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Differential effects of carboxyfullerene on MPP+/MPTP-induced neurotoxicity

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Abstract

The effects of carboxyfullerene on a well-known neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its active metabolite 1-methyl-4-phenyl-pyridinium (MPP^+) were investigated. In chloral hydrate-anesthetized rats, cytosolic cytochrome c was elevated in the infused substantia nigra 4 h after an intranigral infusion of MPP^+. Five days after local application of MPP^+, lipid peroxidation (LP) was elevated in the infused substantia nigra. Furthermore, dopamine content and tyrosine hydroxylase (TH)-positive axons were reduced in the ipsilateral striatum. Concomitant intranigral infusion of carboxyfullerene abolished the elevation in cytochrome c and oxidative injuries induced by MPP^+. In contrast, systemic application of carboxyfullerene did not prevent neurotoxicity induced by intraperitoneal injection of MPTP. In mice, systemic administration of MPTP induced a dose-dependent depletion in striatal dopamine content. Simultaneous injection of carboxyfullerene (10 mg/kg) actually potentiated MPTP-induced reduction in striatal dopamine content. Furthermore, systemic administration of carboxyfullerene (30 mg/kg) caused death in the MPTP-treated mice. An increase in the striatal MPP^+ level and reduction in hepatic P450 level were observed in the carboxyfullerene co-treated mice. These data showed that systemic application of carboxyfullerene appears to potentiate MPTP-induced neurotoxicity while local carboxyfullerene has been suggested as a neuroprotective agent. Furthermore, an increase in striatal MPP^+ level may contribute to the potentiation by carboxyfullerene of MPTP-induced neurotoxicity.

Keywords: Carboxyfullerene; MPTP; MPP^+; Neurotoxicity

1. Introduction

Recently, several water-soluble derivatives of cage molecules, i.e. fullerenes, have been developed and successfully synthesized, including carboxyfullerene (carboxylic derivative of fullerenes), fullerenol (polyhydroxylated fullerene) and hexasulfobutylated C60 (FC4S). Both in vitro and in vivo studies have been performed on water-miscible fullerene derivatives, and the action of these molecules has become a major focus of interest in the field of antioxidative research. For example, carboxyfullerenes has been demonstrated to prevent neurotoxicity evoked by *N*-methyl-D-aspartate (NMDA), by serum deprivation or by exposure to the Alzheimer disease amyloid peptide ($A\beta_{1-42}$) in cortical cell cultures (Dugan et al., 1997). Fullerenol-1 has been found to not only attenuate exsanguination-induced bronchoconstriction (Lai and Chiang, 1997) but also prevent H_2O_2-induced inhibition of population spikes in the rat hippocampal slice preparation (Tsai et al., 1997). Furthermore, chronic infusion of carboxyfullerenes reportedly delayed both functional deterioration and death of transgenic mice carrying the human mutant superoxide dismutase (SOD) gene responsible for familial amyotrophic lateral sclerosis (Dugan et al., 1997). Moreover, our previous study found that intranigral infusion of carboxyfullerene prevented iron-induced neurodegeneration in anesthetized rats (Lin et al., 1999). Recently, systemic FC4S was found to reduce cortical infarction induced by focal ischemia-reperfusion (Huang et al., 2001).

Due to the increasing popularity of water-miscible fullerenes in the neuroprotective research, the toxicity of these molecules has become critical. Our previous study has shown that local application of carboxyfullerene in the substantia nigra did not cause any toxicity in vivo

