

Nicotine Prevents MPTP-induced Dopaminergic Neurodegeneration

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ABSTRACT

Epidemiological studies of Parkinson disease (PD) have found an inverse correlation between cigarette smoking and the risk of developing PD, which suggests that nicotine has a protective effect. Results from animal models of PD are conflicting, raising questions about a protective potential of nicotine. In this study, mice were pretreated with low-dose nicotine before MPTP administration, and examined to determine a neuroprotective potential of nicotine. The schedule of nicotine administration was selected to simulate the future human trials using this putative neuroprotective agent. Male C57Bl/6 mice were pretreated with nicotine for 5 days (0.2 mg/kg/d, i.p.). After the 5-day-pretreatment with nicotine only, nicotine and MPTP (30 mg/kg/d, i.p.) were co-administered for 1 to 5 consecutive days. The total dose of nicotine, 0.2 mg/kg/d for 6 to 10 days, is the lowest one ever studied. Tyrosine hydroxylase (TH) immunohistochemical staining of the nigral sections was performed. Over the experimental period, there was a significant reduction in the TH-positive cells. In the nicotine-MPTP group, the degree of TH neuron depletion was reduced at days 4 and 5 of co-administration. These findings suggest that the nicotinic neurotransmission on the dopaminergic neurons are promising targets for neuroprotective therapy of PD.

Key words: MPTP, nicotine, Parkinson disease, smoking

INTRODUCTION

Parkinson disease (PD) is a progressive neurodegenerative disorder that is characterized by bradykinesia, rigidity and rest tremor. Underlying the pathophysiology of PD is a selective loss of dopaminergic neurons in the substantia nigra pars com-

pacta (SNpc). However, the mechanism of the selective neuronal death in PD is largely unknown. The search for environmental factors or medications that prevent, arrest or slow the progressive degeneration of the dopamine neurons is important.

Interesting findings from clinical and animal studies suggest that cigarette smoking might have a neuroprotective action against PD. First, numerous epidemiological studies of PD have found an inverse correlation between cigarette smoking and the risk of developing PD, which suggests the potential protective effect of smoking (Baron, 1996; Hellenbrand et al., 1997; Chan et al., 1998;

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Smargiassi et al., 1998; Gorell et al., 1999; Hernan et al., 2002). Second, the administration of nicotine in experimental PD animal models has demonstrated that nicotine can counteract the dopamine cell loss (Carr and Rowell, 1990; Shahi et al., 1991; Janson et al., 1992; Janson and Moller, 1993; Maggio et al., 1998; Costa et al., 2001; Ryan et al., 2001).

However, a number of arguments have been raised against the existence of a true protective effect of smoking on the risk of PD (Morens et al., 1995; Riggs, 1996; Quik et al., 2007). Moreover, the results from animal experiments are discordant regarding the effects of nicotine against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- or 6-hydroxydopamine (6-OHDA)- induced dopamine cell loss, and the mesodiencephalic hemitranssection (Sershen et al., 1988; Carr and Rowell, 1990; Fung et al., 1991; Shahi et al., 1991; Behmand and Harik, 1992; Janson et al., 1992; Janson and Moller, 1993; Blum et al., 1996; Maggio et al., 1998; Costa et al., 2001; Ryan et al., 2001; Quik et al., 2007). In some of the previous studies, continuous nicotine administration failed to prevent dopamine depletion in the striatum after MPTP administration and in 6-OHDA lesions (Sershen et al., 1988; Fung et al., 1991; Behmand and Harik, 1992; Janson et al., 1992; Blum et al., 1996). In contrast, the intermittent application protected the dopamine neurons (Carr and Rowell, 1990; Shahi et al., 1991; Janson et al., 1992; Maggio et al., 1998; Costa et al., 2001). However, in other studies, the continuous nicotine administration protected the dopamine neurons (Janson and Moller, 1993; Ryan et al., 2001). An explanation for these discrepant results may be related to the dose and schedule of toxin and nicotine administrations (Quik et al., 2007).

