules may be varied depending on experimental conditions. Although we did not provide direct evidence, the present results combined with other observations carefully suggest that the biological relevance of these two molecules is due to each molecule and/or synergic effects combined with each other.

Although the role of astroglial activation in PD is disputed, several studies using the MPTP model support the idea that astroglial activation can contribute to the degeneration of nigrostriatal DA neurons via generation of neurotoxic substances (47, 48). One such substance expressed in activated astrocytes is MPO, which can use NO₂⁻ to generate RNS and cause DA neuronal death in the MPTP model (5). This increased expression of astroglial MPO mediates neurotoxicity on DA neurons under two possible mechanisms. First, MPO is known as the key enzyme for production of cytotoxic ROS/RNS (49). These astroglial MPO-derived oxidants can be released and then give deleterious effects on adjacent neurons (5). This was supported by evidence that increased levels of 3-chlorotyrosine– and HOCl-modified proteins and MPO-derived oxidative stress markers were observed in MPTP-treated SN (5). Another possible mechanism is the cross talk with other immune cells through MPO secretion (50). MPO can be secreted and then activate neutrophils through binding with CD11b/CD18 integrins. Because microglia also express CD11b/CD18 integrins, MPO secretion may participate in DA neuronal death in the MPTP model of PD via microglial activation. Although the cytokine-like effect of MPO is still unknown, this enzyme may play an important role in the signaling pathway of microglial activation, resulting in neuronal death. This is consistent with our present data showing that MPO was expressed in activated astrocytes in the MPTP-treated SN, as assessed by double-label immunostaining. Additional experiments showed that paroxetine attenuated MPO immunoreactivity in the SN. Taken together, our results suggest that paroxetine rescues DA neuronal death via suppression of MPO expression in activated astrocytes.

The most prominent biochemical change in the denervated STR caused by MPTP injection is a reduction in the levels of striatal dopamine (18). This biochemical deficit in mice yields a characteristic motor dysfunction that leads to increased latency to fall (deteriorated balance) on the accelerating rotarod (9). This notion is consistent with the present demonstration that motor performance on a rotarod is decreased with the loss of striatal TH terminals and, by extension, the consequent reduction of DA levels in the MPTP-treated STR (51). Paroxetine ameliorated these MPTP-induced motor deficits; accompanying this behavioral recovery was an increase in DA levels in the denervated STR. These behavioral and in vivo biochemical effects of paroxetine on the lesioned nigrostriatal DA system, taken together with our demonstration of inhibitory effects of paroxetine on glial activation-mediated oxidative stress, suggest that paroxetine and its analogues may have therapeutic value in the treatment PD symptoms related to neuroinflammation.

Finally, the most frequent neuropsychiatric symptom in PD is depression, which is observed in up to 46% of patients with PD (11). In this context, several clinical reports have concluded that paroxetine is a safe and effective drug for use in treating the depression associated with PD (12, 13). However, paroxetine has also been found to exacerbate parkinsonism symptoms associated with depression (52). Although our results suggest that paroxetine is beneficial in the treatment of aspects of PD related to glial activation, these conflicting clinical data suggest that the use of paroxetine in PD patients warrants caution (53).

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Disclosures

The authors have no financial conflicts of interest.

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the development of dopaminergic neurons via extracellular signal-regulated kinase and Nurr1 activation. 