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(Lin et al., 1999). Systemic carboxyfullerene by osmotic pump (15 mg/kg per day) was not toxic to transgenic mice carrying human SOD gene (Dugan et al., 1997). However, intraperitoneal injection of polyalkylsulfonated C60 reportedly caused nephrotoxicity (Chen et al., 1998). The LD_{50} of fullerenol or polyalkylsulfonated C60 by intraperitoneal injection was 0.6–1.2 g/kg (Chen et al., 1998; Ueng et al., 1997). Therefore, the toxicity of the water-soluble fullerene derivatives renders their usefulness questionable. In the present study, the effects of carboxyfullerene were tested in two well-established animal models for Parkinson's study, i.e. intranigral infusion of 1-methyl-4-phenylpyridinium (MPP^+) in rats or systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice, respectively. In the brain, MPTP is metabolized to MPP^+ by monoamine $oxidase_B$ in astrocytes (Chiba et al., 1984) and accumulates in the dopaminergic neurons via the DA-reuptake system (Javitch et al., 1985). Intracellularly, MPP^+ is concentrated in mitochondria where it may block NADH-CoQ10 reductase (complex I) of the respiratory chain (Bates et al., 1994). Several proposed mechanisms of MPP^+ action have been suggested, including an oxidative stress by free radical generation, inhibition of mitochondrial respiration and energy depletion (Chiueh et al., 1992; Sayre, 1989; Wu et al., 1994). Apoptosis is reportedly responsible for the MPP^+-induced neurotoxicity (Spooren et al., 1998; Tatton and Kish, 1997). Therefore, the lipid peroxidation (LP) and cytosolic cytochrome c in the MPP^+-infused substantia nigra were measured to indicate the involvement of oxidative stress and apoptosis, respectively. Furthermore, functional integrity was evaluated by measuring dopamine content and tyrosine hydroxylase (TH)-positive axons in rat striatum. Our data showed that systemic and local carboxyfullerene differentially altered MPP^+- or MPTP- induced neurotoxicity.

2. Methods

Both 60 adult male Sprague–Dawley rats (250–350 g) and 150 BALB/c mice (20–25 g) supplied by National Laboratory Animal Breeding and Research Center, Taipei, Taiwan, were used. These animals were maintained according to the guidelines established in "Guide For The Care And Use Of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, USA (1985).

2.1. Rat study

2.1.1. Intranigral infusion of MPP^+

Rats were anesthetized with chloral hydrate (450 mg/kg, i.p., Sigma, USA) and stereotaxically immobilized. Holes were drilled above the cortical surface for intranigral infusion of drugs. One microliter of MPP^+ (3 μg per injection site) ± carboxyfullerene (10 μg per injection site) was infused stereotaxically into the substantia nigra (coordinates: 3.2 mm anterior and 2 mm above the interaural zero, 2.1 mm lateral to the midline; mouth bar = −3.5 mm) (Paxinos and Watson, 1986). After the infusion, rats recovered from anesthesia and were placed in home cages.

2.1.2. Western blot analysis of cytochrome c

Four hours after intranigral infusion, rats were decapitated. Substantia nigras were dissected and homogenized with a Dounce tissue grinder in 100 μl ice-cold mitochondrial isolation buffer (70 mM sucrose, 210 mM mannitol, 5 mM Tris–HCl, 1 mM EDTA, 20 μM fluorocitrate, pH 7.4). After homogenization, the suspension was centrifuged at 600 × *g* for 5 min at 4 °C, the supernatant transferred to a chilled Eppendorf tube and centrifuge at 17,000 × *g* for 10 min at 4 °C. The cytosolic fraction was frozen in liquid nitrogen and stored at −80 °C. Purity of the cytosolic fraction was determined by measuring cytochrome c oxidase. The cytosolic protein samples (50 μg) were run on 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto a nitrocellular membrane (Bio-Rad, USA) at 80 V for 75 min. Blots were probed with a mouse monoclonal antibody (7H8.2C12, Pharmingen, USA) against the denatured form of cytochrome c at dilution of 1:500 at 4 °C for 45 min. After primary antibody incubation, the membrane was washed and incubated with horseradish peroxidase-conjugated goat antimouse IgG (Chemicon Int., USA) for 40 min at room temperature. The immunoreaction was visualized using Amersham enhanced chemiluminescence (Amersham Pharmacia Biotech, UK). After this detection, the bound primary and secondary antibodies were stripped by incubating the membrane in stripping buffer (63 mM Tris–base, 0.7% 2-mercaptoethanol, 2% sodium dodecyl sulfate) at 50 °C for 30 min. The membrane was reprobed with a mouse cytochrome c oxidase (subunit IV) antibody and β-actin and then visualized with a procedure identical to that for cytochrome c.