The objective of this study was to determine whether the intermittent administration of low-dose nicotine and a treatment with nicotine before and after nigral damage may be neuroprotective in the MPTP mouse PD model. The schedule of nicotine administration was selected to simulate the future human trials using this putative neuroprotective agent. The mice were pretreated with nicotine (0.2 mg/kg/d, i.p.) for 5 days before administering the MPTP, co-administered the nicotine and MPTP for 1 to 5 days, and examined to determine if nicotine

has a protective effect against MPTP-induced dopamine cell loss. The total dose of nicotine, 0.2 mg/kg/d for 6 to 10 days, is the lowest one ever studied.

MATERIALS AND METHODS

Animals and treatment

This study received approval from the Institutional Animal Care and Use Committee, and conformed to the animal welfare guidelines set in the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80~23, 1985). Ten-week-old male C57Bl/6 mice (23~28 g, National Institute of Toxicological Research, Animal Breeding Laboratory, Seoul, Korea) were housed 4 per cage in a temperature-controlled room under a 12-hour light/dark cycle with access to food and water *ad libitum*. The animals were pretreated with nicotine (0.2 mg/kg/d, i.p.) for 5 days. After a 5-day-pretreatment with nicotine only, both nicotine (0.2 mg/kg/d, i.p.) and MPTP (30 mg/kg/d, i.p.) were co-administered for 1 to 5 consecutive days. At days 1, 2, 3, 4, and 5 of co-administration, the animals were sacrificed 4 hours after co-administering the nicotine and MPTP (nicotine-MPTP group, N=10). For a positive control, the mice were treated with normal saline for 5 days and then MPTP during the following days (saline-MPTP group). The animals treated with normal saline only served as a negative control.

Tyrosine hydroxylase immunohistochemistry

After anesthetizing the animals with ether, they were perfused transcardially with cold normal saline and subsequently cold 4% paraformaldehyde in phosphate buffer saline (0.1 M, pH 7.4). The brains were immediately removed, and immersed in the same fixative for 12 hours and then washed in a series of cold sucrose solutions with an increasing concentration. A block containing the midbrain was taken and was frozen in OCT, and serial transverse 12 μ m cryosections were taken. The sections for immunohistochemical labeling were first incubated for 48 hours at 4°C in phosphate buffer saline containing Triton X-100 (0.3%), bovine serum albumin (0.5 mg/ml), normal goat serum (3 drops/10 ml), and the appropriate dilution of the primary antibody: rabbit anti-mouse tyrosine hydroxylase monoclonal

antibody (Chemicon, USA, Cat. No. MAB318) at a dilution of 1 : 2,000. After removing the primary antibody and washing the sections with phosphate buffer saline, the sample sections were reacted for 90 minutes with the secondary antiserum containing the anti-rabbit IgG conjugated with biotin and Triton X-100 (0.3%). They were then processed using the avidin-biotin complex method (Vectastain, USA), and then reacted with 3,3'-diaminobenzidine. The sections were dried overnight, serially dehydrated in ethanol, and delipidified in xylene. The sections to be compared were treated in the same way throughout the procedure. In order to control nonspecific staining, additional sections were subjected to the immunostaining procedure without the primary antibodies. These sections did not exhibit any of the immunoreactivity described in this report.

Quantitative morphology

The numbers of SNpc dopaminergic neurons were counted. For each mouse, the sections corresponding to the representative mesencephalic plane (in the mouse brain atlas: 0.52 mm plane from the interaural line) were analyzed (Franklin and Paxinos, 1997). The mean number of neurons was cal-

culated by averaging the number of neurons counted from five sections. TH-immunoreactive neurons were counted manually in the SNpc (light microscopy; $\times 400$). The full extent of the structure in each section was examined on both the left and right sides. The SNpc and the ventral tegmental area were separated by white matter bundles, the medial lemniscus and the tractus opticus basalis. The neurons were counted only if they contained a nucleus surrounded by cytoplasm. The number of neurons was determined by researchers who were blinded to the treatment and the time point studied. One-way analyses of variance were used to compare the counts from each section. Two-tailed statistical significance tests were performed, and a p value < 0.05 was considered significant.

