Attenuation of Paraquat-Induced Dopaminergic Toxicity on the Substantia Nigra by (−)-Deprenyl *in Vivo*

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(−)-Deprenyl (DEP) had been shown to slow of progression of Parkinson's disease (PD). The present study sought to determine whether DEP would attenuate the nigrostriatal system damage induced by intranigral administration of the herbicide paraquat (PQ) as a model of parkinsonism *in vivo*. Neurochemical and behavioral observations of Wistar rats were the focus of our study. In the neurochemical observation, the PQ injected in the rats caused dose-dependent depletion of dopamine (DA) in the ipsilateral striata. The coadministration of DEP with PQ partially increased the striatal DA level. The prediction of the striatal DA levels was calculated by regression coefficients obtained from multiple linear regression ($r^2 = 0.82$): DA level (% of control) = 103.34 − 9.58 PQ (nmol) + 0.79 DEP (nmol). It was demonstrated that the high dose of 20 nmol DEP could significant attenuate the PQ (5 nmol)-elicited dopaminergic toxicity ($p < 0.05$). In the behavioral observation, the intranigral injection of PQ into the rats caused a rotation behavior contralateral to the lesioned side in response to apomorphine administration (0.5 mg/kg, sc). This apomorphine-induced rotational behavior could also be attenuated significantly by coadministration of DEP (20 nmol) and PQ (5 nmol) compared with PQ-treated (5 nmol) animals ($p < 0.05$). The above observations indicate that DEP could provide a protective effect on the moderate injury elicited by PQ toxicity of the nigrostrital dopaminergic system. DEP might be a useful therapeutic agent in treating patients with early-stage PD. © 2001 Academic Press

Key Words: paraquat; deprenyl; dopamine; Parkinson's disease.

Progressive loss of nigrostriatal dopaminergic (DAergic) neurons may elicit Parkinson's disease (PD). Increasing evidence supports the hypothesis that environmental factors contribute to PD development (for review, see Tanner, 1989). The discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration results in a syndrome resembling the clinical, biochemical, and pathological features of PD in hu-

mans and experimental animals has stimulated a search for the environmental chemicals resembling MPTP that might cause PD (Langston *et al.*, 1983; Burns *et al.*, 1983; Ballard *et al.*, 1985). MPTP is converted by the action of monoamine oxidase B (MAO-B) to 1-methyl-4-phenylpyridinium ion (MPP^+), the effective neurotoxin (Javitch *et al.*, 1985; Singh *et al.*, 1988). Paraquat (1,1′,-dimethyl-4,4′-bipyridium dichloride; PQ) is a widely used herbicide. Because of the striking similarity in structure between PQ and MPP^+, PQ may cause toxic effects to the DA neurons as MPP^+. From the epidemiological observation, a strong correlation has been reported between the incidence of PD and the level of PQ (Barbeau *et al.*, 1986; Ho *et al.*, 1989; Hertzman *et al.*, 1990; Tanner and Goldman, 1996; Liou *et al.*, 1997). PQ has been shown to induce nigrostriatal neuronal degeneration in rodents (Endo *et al.*, 1988; Fredriksson *et al.*, 1993; Liou *et al.*, 1996; Brooks *et al.*, 1999). These findings suggest that PQ might play an important role in the pathogenesis of PD.

The mechanism of PQ has been suggested to be mediated by a series of free radical reactions (Bus *et al.*, 1976; Trush *et al.*, 1981, Kadiiska *et al.*, 1993). These highly toxic radicals are extremely reactive with macromolecules and may result in multiple organ injuries, leading to death in several species. Recognizing the fact that PQ is a strong redox agent and contributes to the formation of reactive oxygen species (ROS), attempts have been made to explore pharmacological strategies that may reduce the formation of these ROS and/or prevent their toxic effects. Intranigral infusion of Cu-free superoxide dismutase (SOD) prevents PQ-induced behavioral stimulation and electrocortical epileptogenic discharges in rats (Iannone *et al.*, 1991). Melatonin protects against PQ-induced toxicity and genotoxicity is mediated by its free radical scavenging activity (Melchiorri *et al.*, 1996, 1998).

