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The potentiating effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on paraquat-induced neurochemical and behavioral changes in mice

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Abstract

Although the etiology of Parkinson's disease (PD) is not fully understood, there are numerous studies that have linked the increased risk for developing PD to pesticides exposure including paraquat (PQ). Moreover, the exposure to a combination of compounds or chemical mixtures has been suggested to further increase this risk. In the current study, the effects of PQ on the nigrostriatal dopaminergic system in male C57BL6 mice exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were examined to assess the impact of toxic substance mixtures exposure on neurochemical and behavioral changes. In this study, a low non-toxic dose of MPTP (10 mg/kg) was injected once a day for 5 days and was followed by PQ (7 mg/kg) once a day for 6 days (subacute protocol) or once a week for 10 weeks (chronic protocol). The results from the subacute protocol showed that PQ reduced the turnover of dopamine (DA) as indicated by a 21% and a 22.3% decrease in dihydroxyphenyl acetic acid (DOPAC), homovanillic acid and increased *S*-adenosyl methionine/*S*-adenosyl homocysteine index (SAM/SAH) by 100%. However, the administration of PQ to MPTP primed mice resulted in the decrease of DOPAC, HVA, DA, by 35.8%, 35.2% and 22.1%, respectively. In addition, PQ decreased the total number of movements (TM) by 28% but MPTP plus PQ decreased TM by 41%. The SAM/SAH index showed that MPTP increased methylation by 33.3%, but MPTP plus PQ increased methylation by 81%. In the chronic protocol, the data showed that MPTP administration did not affect DA, DOPAC, and HVA levels. The administration of PQ led to significant decrease in DOPAC, HVA, and TD by 31.6%, 19.9%, and 21.2% respectively with no effect on DA levels. The MPTP plus PQ group showed reduced DA, DOPAC, HVA, and total distance traveled by 58.4%, 82.8%, 55.8%, and 83.9%, respectively. Meanwhile, PQ administration caused a reduction in tyrosine hydroxylase immunoreactivity in the substantia nigra, and this effect was more pronounced in MPTP pretreated mice. It was concluded from this study that prior treatment with MPTP potentiated the effects of PQ in reducing DA, DOPAC, HVA, TH immunoreactivity, locomotor activity, and increasing the methylation index. The enhanced effects of PQ following MPTP administration further support the role of toxic substance mixtures in causing Parkinson's disease.

Keywords: Parkinson's disease; Paraquat; Dopamine; Methylation; MPTP

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder, which affects approximately 1.5 million Americans (Standaert and Stern, 1993). The symptoms of PD include tremor, hypokinesia, and rigidity, caused by the degeneration of dopaminergic neurons in the nigrostriatal pathway with subsequent depletion of dopamine (DA) in the neostriatum (Hornykiewicz, 1966). Generally, the cause of non-familial PD is unknown; however, there are numerous studies that linked pesticide exposure to the increased risk of developing this type of PD (Tanner, 1989, 1992; Tanner and Goldman, 1994, 1996). Non-familial PD represents over 90% of all PD cases and its onset typically occurs after 50 years of age (Moghal et al., 1994; Langston, 1998; Olanow and Tatton, 1999; Tanner and Ben-

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Shlomo, 1999). A recent study of male twins has confirmed that genetic heritability is not the basis of sporadic PD with onset over age of 50 (Tanner et al., 1999). Although it has been proposed that PD may be caused by a single toxic environmental exposure (Langston et al., 1983) recently it was suggested that the vast majority of PD cases probably result from interactions between genetic make-up and environmental exposures to certain toxins (Greenamyre et al., 2003). These findings led to the hypothesis that PD may be initiated or precipitated by environmental or endogenous toxins in genetically-predisposed individuals (Matsubara et al., 1995; Corrigan et al., 2000).

The environmental factors hypothesis has been given credence by the identification of the pre-toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This substance was discovered as a chemical contaminant of synthetic heroin that produces an acute parkinsonian syndrome in humans, and in mice similar to idiopathic PD (Davis et al., 1979; Langston et al., 1983; Duvoisin et al., 1986; Freyaldenhoven et al., 1995). Upon entering the brain, MPTP is converted to 1-methyl-4-phenyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase B (Fahn, 1988). MPP^+ is then accumulated into the dopaminergic neurons by the high affinity DA uptake system (Frederickson, 1989). Biochemically, MPTP administration causes damage to the nigrostriatal system, with subsequent DA depletion similar to that seen in PD (Hallman et al., 1985), and therefore, it is possible that compounds similar to MPTP may cause Parkinson's disease.

Paraquat (PQ) (1, 1-dimethyl-4, 4-bipyridium dichloride), a widely used non-selective herbicide, is structurally similar to MPTP and has been correlated with a high incidence of PD in some regions of Canada (Barbeau et al., 1987). It is believed that PQ would not cause PD because of its poor ability to cross the blood–brain barrier to yield consistent results after its systemic administration (Markey et al., 1986; Perry et al., 1986; Bagetta et al., 1992). On the other hand, the entry of PQ to the brain has been demonstrated through its ability to target the dopaminergic system (Lindquist et al., 1988); but causes no significant reductions in dopamine levels (Widdowson et al., 1996).

Since it is known that PQ does not cross the blood–brain barrier with ease and it is extensively used in the environment, there must be other environmental determining factors which influence the ability of this herbicide to cause PD. The exposure to certain mixture of toxic substance has been associated with Parkinson's disease (Miller et al., 1991). In the present study, the effects of PQ for causing dopamine depletion and behavioral impairments in mice which have been exposed to MPTP were examined to assess the impact of toxic substance mixtures. In this study, mice were primed with a low-dose of MPTP (10 mg/kg), which do not lead to significant dopamine depletion and behavioral impairment, and were followed by PQ injection. To assess neurochemical and behavior changes, the levels of dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA) measurements were performed, locomotor activity was evaluated and brain sections for TH-immunoreactivity were stained. In addition, we measured the levels of the *S*-adenosyl methionine (SAM)/*S*-adenosyl homocysteine (SAH) index to determine whether methylation is increased by PQ. This was assessed based on the observation that administration of *S*-adenosyl methionine (SAM), the endogenous methyl donor, causes neurological and biochemical changes that resemble PD (Charlton and Way, 1978; Charlton, 1990; Crowell et al., 1993; Charlton and Mack, 1994). Also MPP+, the toxic metabolite of MPTP, has been shown to increase the effects of SAM (Lee and Charlton, 2001).

2. Materials and methods

2.1. Animals

Male C57BL6 mice 3 to 4 weeks old were purchased from Harlan (Indianapolis, IN). Animals were housed four per cage with food and water available ad libitum in a room maintained under controlled temperature (21±2 °C) and humidity with a 12:12 light–dark cycle. All mice were acclimated for at least 1 week prior to commencement of experiments. All procedures were approved by the Animal Care and Use Committee at Florida A&M University.

2.2. Chemicals

Paraquat, *S*-adenosyl methionine, *S*-adenosyl homocysteine, dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), and EDTA were purchased from Sigma Chemical Co. (St. Louis, MO). Phosphoric acid, perchloric acid, citric acid, octanesulfonic acid sodium salt and methanol were purchased from Fisher (Norcross, GA). MPTP was purchased from RBI (Natick, MA).

2.3. Drug administration

In the subacute study, 24 male C57BL6 mice were divided into four groups (A, B, C, and D). Groups A and B were injected intraperitoneally (i.p) with 10 mg/kg MPTP once a day for 5 days. Groups C and D were injected i.p. with phosphate buffered saline (PBS) once a day for 5 days. This was followed by a 2 day resting period (receiving no injections). After the resting period, groups A and C received i.p. injections with 7 mg/kg PQ once a day for 6 days while groups B and D received i.p. injections with PBS once a day for 6 days. The treatment groups were control (PBS plus PBS); MPTP (PBS plus MPTP), PQ (PBS plus PQ) and MPTP plus PQ. There were a total of 6 mice per treatment group. Four mice per treatment group were used for locomotor activity, and later combined with two additional mice (time matched after injection) per treatment group and used for neurochemical analysis.