RESULTS

Effect of MPTP on the number of TH-immunoreactive cells in the saline-MPTP group

The time course of the changes in the mean number of the TH-immunoreactive neurons per representative mesencephalic plane for the SNpc, following a daily dose of 30 mg/kg of MPTP, is shown

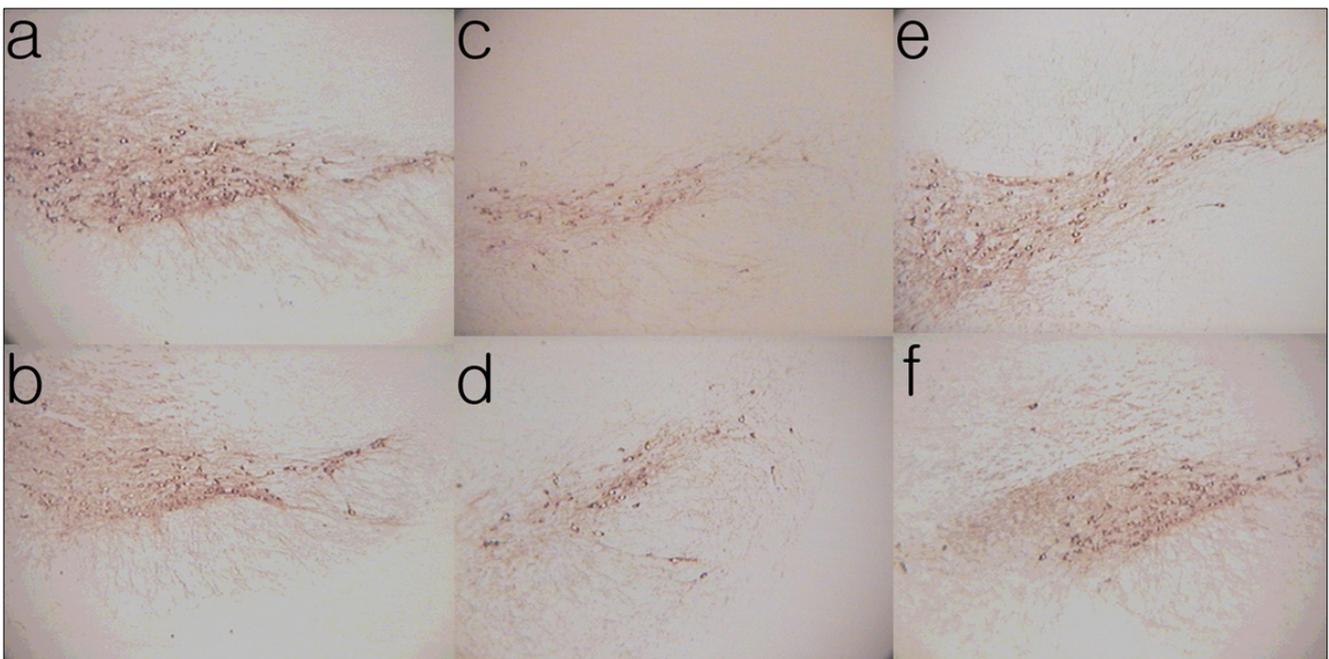


Fig. 1. Representative figures showing TH immunostaining of the nigra sections from the control, the saline-MPTP mice, and the nicotine-MPTP mice. In the saline-MPTP mice, a significant reduction in TH-immunoreactivity was observed at days 3, 4, and 5 (a: control, b: day 3, c: day 4, d: day 5, $\times 100$). In the nicotine-MPTP mice, the degree of TH-immunoreactive neuron depletion was reduced, compared to the saline-MPTP mice (e: day 4, f: day 5, $\times 100$).

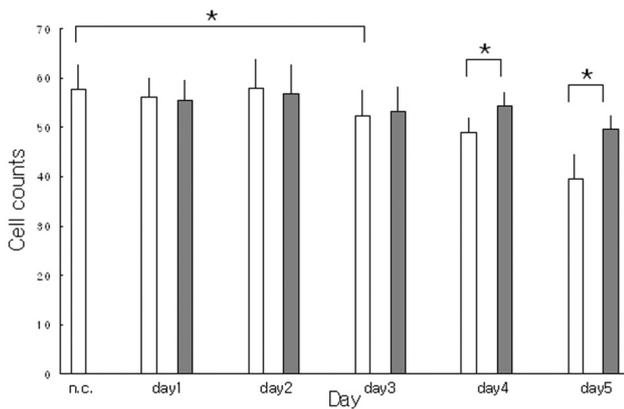


Fig. 2. Counts of the TH-positive neurons. White bars denote the saline-MPTP group, and gray bars the nicotine-MPTP group. In the saline-MPTP mice, a significant reduction in TH-immunoreactivity was observed from day 3. In the nicotine-MPTP mice, the degree of TH-immunoreactive neuron depletion was reduced at days 4 and 5. The data is expressed as a mean \pm standard error of the mean. Asterisks denote the statistical significance ($p < 0.05$).