(−)-Deprenyl (selegiline; DEP), a selective type B monoamine oxidase inhibitor, is an adjuvant to levodopa therapy of PD. DEP has been shown to delay the emergence of disability and the progression of signs and symptoms in the early phase of the disease (Parkinson Study Group, 1993). In addition to its antiparkinsonian actions, DEP has prevented the neurotoxicity of MPTP in monkeys (Cohen *et al.*, 1984) and has protective

effects against toxin-induced neuronal deterioration (Finnegan *et al.*, 1990; Wu *et al.*, 1993, 1996; Salonen *et al.*, 1996). DEP has been reported to reduce the toxic effect of MPP^+ via its antioxidant effect *in vivo* (Wu *et al.*, 1993, 1996). Low doses of DEP administration after the insult have been shown to possess neuronal rescue-like properties with an unknown mechanism, possibly independent of MAO-B inhibition, in different degenerative models both *in vitro* and *in vivo* (Ansari *et al.*, 1993; Wu *et al.*, 1993; Ju *et al.*, 1994; Tang *et al.*, 1998). Furthermore, DEP has shown antiapoptotic properties *in vitro* (Tatton *et al.*, 1994; Wadia *et al.*, 1998; Carlile *et al.*, 2000). DEP is thus considered to be a potential effective neuroprotective agent.

Both MPP^+ and PQ have similar effects on free radical generation (Johannessen *et al.*, 1986; Fallon *et al.*, 1997; Lotharius *et al.*, 1999) and could induce nigrostriatal degeneration. However, The effect of DEP on PQ-elicited nigrostriatal dopaminergic toxicity *in vivo* remains unclear. The primary goal of this study was to examine the possibility of whether DEP could attenuate nigrostriatal system damage by the intranigral administration of the herbicide PQ. Drug-induced behavioral asymmetries observed on the tested animals with unilateral destruction of the nigrostriatal DA system were used as an experimental model to study PD (Ungerstedt and Arbuthnott, 1970) and to evaluate the recovery of DA neuronal function by therapeutic approaches (Perlow *et al.*, 1979; Wang *et al.*, 1995; Hoffman *et al.*, 1997). The present study was performed by direct intracerebral injection of PQ and DEP into the unilateral substantia nigra compacta (SNc) of the rats. The effects of DEP on PQ-induced toxicity in nigrostriatal system were assessed by striatal neurochemistry and behavioral observations *in vivo*.

MATERIALS AND METHODS

Animals. Male Wistar rats (3 months old) were housed 4 per cage in a temperature-regulated room (23 ± 2°C) and maintained on a 12:12 h light: dark cycle (lights on at 0600 h), with food and water available *ad libitum*. The animals were acclimated for at least one week before the surgical procedure. The rats weighed 250–300 g at the time of the stereotaxic operation.

Stereotaxic operation. The rats were anesthetized by pentobarbital sodium (30 mg/kg, ip) and mounted in a stereotaxic apparatus (David Kopf instruments). A 2-cm long incision was made in the midline of scalp to expose the skull and a 0.5-mm-diameter hole was drilled in the calvaria over the right SNc for the insertion of a 30-gauge injection needle by using the Paxinos and Waston (1986) coordinates: AP 5.3, L 2.0, H 7.8 mm. All rats underwent intranigral injection; they were divided into 4 major groups with different drugs administration protocol. Group A received intranigral injection of PQ (2.5, 5, and 10 nmole, respectively, $n = 7$ in each group). Group B received intranigral co-administration of PQ and DEP. They were administrated with the following listed dosage regimen of PQ and DEP (nmole): PQ 2.5 + DEP 5, PQ 2.5 + DEP 20, PQ 5 + DEP 5, PQ 5 + DEP 20, PQ 10 + DEP 5, and PQ 10 + DEP 20, respectively ($n = 7$ in each group). Group C received intranigral injection of DEP 5 and 20 nmole, respectively ($n = 7$ in each group). Group D, the sham-operated control animals, received intranigral injection of saline ($n = 7$). PQ (Sigma, St. Louis, MO, USA) and DEP (RBI, Natick, MA, USA) were dissolved in normal saline and were slowly infused