In the chronic study, 36 male C57BL6 mice were divided into four groups (A, B, C, and D). Groups A and B were injected i.p. with 10 mg/kg MPTP once a day for 5 days. Groups C and D were injected i.p. with PBS once a day for 5 days. This was followed by a 2 day resting period (receiving no injections). After the resting period, groups A and C received i.p. injections

with 7 mg/kg PQ once a week for 10 weeks while groups B and D received i.p. injections with PBS once a week for 10 weeks. The treatment groups were control (PBS plus PBS); MPTP (PBS plus MPTP), PQ (PBS plus PQ) and MPTP plus PQ. There were a total of 9 mice per treatment group. Four mice per treatment group were used for locomotor activity, and later combined with two additional mice per treatment group (time matched after injection) and used for neurochemical analysis. The remaining mice ($n=3$ per treatment group) were used for tyrosine hydroxylase immunohistochemistry.

2.4. Measurements of locomotor activity

The Digiscan animal activity monitoring system (Ominetech Electronics Inc., Sydney, Australia) consisting of four chambers (one animal per chamber) was used to measure locomotor activity. Locomotor activity counts were assessed 1 h after the last injection ($n=4$ per treatment group). The locomotor activity was recorded as total number of movements (TM) and total distance traveled (TD).

2.5. Biochemical analysis

Mice ($n=4$ per treatment group) were sacrificed immediately after the measurement of locomotor activities (2 h after the last injection) and an additional 2 animals per treatment group were sacrificed (2 h after the last injection) and were combined to make a final sample size of 6 animals per treatment group for biochemical analysis. Striatum of mice were dissected and placed in 0.4 M perchloric acid and the tissues were homogenized and then centrifuged at 4 °C for 15 min at 12,000×*g*. The supernatants were filtered in 0.2 μm filters (Fisher) for 2 min at 10,000×*g*. DA, DOPAC, HVA were analyzed using high performance liquid chromatography (HPLC, Shimadzu) with a Coulochem II electrochemical detector (ESA) with a guard cell set at +350 mV; channel 1 set at −150 mV; channel 2 set at +220 mV. SAH and SAM were detected using HPLC equipped with a UV detector at a wavelength of 260 nm. The mobile phase consisted of w/v 0.84% sodium acetate, 1.2% citric acid, 0.015% octanesulfonic acid sodium salt, 0.02% disodium EDTA (w/v) in 15% methanol in water and pH adjusted to 3.5 with phosphoric acid. The system included a solvent delivery system and an auto-injector coupled to a pre-column, and a Whatman C18 (5 μm, 250×4.6 mm) column. Concentrations of DA, DOPAC, HVA, SAM, and SAH were determined by assaying standards of known amounts of each compound and extrapolating from a standard curve.

2.6. Immunohistochemistry for tyrosine hydroxylase (TH)

Two hours after the last injection of the chronic study, mice ($n=3$ per treatment group) were anesthetized with chloral hydrate and perfused intracardially with phosphate buffered saline followed by 4% paraformaldehyde (PFA). Brains were harvested and post-fixed with 15% sucrose in 4% PFA for 2 days at 4 °C. The brains were then cut on a freezing sliding microtome at a thickness of 25 μm. The sections were collected in 0.1 M phosphate buffer and stored at 4 °C for at least 1 week before TH staining. Sections were stained for TH positive cell bodies as described by Charlton and Mack (1994), with slight modifications. Briefly, loose sections were washed three times in 0.3% Triton X-100 in phosphate buffer for 5 min. Sections were then incubated for 1 h at room temperature in 0.5% Triton X-100 in phosphate buffer containing 10% goat serum. The sections were then washed twice in phosphate buffer and once in 0.3% Triton X-100, in phosphate buffer, for 5 min each wash.

The sections were incubated with primary antibody solution containing a 1:200 dilution of rabbit anti-tyrosine hydroxylase (Chemicon International, Temecula, CA) for 1 h at room temperature and then covered and placed in the refrigerator maintained at 4 °C for 72 h. The sections were washed three times in 0.1 M phosphate buffer for 5 min each wash. Sections were then incubated for 1 h at room temperature with secondary antibody solution containing a 1:100 dilution of fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit IgG (Chemicon International, Temecula, CA) in 0.3% Triton X-100. The sections were then washed three times in 0.1 M phosphate buffer for 5 min each wash. Sections were mounted on 1% gelatin coated slides. The slides were air dried on a slide warmer at 50 °C for at least 20 min. The dry slides were cleared by immersion in xylene for 1 min before covering with coverslip (Corning Cover No. 1, 24×60 mm) with DPX (Fluka, Milwaukee, WI). TH positive cells were visualized with an epifluorescent microscope (Nikon) with blue (450–490 nm) excitation light.

2.7. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test. All values represent the mean±S.E.M. *P* values (less than 0.05) were considered significant.

3. Results

3.1. The effect of subacute and chronic treatment with PQ following MPTP treatment on locomotor activity

The results from the subacute study show that there was a significant effect of treatments on locomotor activities, total number of movements $F(3, 15)=9.610$, $P<0.0016$; total distance traveled $F(3, 15)=33.40$, $P<0.0001$. The results show that PQ significantly decrease ($P<0.05$) the total number of movements (TM) by 28% (Fig. 1A) when compared to the control. The administration of MPTP and then PQ decreased TM by 41% ($P<0.01$) and by 38.8% ($P<0.01$) when compared to the control and MPTP, respectively (Fig. 1A). PQ decreased the total distance traveled by 64.1% ($P<0.001$), when compared to the control (Fig. 1B). Meanwhile, the administration of MPTP and then PQ decreased TD by 78.1% ($P<0.001$); by 72.7% ($P<0.001$) and by 38.9% ($P<0.01$) as compared to the control, MPTP and PQ groups, respectively (Fig. 1B).

In the chronic protocol, ANOVA revealed a significant effect of treatments on locomotor activities, Total number of

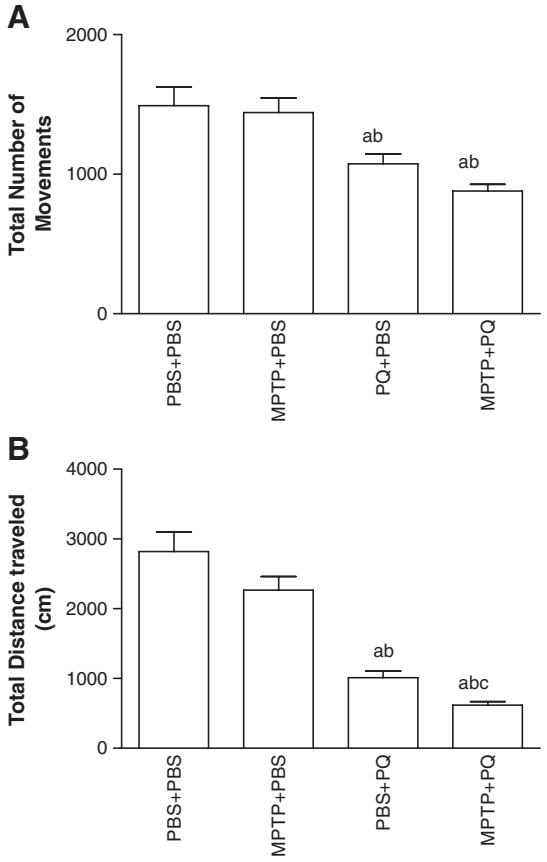

Fig. 1. Effects of the i.p. administration of 7 mg/kg PQ once a day for 6 days following priming with 10 mg/kg MPTP (once a day for 5 days) on mouse locomotor activity. The total number of movements and total distance traveled by mice was measured 1 h after the last PQ injection. Values are expressed as mean ± S.E.M. Statistical comparison was performed using ANOVA with subsequent Newman–Keuls post-hoc test. Total number of movements ($n=4$ per treatment group): [a]significantly different from control (PBS plus PBS) (PBS+PBS vs. PQ+PBS, $P<0.05$; PBS+PBS vs. MPTP+PQ, $P<0.01$); [b]significantly different from MPTP plus PBS (vs. PQ+PBS, $P<0.05$; vs. MPTP+PQ, $P<0.01$). [c]Significantly different from PQ plus PBS group. Total distance traveled: [a]significantly different from control (PBS plus PBS) (PBS+PBS vs. PQ+PBS $P<0.001$; PBS+PBS vs. MPTP+PQ, $P<0.001$); [b]significantly different from MPTP plus PBS (vs. PQ+PBS, $P<0.001$; vs. MPTP+PQ, $P<0.001$); [c]significantly different from PQ plus PBS group (vs. MPTP+PQ, $P<0.05$).