in Fig. 1. Over the experimental period, there was a significant reduction in the TH-positive cells in the representative plane for the SNpc. The TH-positive cells of the saline-MPTP group were 56.0 ± 4.2 , 57.9 ± 6.1 , 52.3 ± 5.3 , 49.0 ± 3.1 , and 39.4 ± 5.2 at days 1, 2, 3, 4, and 5, respectively (saline-only controls: 57.6 ± 5.2 , Fig. 2). After three doses of MPTP, the TH immunoreactivity tended to decrease ($p < 0.05$).

Effect of MPTP on the number of TH-immunoreactive cells in the nicotine-MPTP group

An examination of the nicotine-MPTP group showed the protective effects of nicotine (Fig. 1). At days 4 and 5, the degree of TH-immunoreactive neuron depletion was reduced. The number of TH-positive cells was 54.3 ± 2.9 , and 49.5 ± 3.1 at days 4 and 5 (Fig. 2).

DISCUSSION

Determining the number of TH-immunoreactive neurons demonstrated that a treatment with a low dose of nicotine prior to and after inducing lesions in the SNpc dopaminergic neurons with MPTP reduced the resulting decrease in the number of TH-immunoreactive neurons. At 3 day after the injection, the number of SNpc dopamine neurons began to decrease. However, at days 4 and 5, the TH-immunoreactivity was increased in the mice

treated with nicotine. The dose selected in this study was the lowest one ever studied, and acted effectively. In previous studies, the intermittent or acute administration prior to administering the toxins proved to be critical for the neuroprotective effects (Carr and Rowell, 1990; Shahi et al., 1991; Janson et al., 1992; Maggio et al., 1998; Costa et al., 2001).

Several mechanisms for protecting the SNpc dopamine neurons by nicotine have been proposed. Nicotine prevents the N-methyl-D-aspartate receptor-mediated neurotoxicity in striatal neurons, and induces the increased mRNA levels for both the fibroblast growth factor-2 and the brain-derived neurotrophic factor, resulting in higher levels of these neurotrophins in the striatum (Marin et al., 1994; Maggio et al., 1998). Furthermore, nicotine may act as an antioxidant by inhibiting the Fenton reaction (Linert et al., 1999). As a novel neuroprotective agent in PD, the optimal dosage and administration schedule of nicotine needs to be determined. The neuroprotective mechanisms of nicotine, which have already been proposed or is waiting to be uncovered, should influence the selection of the optimal dose and schedule.

One of the pharmacological effects of nicotine is the change in the locomotor activity. The locomotor activity in normal rats was initially depressed, which then increased following the administration of nicotine (Clarke and Kumar, 1983). Tolerance to the depressant action develops with repeated exposure, but the stimulant effects persist. Similar effects have been noted following nicotine administration in mice with parkinsonism induced by MPTP (Sershen et al., 1987). Nicotinic acetylcholine receptors exist on the SNpc dopaminergic neurons (Sorenson et al., 1998). Stimulating these receptors leads to acetylcholine release and the regulation of the other neurotransmitters including dopamine (Newhouse et al., 1997). The amount of dopamine released is increased in the striatum of rats following the subcutaneous nicotine administration (Seppa and Ahtee, 2000). The situation, in which nicotine has a neuroprotective potential and increases the dopamine release, is akin to deprenyl (The Parkinson Study Group, 1993).

In summary, these results show that the administration of low-dose nicotine before and after the

nigral damage can support neuronal survival in the MPTP mouse PD model. These findings suggest that the nicotinic neurotransmission on the dopaminergic neurons are promising targets for neuroprotective therapy of PD.

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