(0.2 μl/min) by an infusion pump through a 30-gauge stainless steel needle into the right SNc. The injection needle was left in position for a further 2 min following the infusion of total 1 μl of drug solution. The position of the injection site was verified histologically at postmortem.

Striatal dopamine level assayed by HPLC. Animals were scarified by decapitation two weeks after intranigral injection of substance. The brains were quickly removed and immediately frozen in isopentane at–20°C. Serial cryostat sections (200 μm thick) were cut in the frontal plane, the tissues of the striatum micropunched (Palkovits, 1973), and the micropunched tissues homogenized in perchloric acid (0.1 M), using an ultrasonic cell disruptor (Heat System Ultrasonic, USA), then centrifuged at 3000 g for 3 min. The supernatants were removed and assayed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) using high-pressure liquid chromatography (HPLC) with electrochemical detection. A C-18 reverse phase column (5 μm, 12.5 cm long, Waters Chromatography Division, USA) was connected to a carbon electrode set at a potential of 0.75 V relative to the Ag/AgCl reference electrode, together with an LC-4 amperometric detector (Bioanalytic System Inc., USA) in the HPLC system (Tsai *et al.*, 1994; Liou *et al.*, 1996).

Each liter of the mobile phase used in the experiment contained heptanesulfonic acid (1.75 g), disodium EDTA (0.1 g), triethylamine (3.5 ml), phosphoric acid (4 ml) and acetonitrile (40 ml) in distilled water (Tsai *et al.*, 1994; Liou *et al.*, 1996). It was filtered and degassed just prior to use. External standards of DA, DOPAC and HVA were dissolved in perchloric acid (0.1 M) and run at the same time as the experimental samples. The protein contents were assayed from tissue pellets solubilized in NaOH (0.5 M) by the method of Lowry *et al.* (1951).

Behavioral observations and pharmacological tests. The spontaneous behavior of rats was observed after unilateral intranigral injection of substances, when left undisturbed in their cages or when disturbed by handling, pinching of their tails, and sudden noise. Apomorphine-induced rotational behavior was assessed utilizing a computerized rotometer system (Hudson *et al.*, 1993) described by Ungerstedt and Arbuthnott (1970). Rats were tested 10 days after intranigral injection of the test substance or saline. The rats were placed in rotometer bowls and secured to the counting head by a thoracic harness. After acclimation for at least 10 min, an injection of apomorphine (Sigma, St. Louis, MO, USA, 0.5 mg/kg, *sc*) was administered. Animals that had completed a 360° circle towards the intact (contralateral) side and/or the lesion (ipsilateral) side were monitored and recorded by computer every minute for 2 h continuously (Hudson *et al.*, 1993; Wang *et al.*, 1995).

Statistical methods. All observed values are expressed as the mean ± SE. The overall analyses for comparison of mean values were performed by one-way analysis of variance (ANOVA). Statistical significant level (α) was set up at 0.05. Post-hoc tests (i.e., Scheffe's method) were used to examine the specific difference between any of two groups while the results of the overall analysis reach statistical significance. The relationship of DA to PQ and DEP was analyzed by the use of multiple linear regression. The association between the rotational behavior and striatal DA levels was analyzed by simple linear regression. The strength of this relationship was quantified by square of correlation coefficient (r^2).