movements (TM) $F(3, 15)=27.77$, $P<0.0001$; Total distance traveled (TD) $F(3, 15)=8.879$, $P<0.0023$. Animals receiving MPTP and then PQ had a decrease in TM by 70.4% ($P<0.001$) by 68.1% ($P<0.001$) and by 67.8% ($P<0.001$) as compared to the control, MPTP and PQ, respectively (Fig. 2A). The same group of animals showed a decrease in TD by 83.9% ($P<0.01$); by 81.63% ($P<0.01$); and by 79.5% ($P<0.01$) when compared to the control, MPTP and PQ groups, respectively (Fig. 2B).

3.2. The effects of subacute and chronic treatment with PQ following MPTP treatment on DA, DOPAC, HVA

In the subacute study, ANOVA demonstrated a significant effect of treatment on the levels DA and its metabolites (DA: $F(3, 23)=38.30$, $P<0.0001$; DOPAC: $F(3, 23)=50.40$, $P<0.0001$; HVA: $F(3, 23)=47.97$, $P<0.0001$). The PQ treatment and the MPTP treatment groups did not have any significant changes in DA levels when compared to the control group (Fig. 3A). However, the MPTP plus PQ group showed a 22.1% decrease in DA levels when compared to that of the control ($P<0.01$) (Fig. 3A). The PQ group showed a significant decline ($P<0.01$) in DOPAC levels by 21%, whereas MPTP plus PQ significantly decreased DOPAC by 35.8% ($P<0.01$); by 29.7% ($P<0.01$) as compared to the control or the MPTP groups, respectively (Fig. 3B). Meanwhile, the administration of PQ caused a significant decline in HVA levels by 21.6% ($P<0.01$) as compared to the control (Fig. 3C). Animals receiving MPTP plus PQ had a 35.2% significant decline in HVA ($P<0.01$) when compared to the control; and by 32.4% ($P<0.01$) when compared to the MPTP group (Fig. 3C).

Animals in the chronic protocol displayed a significant effect of treatment on the levels of DA and its metabolites (DA: $F(3,$

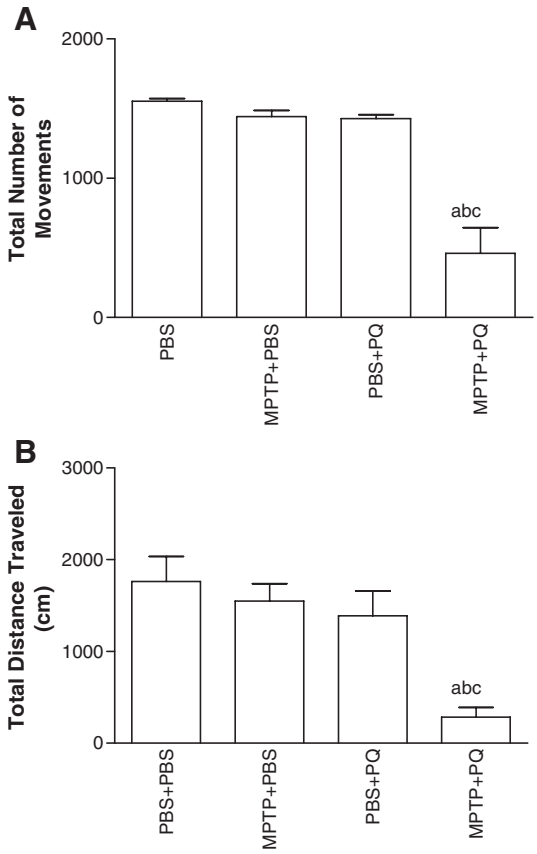

Fig. 2. Effects of the i.p. administration of 7 mg/kg PQ once a week for 8 weeks following priming with 10 mg/kg MPTP (once a day for 5 days) on mouse locomotor activity. The total number of movements and total distance traveled by mice was measured 1 h after the last PQ injection. Values are expressed as mean ± S.E.M. Statistical comparison was performed using ANOVA with subsequent Newman–Keuls post-hoc test ($n=4$ per treatment group). Total number of movements, [a]significantly different from the control group (PBS plus PBS) $P<0.001$; [b]significantly different from MPTP plus PBS, $P<0.001$; [c]significantly different from PQ plus PBS, $P<0.001$. Total distance traveled, [a]significantly different from the control group (PBS plus PBS), $P<0.01$; [b]significantly different from MPTP plus PBS, $P<0.01$; [c]significantly different from PQ plus PBS, $P<0.01$.

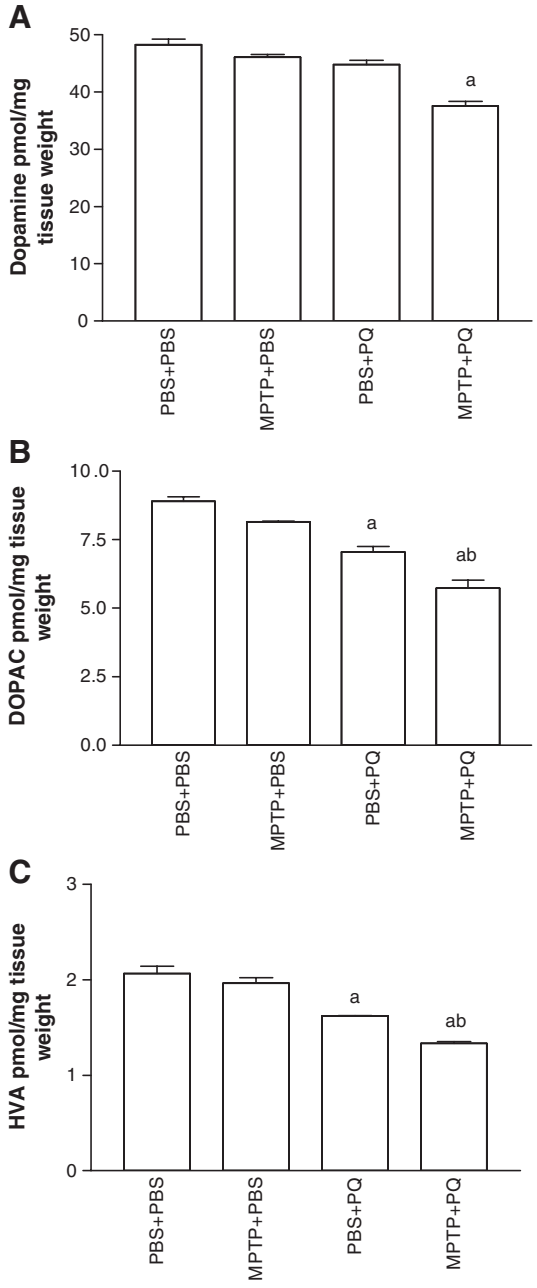

Fig. 3. Effects of the i.p. administration of 7 mg/kg PQ once a day for 6 days following priming with 10 mg/kg MPTP (once a day for 5 days) on the levels of dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA). Mice were sacrificed 2 h after the last PQ injection. Post-mortem striatal levels of DA, DOPAC and HVA were measured using HPLC. Values are expressed as mean ± S.E.M.; pmol/mg wet tissue weight. Statistical comparison was performed using ANOVA with subsequent Newman–Keuls post-hoc test ($n=6$ per treatment group). [a]Significantly different from the control group (PBS plus PBS), $P<0.01$. [b]Significantly different from MPTP plus PBS, $P<0.01$. [c]Significantly different from PQ plus PBS, $P<0.01$.