RESULTS

Striatal Dopamine Content Assayed by HPLC

The injection of PQ into the SNc resulted in reducing the striatal DA levels. The effect depended on the dose of PQ injected. Following intranigral administration of PQ (2.5, 5, and 10 nmole), the DA levels in the ipsilateral striata were decreased to 81.6, 47.9 and 13.5% of saline-treated control. PQ (5 and 10 nmole) significantly decreased the striatal DA levels compared with controls ($p < 0.05$).
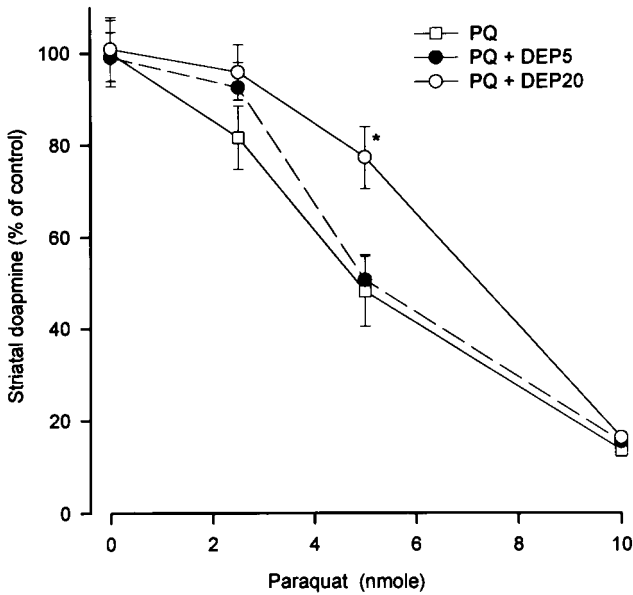
FIG. 1. Effects of unilateral intranigral administration of (–)-deprenyl (DEP) and/or paraquat (PQ) on dopamine (DA) contents of the ipsilateral striatum. Animals were scarified two weeks after treatment ($n = 7$, in each dosage regimen). The striata were micropunched and assayed for DA level by HPLC. The striatal DA contents were expressed as percentage of controls. It was notable that the animals which co-administrated DEP (20 nmole) and PQ (5 nmole) were significantly increased in the striatal DA levels compared with those of PQ (5 nmloe) ($p < 0.05$). PQ + DEP 5: co-administration of PQ and 5 nmole DEP, PQ + DEP 20: coadministration of PQ and 20 nmole DEP.

The effects of DEP on PQ-elicited striatal DA toxicity were showed in Fig. 1. Co-administration of low dose of PQ (2.5 nmole) and DEP (5 and 20 nmole) into SNc attenuated the PQ induced toxicity manifested as the ipsilateral striatal DA levels to be increased to 92.4% and 95.8% of control. In the animals which received PQ (5 nmole) elicited moderate striatal DA toxicity (47.9%), co-administration of PQ (5 nmole) and DEP (5 and 20 nmole) increased the DA levels to 50.4% and 77.2% of control. Co-administration of PQ (10 nmole) and DEP (5 and 20 nmole) increased the striatal DA contents to 16.5% and 16.2% of control. Results of ANOVA showed only the effect of DEP on PQ (5 nmole) on DA level reach statistic significance (F $_{(2,18)}$ =5.84, $p = 0.01$). Subsequent Scheffe's post-hoc assessment showed that the DA levels in the group of co-administration of DEP (20 nmole) and PQ (5 nmole) were significant higher than those in PQ (5 nmole) and co-administration of PQ (5 nmole) and DEP (5 nmole) ($p < 0.05$). PQ (5 nmole) elicited moderated DA toxicity was significantly attenuated by co-administration of high dose of DEP (20 nmole). When the striatal DA level depleted more than 85% by PQ (10 nmole), co-administration DEP (5 and 20 nmole) could not attenuate the PQ elicited severe striatal DA toxicity in rats.

We further analyzed the dose-response effects between the PQ and DEP in the striatal DA level. The prediction of striatal DA levels was calculated by regression coefficients obtained from multiple linear regression ($r^2 = 0.82$): striatal DA level (% of control) = 103.34 –9.58 PQ (nmole) + 0.79 DEP (nmole).

Table 1 showed the effects of DEP on PQ-treated animals on levels of striatal DA and its metabolites. It was demonstrated that DEP in various doses led to a recovery of DA, DOPAC and HVA in the neostriatum. The PQ-induced increase in DA utilization as seen from the (DOPAC+HVA)/DA ratio was not counteracted by the DEP treatment.

Behavioral Studies

Figure 2 showed the apomorphine (0.5 mg/kg, *sc*) induced rotational behavior in rats 10 days after unilateral intranigral

TABLE 1
Effects of (−)-Deprenyl on Paraquat-Treated Animals on Levels of Striatal Dopamine and Its Metabolites

	DOPAC (pg/μg)	DA (pg/μg)	HVA (pg/μg)	DOPAC/DA	HVA/DA
Control	27.94 ± 2.58	184.64 ± 13.26	9.24 ± 1.12	0.14 ± 0.01	0.05 ± 0.01
DEP (5)	28.69 ± 2.72	182.72 ± 10.37	10.05 ± 1.67	0.14 ± 0.01	0.05 ± 0.01
DEP (20)	31.29 ± 4.36	186.26 ± 12.79	11.51 ± 1.70	0.13 ± 0.01	0.05 ± 0.01
PQ (2.5)	24.42 ± 3.15	150.62 ± 12.67	6.38 ± 1.27	0.16 ± 0.01	0.04 ± 0.01
PQ (2.5) + DEP (5)	22.04 ± 2.59	170.69 ± 10.04	7.05 ± 1.71	0.13 ± 0.01	0.04 ± 0.01
PQ (2.5) + DEP (20)	23.08 ± 4.21	176.84 ± 11.02	7.21 ± 1.26	0.13 ± 0.01	0.05 ± 0.01
PQ (5)	20.17 ± 2.51	88.62 ± 14.17	5.14 ± 0.94	0.22 ± 0.04	0.06 ± 0.01
PQ (5) + DEP (5)	20.08 ± 3.01	93.12 ± 10.21	5.09 ± 0.76	0.21 ± 0.03	0.06 ± 0.01
PQ (5) + DEP (20)	25.26 ± 3.28*	142.48 ± 12.39*	6.20 ± 0.81*	0.19 ± 0.02	0.05 ± 0.01
PQ (10)	6.94 ± 1.24	24.84 ± 2.76	2.70 ± 0.24	0.25 ± 0.04	0.11 ± 0.01
PQ (10) + DEP (5)	7.32 ± 1.84	30.19 ± 2.41	2.19 ± 0.76	0.24 ± 0.04	0.10 ± 0.01
PQ (10) + DEP (20)	7.20 ± 1.64	29.92 ± 2.10	2.41 ± 0.92	0.24 ± 0.03	0.08 ± 0.01

Note. Concentrations (in pg/μg protein) of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the striatum 2 weeks after unilateral intranigral injection of (−)-deprenyl (DEP) and/or paraquat (PQ) were listed. Parentheses show the concentration (nmole) of injected PQ and/or DEP. Values are expressed as the mean ± SE for each group ($n = 7$).