23) = 360.6, $P<0.0001$; DOPAC: $F(3, 23) = 54.04$, $P<0.0001$; HVA: $F(3, 23) = 109.8$, $P<0.0001$). Similar effects as the subacute study were observed in that the MPTP and the PQ groups when administered alone did not show any significant changes in DA levels when compared to the control group. However, the MPTP plus PQ group exhibited significant decrease ($P<0.001$) in DA by 58.9%, by 56.3% and by 58.7% as compared to the control, MPTP or PQ groups, respectively

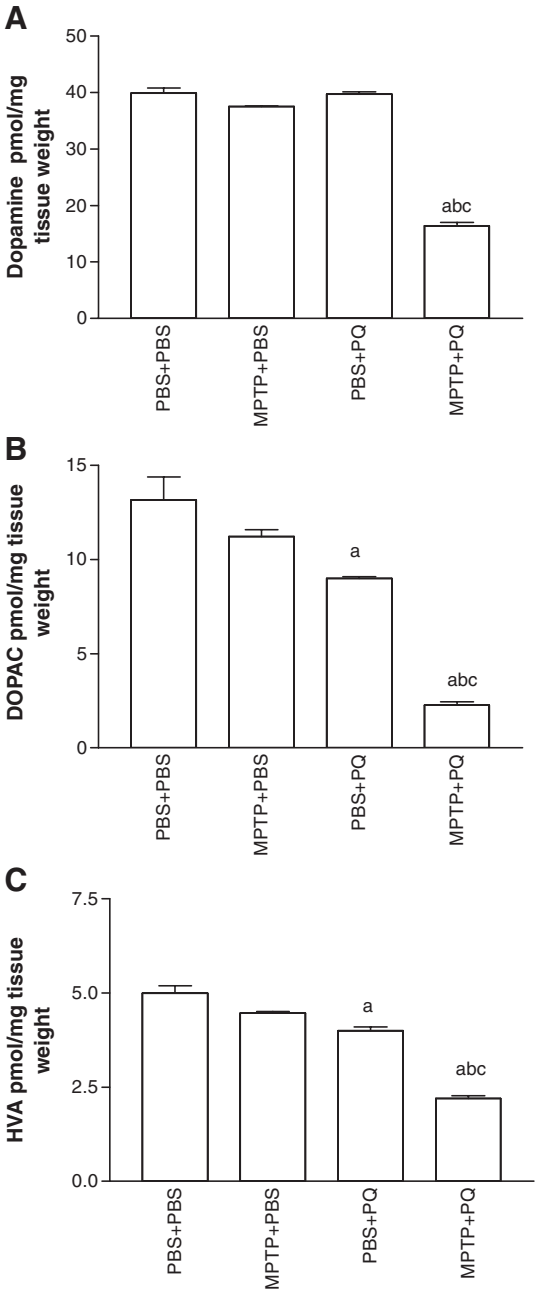

Fig. 4. Effects of the i.p. administration of 7 mg/kg PQ once a week for 8 weeks following priming with 10 mg/kg MPTP (once a day for 5 days) on the levels of dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA). Mice were sacrificed 2 h after the last PQ injection. Post-mortem striatal levels of DA, DOPAC and HVA were measured using HPLC. Values are expressed as mean ± S.E.M.; pmol/mg wet tissue weight. Statistical comparison was performed using ANOVA with subsequent Newman–Keuls post-hoc test ($n=6$ per treatment group). [a]Significantly different from the control group (PBS plus PBS). [b]Significantly different from MPTP plus PBS. [c]Significantly different from PQ. DA: (MPTP plus PQ vs. control, $P<0.001$; vs. MPTP plus PBS, $P<0.001$; vs. PQ plus PBS, $P<0.001$). DOPAC: (PQ plus PBS vs. control, $P<0.01$; MPTP plus PQ vs. control, MPTP plus PBS, PQ plus PBS, $P<0.001$). HVA: (PQ plus PBS vs. control, $P<0.05$; MPTP plus PQ vs. control, MPTP plus PBS, PQ plus PBS, $P<0.001$).

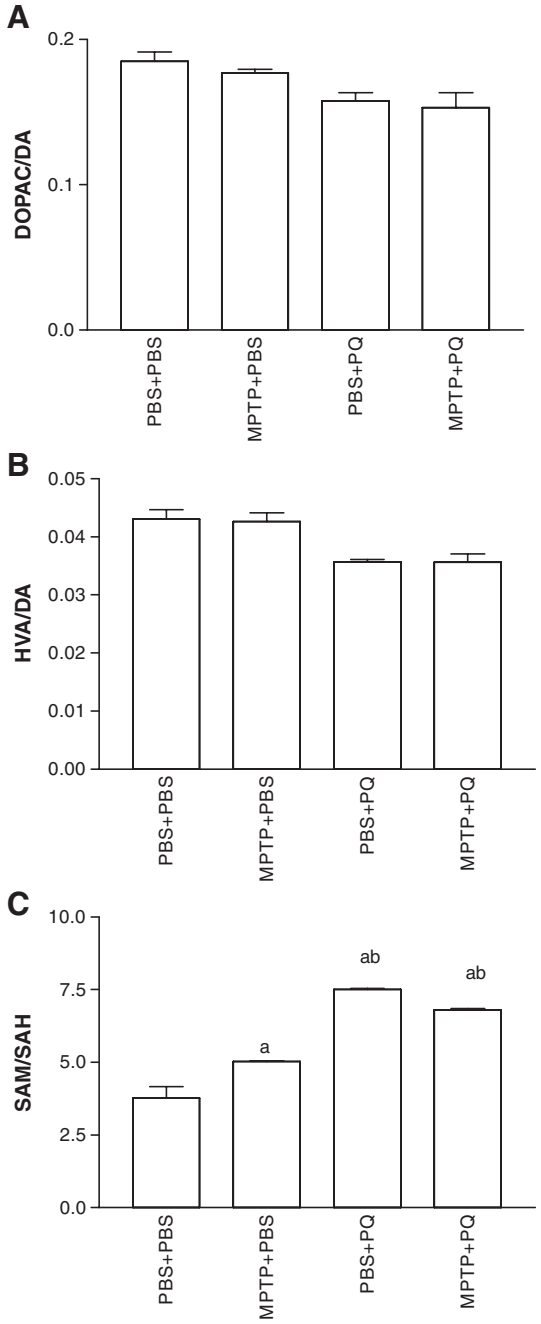

Fig. 5. Effects of the i.p. administration of 7 mg/kg PQ once a day for 6 days following priming with 10 mg/kg MPTP (once a day for 5 days) on dopamine turnover and methylation. Values for DOPAC/DA, HVA/DA, SAM/SAH are expressed as mean±S.E.M. Statistical comparison was performed using ANOVA with subsequent Newman–Keuls post-hoc test. $P<0.05$ was considered significant ($n=6$ per treatment group). [a]Significantly different from the control group. [b]Significantly different from MPTP. [c]Significantly different from PQ. SAM/SAH: (MPTP plus PBS vs. control, $P<0.01$; PQ plus PBS vs. control, $P<0.001$; PQ plus PBS vs. MPTP plus PBS, $P<0.01$; MPTP plus PQ vs. control, $P<0.001$; MPTP plus PQ vs. MPTP plus PBS, $P<0.01$).