* Statistically significant difference compared with the same dose of PQ-treated animals and the coadministration with DEP and PQ-treated rats ($p < 0.05$).
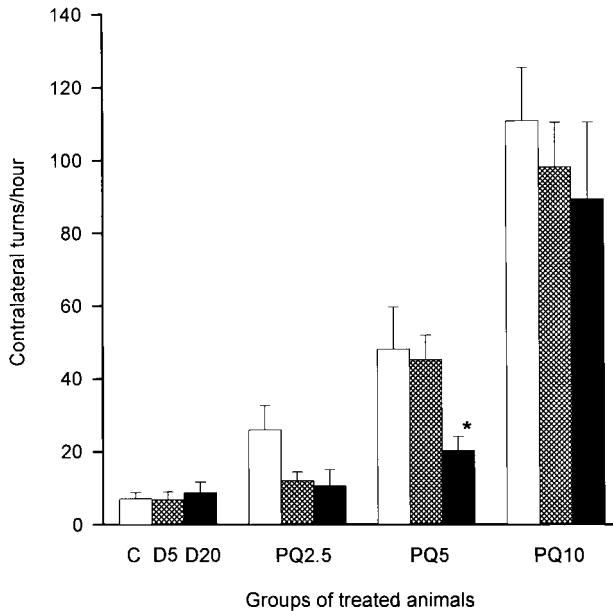
FIG. 2. Effects of apomorphine (0.5 mg/kg, *sc*) on contralateral rotational behavior in rats 10 days after unilateral intranigral injection of (–)-deprenyl (DEP) and/or paraquat (PQ). Each column showed the mean ± SE from 7 rats in each group of treatment. At groups C, D5, and D20, the rats received intranigral injection of saline (control), DEP 5 nmole, and 20 nmole, respectively. At PQ 2.5, PQ 5, and PQ 10, the rats received intranigral administration of PQ 2.5 nmole, 5 nmole, and 10 nmole with or without co-administration of DEP. White columns represented the rats which did not co-administrate with DEP. The hatch columns represented the rats which co-administrated with DEP 5 nmole, and the black columns represented the rats which co-administrated DEP 20 nmole. The statistical significant difference in the numbers of contralateral turning per hour compared with the same dose of PQ- and DEP co-administration with PQ-treated group were shown, $p < 0.05$ (*). Noted that DEP (20 nmole) co-administration with PQ (5 nmole) significantly attenuated the PQ-elicited (5 nmole) rotational behavioral after apomorphine treatment.

injection of DEP and/or PQ. Apomorphine induced rare contralateral rotational behavior in the saline-treated controls (7 ± 2 turns/h, $n = 7$) and DEP-treated (5 and 20 nmole) rats (7 ± 2 and 9 ± 4 turns/h, $n = 7$). In rats receiving intranigral injection of PQ (2.5, 5 and 10 nmole), the apomorphine elicited contralateral rotational behavior at a rate of 26 ± 8, 48 ± 12, and 110 ± 14 turns/h ($n = 7$ in each dosage), which was statistically significant compared with saline- and DEP-treated animals ($p < 0.05$).

Co-administration of PQ and DEP into SNc could attenuate the apomorphine induced contralateral rotational behavior in rats treated with PQ dose-dependently. Apomorphine induced contralateral rotational behavior in the rats that received co-administrated low dose of PQ (2.5 nmole) and DEP (5 and 20 nmole) at the rates of 12 ± 2 and 11 ± 5 ($n = 7$). In the rats injected with the moderate dose of PQ (5 nmole) and DEP (5 and 20 nmole), apomorphine elicited contralateral rotational behavior in rats at the rates of 45 ± 7 and 20 ± 4 ($n = 7$). Likewise, apomorphine elicited rotational behavior in the rats treated with high dose of PQ (10 nmole) and DEP (5 and 20

nmole) at the rates of 97 ± 12 and 89 ± 23 ($n = 7$). Among these treated rats, co-administration of DEP (20 nmole) and PQ (5 nmole) revealed significantly that decreased apomorphine elicited rotational behavior compared with those rats that received PQ (5 nmole) ($p < 0.05$).

There is a well correlation between the change in striatal DA levels and behavior measure of rotation using individual animal data ($r^2 = 0.73$). A simple linear regression equation for the relationship between rotational behavior and striatal DA levels was: animal total contralateral turns/h = –0.56 (striatal DA levels) + 108.26.

DISCUSSION

The present study demonstrated that DEP could attenuate the toxic effects elicited by PQ on striatal DA and its metabolites. In accordance with neurochemical findings, DEP caused significant diminution of apomorphine-induced rotational behavior in rats whose striata had been unilateral lesioned with PQ. These neurochemical and behavioral results suggest that DEP could protect nigrostriatal neurons from PQ-induced DA-ergic toxicity.

PQ has been extensively studied as a both a pulmonary and neurotoxicant. Unlike the accidental and high-level PQ exposure that produced acute pulmonary toxicity, it was presumed that the chronic low-level nonpulmonary toxic doses could produce a different syndrome defined by damage to basal ganglia and parkinsonism. Little is actually known about actual human exposure levels to PQ and the routes by which they occur, although it is likely that they would include inhalation, per oral ingestion or through transdermal absorption. Low levels of PQ appear to be retained in tissue such as muscle after subcutaneous exposures from where it can then be slowly released into blood (Sharp *et al.*, 1972). Several investigators suggest PQ was a causal factor for PD (Barbeau *et al.*, 1986; Ho *et al.*, 1989; Hertzman *et al.*, 1990; Tanner and Goldman, 1996; Liou *et al.*, 1997). Bocchetta and Corsini (1986) reported two patients believed to suffer from PQ-induced parkinsonism. Sanchez-Ramos *et al.* (1987) reported a young farmer who had been exposed to PQ and affected with PD. Animal studies also showed that repeated systemic injection of PQ was sufficient to reduce locomotor activity and the decline of DA-ergic neurons in mice (Brooks *et al.*, 1999). Systemical administration of [14C] PQ indicated that the herbicide did partition in the CNS presumably by penetration of the endothelium comprising the blood–brain barrier (Lindquist *et al.*, 1988). The autoradiographic studies with systemically applied [14C] PQ also demonstrated that the compound was most highly confined to neuromelanin producing cells such as those in the SNc (Lindquist *et al.*, 1988). Such a pattern of distribution suggests that PQ can preferentially enter and/or be maintained in cells which elaborate neuromelanin.

PQ possesses marked neurotoxicity for the nigro-stritatal DA-ergic system (Endo *et al.*, 1988; Fredriksson *et al.*, 1993;

Liou *et al.*,1996; Yang and Sun, 1998; Brooks *et al.*, 1999). We found that the intranigral administration of PQ caused a dose-dependent decrease of DA in the major terminal area, the striatum. Similar pharmacological results were seen in MPP[+]-treated rats (Bradbury *et al.*, 1986; Sirinathsinghji *et al.*, 1988; Sun *et al.*, 1988). Our results revealed that DEP could provide protective effect against PQ-induced moderate nigral injury as reflected by a 50% or less depletion of DA in the striatum. As the dose of PQ elicited more than 85% depletion of striatal DA, DEP could not exert its protective effect. These observations were consistent with previous findings on the effect of DEP in MPP[+]-treated rats *in vivo* (Wu *et al.*, 1993, 1996).

Unilateral lesions within the nigrostriatal DA-ergic system of the rats induced a rotational behavior, which reflected an imbalance of DA-ergic activity in the striata (Ungerstedt and Arbuthnott, 1970; Creese *et al.*, 1977). Behavioral supersensitivity was manifested by the rats rotating in a direction contralateral to the side of the lesion following the systemic administration of a DA agonist, such as apomorphine, and appeared to be caused by supersensitivity of the denervated striatal DA receptors (Ungerstedt and Arbuthnott, 1970; Creese *et al.*, 1977; Hudson *et al.*, 1993). This behavior correlated with the extent of DA depletion and thus was utilized as a functional index for the recovery of DA-containing neurons following therapeutic interventions (Perlow *et al.*, 1979; Wang *et al.*, 1995; Hoffman *et al.*, 1997). The present experiment showed that the apomorphine could induce a degree of vigorous contralateral rotational behavior in rats after unilateral intranigral injection of PQ in a dose-dependent manner. These rotational behaviors were significantly reduction by administration of DEP (20 nmole) in the PQ-treated (5 nmole) animals. This restored rotational behavior manifestation was similar to the fetal DA homografts in unilateral 6-hydroxy-dopamine (6-OHDA) lesioned rats (Wang *et al.*, 1995).