(Fig. 4A). The PQ had a decreased DOPAC by 31.7% ($P<0.01$) when compared to the control (Fig. 4B). In the MPTP plus PQ group there was a significant decrease in DOPAC levels by 82.8% ($P<0.001$) when compared to the control (Fig. 4B). Moreover, the PQ group had a reduced HVA level by 20% ($P<0.05$) when compared to the control (Fig. 4C). However, MPTP plus PQ exposure was associated

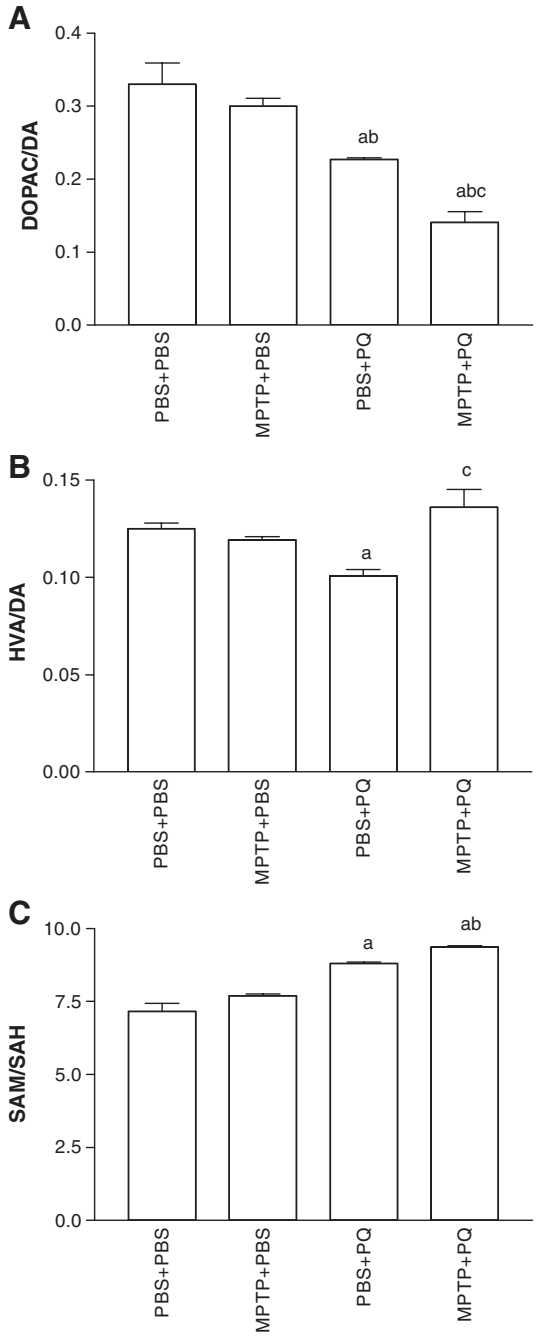

Fig. 6. Effects of the i.p. administration of 7 mg/kg PQ once a week for 8 weeks following priming with 10 mg/kg MPTP (once a day for 5 days) on dopamine turnover and methylation. Values for DOPAC/DA, HVA/DA, SAM/SAH are expressed as mean±S.E.M. Statistical comparison was performed using ANOVA with subsequent Newman–Keuls post-hoc test. $P<0.05$ was considered significant ($n=6$ per treatment group). [a]Significantly different from the control group. [b]Significantly different from MPTP. [c]Significantly different from PQ. DOPAC/DA: (PQ plus PBS vs. control, $P<0.01$; PQ plus PBS vs. MPTP plus PBS, $P<0.01$; MPTP plus PQ vs. control, $P<0.001$; MPTP plus PQ vs. MPTP plus PBS, $P<0.001$; MPTP plus PQ vs. PQ plus PBS, $P<0.01$). HVA/DA: (PQ plus PBS vs. control, $P<0.01$; MPTP plus PQ vs. PQ plus PBS, $P<0.01$). SAM/SAH: (PQ plus PBS vs. control, $P<0.05$; MPTP plus PQ vs. control, $P<0.01$; MPTP plus PQ vs. MPTP plus PBS, $P<0.05$).

with a decline in HVA levels by 56% ($P<0.001$) when compared to the control (Fig. 4C).

3.3. The effect of subacute and chronic treatment with PQ following MPTP administration on DA turnover and methylation

The DOPAC/DA and the HVA/DA ratios are markers of DA turnover. The results from the subacute study showed that PQ and MPTP plus PQ treatments did not have any significant effects on DOPAC/DA and the HVA/DA indexes (Fig. 5A and B). *S*-adenosyl methionine (SAM)/*S*-adenosyl homocysteine (SAH) index is a measure of methylation. In the subacute study ANOVA revealed a significant effect on methylation ($F(3,23)=71.46$; $P<0.0001$).The results show that MPTP, PQ or MPT plus PQ treatments significantly increased methylation by 33.3%, 100% and 81%, respectively when compared to the control (Fig. 5C). MPTP plus PQ increased methylation by 35.7% ($P<0.01$) when compared to the MPTP treatment group, demonstrating the potentiated effects of PQ on methylation (Fig. 5C).

In the chronic study, ANOVA demonstrated a significant effect of treatments on DOPAC/DA, HVA/DA and SAM/SAH (DOPAC/DA $F(3, 23)=23.86$, $P<0.0001$; HVA/DA $F(3, 23)=8.263$, $P<0.0009$; SAM/SAH $F(3, 23)=48.91$, $P<0.0001$). PQ treatment caused a decrease in DOPAC/DA ratio by 31.8% ($P<0.01$) when compared to the control (Fig. 6A). Meanwhile, MPTP plus PQ treatment was associated with a decrease in DOPAC/DA ratio by 57.7% ($P<0.001$) when compared to the control (Fig. 6A). MPTP plus PQ treatment caused an increase in HVA/DA by 34.7% ($P<0.01$) when compared to the PQ treated group (Fig. 6B). The MPTP, PQ, MPTP plus PQ treatment groups increased SAM/SAH by 7.7%, 23.1% ($P<0.05$) and 30.9% ($P<0.01$) respectively when compared to the control (Fig. 6C). In addition, MPTP plus PQ increased SAM/SAH by 21.6% ($P<0.05$) when compared to MPTP demonstrating the potentiated effects of PQ on methylation (Fig. 6C). Furthermore, as indicated by the white arrows, there is a clear defined staining of substantia nigra pars compacta (SN) neurons in the control animals (Fig. 7). MPTP appeared to cause a slight decline in tyrosine hydroxylase (TH) immunoreactivity of the substantia nigra (SN) (Fig. 7). This effect appears to be more pronounced in the PQ and PQ plus MPTP treatment groups as indicated by the white arrows (Fig. 7).

4. Discussion

Epidemiological studies have linked pesticide exposure with the increase in the incidence of PD (Lewin, 1985; Liou et al., 1997). In fact, PD mortality rate distribution by geographical regions has also been interpreted as consistent with the environmental exposure to pesticides (Imaizumi, 1995; Lanska, 1997). Sanchez-Ramos et al. (1987) reported on a young farmer who had been exposed to PQ and affected with PD. Although pesticides are common to the environment, there must be other determining factors such as the genetic make-up which influence the ability of pesticides to cause PD and the associated dopamine depletion. The present study examined the ability of PQ to induce DA depletion and behavioral impairments in mice exposed to a low dose of MPTP.