The present study showed that apomorphine-induced contralateral circling behavior seen after unilateral intranigral injection of PQ correlated well with the decrease in striatal DA levels, and the neurochemical and behavioral changes induced by PQ were attenuated by administration of DEP. It suggested that the decrease in turning was related to the protective action of DEP on PQ-induced DA depletion.

The increases in the ratio of DA metabolites to DA indicated that the compensatory mechanisms could be aroused to increase the DA release in the brain of PD or in brain of the tested animals that had been treated with 6-OHDA, MPTP and PQ (Palkovits, 1973; Sirinathsinghji *et al.*, 1988; Liou *et al.*, 1996). The present experiment showed no change in the ratio of DA metabolites to DA between the PQ-treated and co-administrated with PQ and DEP-treated rats. These data indicated that DEP might not increase striatal DA levels via its metabolites such as methamphetamine or amphetamine (ThyagaRajan *et al.*, 1999), which had been shown to enhance DA release or alter the DA turnover (reflected as the ratio of DOPAC/DA) by its inhibitory action of MAO-B activity.

Although the mechanisms of how DEP operated its effects on PQ were not known for certain, several lines of evidence indicated that DEP might exert its protective actions via its antioxidative properties. DEP had been reported to suppress the formation of free radicals from MPTP and its analogues (Chiueh *et al.,* 1992; Wu *et al.*, 1996) and it could scavenge hydroxyl and peroxyl radicals both *in vivo* and *in vitro* (Cohen and Spina, 1989; Wu *et al.*, 1993, 1996; Thomas *et al.*, 1997). Besides, DEP could prevent quinolinic acid-induced hippocampal damage by a mechanism of interfering ROS generation (Behan *et al.*, 1999). In addition to the putative antioxidant properties, the neuroprotection of DEP had been considered to be associated with several intracellular mechanisms, including enhancement of antioxidant enzymes, such as SOD and catalase (Carrillo *et al.*, 1992), inhibition of the uptake of DA (Zsilla *et al.*, 1986), preservation of mitochondrial membrane potential (Wadia *et al.*, 1998), activation of antiapopotic system (Tatton *et al.*, 1994; Maruyama and Naoi, 1999; Carlile *et al.*, 2000), and increase in mRNA of trophic factors, such as BDNF (Tang *et al.*, 1998), NGF (Semkov *et al.*, 1996) and CTNF (Seniuk *et al.*, 1994). DEP might probably act like an antioxidant and/or scavenger, inhibiting the free radical formation and oxidative injury elicited by PQ in the nigrostriatal system in this experimental paradigm, but the exact mechanism remained to be elucidated.

PQ is a generator of superoxide anions which were known to induce cell damage, either directly by blocking cell respiratory chain (Patel *et al.*, 1996), or indirectly by activation of cholinergic (Seto and Shinohara 1988) and glutamatergic (Pellegrini-Giampietro *et al.*, 1990; Bagetta *et al.*, 1992) transmission that causes neuronal death via an excitotoxic mechanism. Polyamine metabolism was a secondary target for PQ toxicity. PQ can interfere with polyamine synthesis and uptake, producing arrested growth and eventually cell death (Masek and Richards, 1990; Bayoumi *et al.*, 2000). The intracellular spermidine and spermine pools were negatively affected with PQ in a dose-response manner (Bayoumi *et al.*, 2000). Our results illustrated that DEP apparently had protective effect on rats which were given a moderate, but not high, dose of PQ elicited neurotoxicity. As to whether a high dose of PQ might possess more potent toxicity to generate mechanism that was beyond the neuroprotective action of DEP *in vivo*, it deserves a further investigation.

In conclusion, DEP could reduce the moderate neurotoxic effect of MPP[+] and PQ in rats. These data revealed that DEP might be a useful therapeutic agent in treating the early stage of PD patients.

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