In the brain, DA is metabolized to DOPAC via monoamine oxidase (Oreland, 1991) and/or HVA via catechol-*o*-methyltransferase (COMT) with the transfer of the methyl group of *S*-adenosyl-L-methionine to the phenolic groups of the catechol substrate in the presence of Mg^{2+} (Mannisto and Kaakkola, 1999). Thus DA, DOPAC, and HVA levels reflect the activity of dopaminergic neurons. Results obtained from the subacute study showed that PQ impaired dopaminergic function as indicated by the decrease levels of dopamine metabolites HVA and DOPAC in the striatum. These changes in DA neuronal function are also reflected by the decrease in motor activity. PQ-induced reductions in HVA and DOPAC in the subacute study

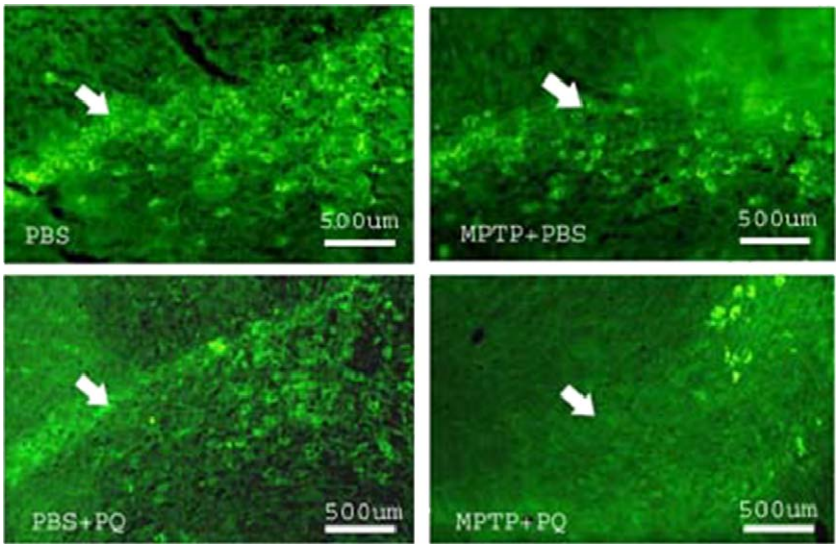

Fig. 7. Effects of the i.p. administration of 7 mg/kg PQ once a week for 8 weeks following priming with 10 mg/kg MPTP (once a day for 5 days) on tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra of mice. Arrows indicate staining of TH neurons.

may be due to the ability of PQ to alter DA release and/or inhibit DA reuptake. This notion is supported by the results which indicate that a single injection of PQ induces DA overflow, and pretreatment with the dopamine transporter inhibitor, GBR 12909, significantly reduced PQ uptake into striatal tissues (Shimizu et al., 2003).

If neuronal integrity and compensatory mechanism are intact, an initial increase in DA release or blockade of DA uptake will result in activation of autoreceptors with a resultant decrease in DA release, leading to the decrease in the availability of DA in the synaptic cleft to undergo methylation by *S*-adenosyl methionine (SAM). This in turn could be associated with a decrease in SAM utilization resulting in the decrease of SAM clearance from the synaptic cleft. This cascade of events may explain the unaltered DA levels, DOPAC/DA, HVA/DA indexes, and increased methylation seen after PQ administration in the subacute study. However, the results of the PQ group in the subacute study were obtained 2 h after receiving a total of six injections of PQ, and are likely to be influenced by PQ remaining in the brain, since the biological half-life of PQ was estimated to be 40.9 h (Dey et al., 1990). The findings obtained from the subacute study suggest that the neurochemical effects of PQ would depend on the dose and the time after administration.

The results from the chronic study showed that PQ significantly reduced DOPAC and HVA levels when compared to the control. This effect was associated with apparent changes in the substantia nigra (SN) as demonstrated by an apparent decline in tyrosine hydroxylase immunoreactivity. In the chronic study, it is not known if the effects of PQ on TH immunoreactivity reflect actual loss of DA neurons. However, previous studies have reported that using mice of the same strain and age treated with a similar dose of PQ once a week for 4 weeks consistently destroyed DA substantia nigra (SN) neurons as determined by cell counting of TH-immunoreactive cells in the SN using stereological techniques and counterstained for Nissl substances (Fredriksson et al., 1993; Brooks et al., 1999; McCormack et al., 2002). This dosage regimen produced approximately 25% of cell loss (McCormack et al., 2002). In the chronic study, mice were treated for a longer period of time (10 weeks) with a similar dose, thus it is likely that PQ effect might be related to DA neurons damage. However, PQ-induced neuronal damage of SN may not produce dopamine depletion (McCormack et al., 2002; Thiruchelvam et al., 2000, 2003). This is different from mice treated with MPTP where nigral cell damage is consistently accompanied by a substantial decrease in striatal dopamine (DA). In addition, the extent of MPTP-induced DA depletion is usually much greater than the extent of neuronal loss (Chan et al., 1997; Di Monte et al., 2000). In the chronic study, PQ failed to decrease DA levels, which suggest that PQ may preferentially target DA cell bodies to a higher degree than the terminals. This suggestion is supported by the finding that methamphetamine damages nerve terminals and causes dopamine depletion, but does not affect substantia nigra cell bodies (Harvey et al., 2000). Whereas, the administration of high doses of MPTP ($\geq$20 mg/kg) were associated with nerve terminals (resulting in dopamine depletion) and the substantia nigra cell bodies damage (Thomas et al., 2004).

The results from the chronic study demonstrate that mice pretreated with 10 mg/kg MPTP did not produce any significant changes in dopamine, DOPAC, HVA, dopamine turnover or behavioral parameters. This is consistent with the results published from other investigators with similar doses of MPTP (Walters et al., 1999). The observation that mice pretreated with MPTP and later injected with PQ produced more dramatic effects on DA levels, tyrosine hydroxylase immunoreactivity, and locomotor activity when compared to the control or PQ demonstrates that the dose of MPTP used in the chronic study is suitable for priming the nigrostriatal system to the neurotoxic actions of PQ in these animals.

The results are consistent with other studies which have shown that low doses of MPTP have been shown to potentiate the effects of dithiocarbamate pesticides (McGrew et al., 2000). The current finding from the chronic study suggest that exposure to a single agent such as MPTP might functionally injure the SN neurons without structural changes, however when another exposure occur (in this case PQ), it may lead to neuronal changes. The notion of potentiated effects of PQ on the dopaminergic system with nontoxic chemical mixtures with different modes of toxicity is supported by the observation that PQ potentiates the effects of the widely used herbicide maneb (Thiruchelvam et al., 2000).

The mechanism of PQ toxicity remains unclear; however, the involvement of cyclic reduction/reoxidation and production of oxygen free radicals has been proposed (Bus et al., 1976; Trush et al., 1981; Kadiiska et al., 1993; Hochman et al., 1998). This production of free radicals subsequently might cause lipid peroxidation and promotion of cellular death and apoptosis (Fabisiak et al., 1997; Shimada et al., 1998; Yang and Sun, 1998). Meanwhile, the turnover/metabolism of dopamine has been associated with the generation of free radicals (Berman and Hastings, 1999). In the striatum, DOPAC/DA ratio is an index of DA neuron activity associated with DA release, reuptake, and oxidative metabolism (Westerink and Spaan, 1982, DeMaria et al., 1999), while the HVA/DA ratio reflects the index of extraneuronal methylated metabolism of DA which does not undergo reuptake by DA neurons.

In the chronic protocol, PQ treated group had a decrease in DOPAC/DA and HVA/DA, and an increase in SAM/SAH. Whereas, the MPTP plus PQ group had an increase in the HVA/DA index when compared to the MPTP and PQ treated groups, demonstrating the potentiation of methylation by these compounds. However, MPTP plus PQ significantly reduced the DOPAC/DA index when compared to the control, MPTP treatment groups, further demonstrating the potentiated effects of MPTP. This decrease in DOPAC/DA and increase in HVA/DA induced by MPTP plus PQ treatment is probably a result of striatal terminals damage which leads to DA uptake sites reduction and possible increase in DOPAC diffusion into the synaptic cleft to undergo methylated metabolism to homovanillic acid. Thus, the increase in the methylation index as indicated by the increase in SAM/SAH ratio in PQ and MPTP

plus PQ groups may have resulted from the increase demand for SAM needed to methylate DA or DOPAC. Meanwhile, the role of methylation in DA catabolism has been demonstrated earlier (Yassin et al., 1998).

SAM is required for growth and development and to maintain basal metabolic functions, but both the synthesis and the utilization of SAM are increased during aging (Mays et al., 1973; Stramentinoli et al., 1977; Tuomisto, 1977; Gharib et al., 1982; Sellinger et al., 1988). This is of considerable significance since PD is an age-related disorder and phospholipid methylation activity increases in aging animals (Crews et al., 1980). The increased product of phospholipid methylation, lysophosphatidylcholine (lyso-PTC) a detergent-like, cytotoxic product, can impair various biological functions such as plasmalemma and vesicular membrane integrity, and may eventually cause cell death. Results from our laboratory have shown that lyso-PTC impaired locomotor activity and DA neurotransmission (Lee et al., 2004).

Therefore, exposure to a slightly toxic endogenous and/or exogenous agent followed by secondary exposure to a moderately toxic environmental compound can increase SAM dependent methylation and may precipitate the PD symptoms. This notion is substantiated by the many results supporting relationship between methylation and parkinsonism. For example, a high *N*-methylation activity was found in PD patients (Williams et al., 1993), and two endogenous methylated compounds that induce neurotoxicity were found in the parkinsonian brain and were structurally related to MPTP (Collins et al., 1992; McNaught et al., 1995). Therefore, it is plausible that in the current study the reported changes following PQ may be related to the increase in methylation. In addition, the experimental design used in these experiments may be of significance with respect to human exposures in that the MPTP plus PQ group decreased DA levels by 58.4% and altered turnover indexes in the chronic exposure study, while decreasing DA by 20.6% and not altering the turnover indexes in the subacute exposure study. Furthermore, these finding demonstrate that PQ may act as a slow-acting or delayed dopaminergic toxicant, in which its effects increase with duration of exposure. If this is the case, low level chronic exposure paradigm vs. a subacute exposure paradigm may be more suitable when accessing PQ induced dopaminergic neurotoxicity, and may explain the greater effects seen in the chronic protocol vs. the subacute protocol. In addition, a chronic low level exposure paradigm may be a better reflection of human exposures in that individuals are more likely to be exposed to very low amounts of pesticides in the environment over a long period of time.

In summary, the current study shows that PQ can alter DA turnover, locomotor activity, and increase methylation. The results obtained from the present study provides further evidence that PQ has a central effect and exert biochemical changes after its systemic administration. These effects were more pronounced when the animals were primed with MPTP. Additionally, the findings from these studies may demonstrate how a moderately toxic agent may influence the precipitation of PD symptoms and further support the concept of chemical mixtures induced parkinsonism. Thus the exposure of environmental compounds after the administration of a non-toxic dose of MPTP may be utilized as a useful model for identifying potential genetic, biochemical, or neurological markers responsible for the sensitizing effects of MPTP, and possibly unmasking the mechanism(s) associated with PD.

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References

Bagetta G, Corasaniti MI, Iannone M, Nistico G, Stephenson JD. Production of limbic motor seizures and brain damage by systemic and intracerebral injections of paraquat in rats. Pharmacol Toxicol 1992;71:443–8.

Barbeau A, Roy M, Cloutier T, Plasse L, Paris S. Environmental and genetic factors in the etiology of Parkinson's disease. Adv Neurol 1987;45: 299–306.

Berman SB, Hastings TG. Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J Neurochem 1999;73:1127–37.

Brooks AI, Chadwick CA, Gelbard HA, Cory-Slechta DA, Federoff HJ. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res 1999;823:1–10.

Bus JS, Cagen SZ, Olgaard M, Gibson JE. A mechanism of paraquat toxicity in mice and rats. Toxicol Appl Pharmacol 1976;35:501–13.

Chan P, Di Monte DA, Langston JW, Janson AM. (+) MK-801 does not prevent MPTP-induced loss of nigral neurons in mice. J Pharmacol Exp Ther 1997;280:439–46.

Charlton CG. A parallel relationship between Parkinson's disease and an excess of *S*-adenosyl methionine-dependent biological methylation in the brain. In: Nagatsu T, Fisher A, Yoshida M, editors. Basic clinical and therapeutic aspects of Alzheimer's and Parkinson's disease, vol. 1. New York: Plenum; 1990. p. 333–9.

Charlton CG, Mack J. Substantia nigra degeneration and tyrosine hydroxylase depletion caused by excess *S*-adenosyl methionine in the rat brain. Mol Neurobiol 1994;9:149–61.

Charlton CG, Way EL. Tremor induced by *S*-adenosyl-L-methionine: possible relation to L-DOPA effects. J Pharm Pharmacol 1978;30:819–20.

Collins MA, Neafsey EJ, Matsubara K, Cobuzzi Jr RJ, Rollema H. Indole-*N*-methylated B-carbolinium ions as potential brain-bioactivated neurotoxins. Brain Res 1992;570:154–60.

Corrigan FM, Wienburg CL, Shore RF, Daniel SE, Mann D. Organochlorine insecticides in substantia nigra in Parkinson's disease. J Toxicol Environ Health A 2000;59:229–34.

Crews FT, Hirata F, Axelrod J. Identification and properties of methyltransferases that synthesize phosphatidylcholine in rat brain synaptosomes. J Neurochem 1980;34:1491–8.

Crowell Jr BG, Benson R, Shockley D, Charlton CG. *S*-adenosyl-L-methionine decreases motor activity in the rat: similar to Parkinson's disease-like symptoms. Behav Neural Biol 1993;59:186–93.

Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, et al. Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res 1979;1:9249–54.

DeMaria JE, Lerant AA, Freeman ME. Prolactin activates all three populations of hypothalamic neuroendocrine dopaminergic neurons in ovariectomized rats. Brain Res 1999;837:236–41.

Dey MS, Breeze RG, Hayton WL, Karara AH, Krieger RI. Paraquat pharmacokinetics using a subcutaneous toxic low-dose in the rat. Fundam Appl Toxicol 1990;14:208–16.

Di Monte DA, McCormack A, Petzinger G, Janson AM, Quik M, Langston JW. Relationship among nigrostriatal denervation, parkinsonism, and dyskinesias in the MPTP primate model. Mov Disord 2000;15:459–66.

Duvoisin RC, Heikkila RE, Nicklas WJ, Hess A. Dopaminergic neurotoxicity of MPTP in the mouse: a murine model of Parkinsonism. In: Fahn S, editor. Recent development in Parkinson's disease. New York: Raven Press; 1986. p. 147–54.

Fabisiak JP, Kagan VE, Ritov VB, Johnson DE, Lazo JS. Bcl-2 inhibits selective oxidation and externalization of phosphatidylserine during paraquat-induced apoptosis. Am J Physiol 1997;272:C675–84 [Pt2].

Fahn S. Parkinsonism. In: Wyngaarden JB, Smith Jr LH, editors. Cecil's textbook of medicine. Philadelphia [PA]: Saunders; 1988. p. 2143–7.

Frederickson CJ. Neurobiology of zinc and zinc-containing neurons. Int Rev Neurobiol 1989;31:145–238.

Fredriksson A, Fredriksson M, Eriksson P. Neonatal exposure to paraquat or MPTP induces permanent changes in striatum dopamine and behavior in adult mice. Toxicol Appl Pharmacol 1993;122:258–64.

Freyaldenhoven TE, Ali SF, Hart RW. MPTP− and MPP+-induced effects on body temperature exhibit age-and strain-dependence in mice. Brain Res 1995;688:161–70.

Gharib A, Sarda N, Chabannes B, Cronenberger L, Pacheco H. The regional concentrations of *s*-adenosyl-L-methionine, *s*-adenosyl-L-homocysteine, and adenosine in rat brain. J Neurochem 1982;38:810–5.

Greenamyre JT, Betarbet R, Sherer TB. The rotenone model of Parkinson's disease: genes, environment and mitochondria. Parkinsonism Relat Disord 2003;9:S59–64.

Hallman H, Lange J, Olson L, Stromberg I, Jonsson G. Neurochemical and histochemical characterization of neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurons in the mouse. J Neurochem 1985;44:117–27.

Harvey DC, Lacan G, Tanious SP, Melega WP. Recovery from methamphetamine induced long-term nigrostriatal dopaminergic deficits without substantia nigra cell loss. Brain Res 2000;871:259–70.

Hochman A, Sternin H, Gorodin S, Korsmeyer S, Ziv I, Melamed E, et al. Enhanced oxidative stress and altered antioxidants in brains of Bcl-2 deficient mice. J Neurochem 1998;71:741–8.

Hornykiewicz O. Dopamine (3-hydroxytyramine) and brain function. Pharmacol Rev 1966;18:925–64.

Imaizumi Y. Geographical variations in mortality from Parkinson's disease in Japan, 1977–1985. Acta Neurol Scand 1995;91:311–6.

Kadiiska MB, Hanna PM, Mason RP. In vivo ESR spin trapping evidence for hydroxyl radical-mediated toxicity of paraquat and copper in rats. Toxicol Appl Pharmacol 1993;123:187–92.

Langston JW. Epidemiology versus genetics in Parkinson's disease: progress in resolving an age-old debate. Ann Neurol 1998;44:S45–52.

Langston JW, Ballard PA, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219:979–80.

Lanska DJ. The geographic distribution of Parkinson's disease mortality in the United States. J Neurol Sci 1997;150:63–70.

Lee ES, Charlton C. 1-Methyl-4-phenyl-pyridinium increases *S*-adenosyl-L-methionine dependent phospholipid methylation. Pharmacol Biochem Behav 2001;70:105–14.

Lee ES, Chen H, Shepherd KR, Lamango NS, Soliman KF, Charlton CG. Inhibitory effect of Lysophosphatidylcholine on the dopaminergic system. Neurochem Res 2004;29:1333–42.

Lewin R. Parkinson's disease: an environmental cause? Science 1985; 229:257–8.

Lindquist NG, Larsson BS, Lyden-Sokolowski A. Autoradiography of 14C Paraquat or 14C Diquat in frogs and mice: accumulation in neuromelanin. Neurosci Lett 1988;93:1–6.

Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, et al. Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. Neurology 1997;48:1583–8.

Mannisto PT, Kaakkola S. Catechol-*O*-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev 1999;51:593–628.

Markey SP, Weisz A, Bacon JP. Reduced paraquat does not exhibit MPTP-like neurotoxicity. J Anal Toxicol 1986;10:257.

Matsubara K, Kobayashi S, Kobayashi Y, Yamashita K, Koide H, Hatta M, et al. B-carbolinium cations, endogenous MPP+ analogs, in the lumbar cerebrospinal fluid of parkinsonian patients. Neurology 1995;45:2240–5.

Mays LL, Borek E, Finch CE. Glycine *N*-methyltransferase is a regulatory enzyme which increases in ageing animals. Nature 1973;243(5407):411–3.

McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, et al. Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis 2002;10:119–27.

McGrew DM, Irwin I, Langston JW. Ethylenebisdithiocarbamate enhances MPTP-induced striatal dopamine depletion in mice. Neurotoxicology 2000;21:309–12.

McNaught KS, Thull U, Carrup A, Altomare C, Cellamare S, Carotti A, et al. Inhibition of complex I by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). Biochem Pharmacol 1995;50:1903–11.

Miller DB, Reinhard JF, Daniels AJ, O'Callaghan JP. Diethyldithiocarbamate potentiates the neurotoxicity of in vivo 1-methyl-4-phenyl-1,2,3,6-tettrahydropyridine and of in vitro 1-methyl-4-phenylpyridinium. J Neurochem 1991;57:541–9.

Moghal S, Rajput AH, D'Arcy C, Rajput R. Prevalence of movement disorders in elderly community residents. Neuroepidemiology 1994;13:175–8.

Olanow CW, Tatton WG. Etiology and pathogenesis of Parkinson's disease. Annu Rev Neurosci 1999;22:123–44.

Oreland L. Monoamine oxidase, dopamine and Parkinson's disease. Acta Neurol Scand 1991;84(Suppl. 136):60–5.

Perry TL, Yong VW, Wall RA, Jones K. Paraquat and two endogenous analogues of the neurotoxic substance *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine do not damage dopaminergic nigrostriatal neurons in the mouse. Neurosci Lett 1986;69:285–9.

Sanchez-Ramos JR, Hefti F, Weiner WJ. Paraquat and Parkinson's disease. Neurology 1987;37:728.

Sellinger OZ, Kramer CM, Conger A, Duboff GS. The carboxylmethylation of cerebral membrane-bound proteins increases with age. Mech Ageing Dev 1988;43:161–73.

Shimada H, Hirai KI, Simamura E, Pan J. Mitochondrial NADH-quinone oxidoreductase of the outer membrane is responsible for paraquat cytotoxicity in rat livers. Arch Biochem Biophys 1998;351:75–81.

Shimizu K, Matsubara K, Ohtaki K, Fujimaru S, Saito O, Shiono H. Paraquat induces long-lasting dopamine overflow through the excitotoxic pathway in the striatum of freely moving rats. Brain 2003:976243–52.

Standaert DG, Stern MB. Update on the management of Parkinson's disease. Med Clin North Am 1993;77:169.

Stramentinoli G, Gualano M, Catto E, Algeri SJ. Tissue levels of *s*-adenosyl methionine in ageing rats. J Gerontol 1977;32:392–4.

Tanner CM. The role of environmental toxins in the etiology of Parkinson's disease. Brain Res 1989;12:49–54.

Tanner CM. Occupational and environmental causes of Parkinsonism. Occup Med 1992;7:503–13.

Tanner CM, Ben-Shlomo. Epidemiology of Parkinson's disease. Adv Neurol 1999;80:153–9.

Tanner CM, Goldman SM. Epidemiology of movement disorders. Curr Opin Neurol 1994;7:340–5.

Tanner CM, Goldman SM. Epidemiology of Parkinson's disease. Neuroepidemiology 1996;14:317–35.

Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, et al. J Am Med Assoc 1999;281:341–6.

Thiruchelvam M, Brockel BJ, Richfield EK, Baggs RB, Cory-Slechta DA. Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease. Brain Res 2000;873:225–34.

Thiruchelvam M, McCormack A, Richfield EK, Baggs RB, Tank AW, Di Monte DA, et al. Age-related irreversible progressive nigrostriatal

dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. Eur J Neurosci 2003;18:589–600.

Thomas DM, Walker PD, Benjamins JA, Geddes TJ, Kuhn DM. Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. J Pharmacol Exp Ther 2004;31(1):1–7.

Trush MA, Mimnaugh EG, Ginsburg E, Gram TE. In vitro stimulation by paraquat of reactive oxygen-mediated lipid peroxidation in rat lung. Toxicol Appl Pharmacol 1981;60:279–86.

Tuomisto L. Ontogenesis and regional distribution of histamine and histamine-*N*-methyltransferase in guinea pig brain. J Neurochem 1977;28:271–6.

Walters TL, Irwin I, Delfani K, Langston JW, Janson AM. Diethyldithiocarbamate causes nigral cell loss and dopamine depletion with nontoxic doses of MPTP. Exp Neurol 1999;156:62–70.

Westerink BH, Spaan SJ. Simultaneous determination of the formation rate of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in various rat brain areas. Brain Res 1982;252:239–425.

Widdowson PS, Farnworth MJ, Simpson MG, Lock EA. Influence on age on the passage of paraquat through the blood–brain barrier in rats: a distribution and pathological examination. Hum Exp Toxicol 1996;15:231–6.

Williams AC, Pall HS, Steventon GB, Green S, Buttrum S, Molloy H, et al. *N*-methylation of pyridines and Parkinson's disease. Adv Neurol 1993; 60:194–6.

Yang WL, Sun AY. Paraquat-induced free radical reaction in mouse brain microsomes. Neurochem Res 1998;23:47–53.

Yassin MS, Cheng H, Ekblom J, Oreland L. Inhibitors of catecholamine metabolizing enzymes cause changes in *S*-adenosyl methionine and *S*-adenosyl homocysteine in the rat brain. Neurochemistry 1998:3253–9.