2.1.3. Fluorescence assay of lipid peroxidation in substantia nigra

Five days after intranigral infusion, rats were sacrificed by decapitation. Substantia nigras were dissected from both hemispheres and homogenized in chilled 400 μl chloroform and 200 μl methanol. After centrifugation, an aliquot of the chloroform and methanol layer was scanned using a spectrofluorometer (Aminco Bowman-2, USA). Lipid peroxidation was determined by measuring the levels of malondialdehyde and its dihydropyridine polymers, which emit fluorescence at 426 nm when activated by UV at 356 nm (Kikugawa et al., 1989; Mokanakumar et al., 1994).

2.1.4. HPLC–EC analysis of striatal dopamine content

Five days after infusion, rats were sacrificed by decapitation. Striata were dissected and immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

An HPLC with EC detection procedure was used to quantify dopamine content in striatum (Chiueh et al., 1983).

2.1.5. TH immunohistochemical study

For TH immunohistochemistry, rats were perfused transcardially using 0.9% saline followed by a fixative consisting of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brain were removed and placed in 30% sucrose buffer solution overnight and frozen-sectioned coronally at 45 μm using a cryostat. Brain sections were collected for avidin-biotin immunohistochemistry (ABC Elite Kit, Vector, USA). The primary antibody and secondary antiobodies were diluted with PBS containing 0.3% Triton X-100 (Sigma, USA) and 1% normal goat serum (Vector Lab. Inc., USA). The primary antibody against TH (TE101, Eugene Tech, USA) was incubated with sections for 24 h at 25 °C; the corresponding secondary (goat–anti-rabbit IgG PK-6001, Vector Lab. Inc., USA) was later applied for 45 min. Sections were incubated with the avidin-biotin complex substrate (P-4000, Vector Lab. Inc., USA) for 45 min and then treated with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma, USA) and 0.03% hydrogen peroxide in 0.05 M Tris–buffer (pH 7.6) for 7–10 min. After immunohistochemical reaction, sections were mounted, dehydrated, counter-stained and observed under light microscopes.

2.2. Mice study

2.2.1. Systemic administration of MPTP

Mice were divided into four groups: one group received vehicle injection (0.1 M PBS, i.p.) as the control; a second group was treated with MPTP (10, 20 or 30 mg/kg, i.p.) per day for 4 consecutive days; a third group received MPTP (10, 20 or 30 mg/kg, i.p.) plus carboxyfullerene (10 or 30 mg/kg, i.p.) and a fourth group was treated with carboxyfullerene alone. Animals were sacrificed 24 h after the last injection (i.e. on the fifth day). Striata were dissected and immediately frozen in liquid nitrogen and stored at −80 °C until analysis as previously mentioned. For measuring the striatal dopamine content, an HPLC with EC detection procedure was used to quantify dopamine content in striatum (Chiueh et al., 1983).

2.2.2. Striatal MPP^+ assay

Twenty four hours after the last injection, mice were sacrificed. Striata were dissected and homogenized in ice-cold 0.1N perchloric acid and the homogenates were centrifuged. MPP^+ was measured by HPLC coupled with a spectrophotometric detector (Wu et al., 1994). The mobile phase, composed of 0.1 M acetic acid/acetonitrile (70:30) and 0.1% of triethylamine, was delivered at a flow rate of 1 ml/min. The column was cosmosilpack 5C18, 4.6 mm × 250 mm (Nacalai Tesque Inc., Japan). The wavelength was set to 290 nm.

2.2.3. Microsomal cytochrome P450 assay

Twenty-four hours after the last injection of MPTP, mice were sacrificed and the mouse liver was removed for microsomal preparation as described elsewhere (Ueng et al., 1997). Microsomal cytochrome P450 content was determined by the method of Omura and Sato (Omura and Sato, 1964). Protein concentration was determined using bovine serum albumin as a standard (Lowry et al., 1951).

2.2.4. Statistics

Data were expressed as the mean ± S.E.M. Statistical comparisons were made using one tail Student's *t*-test (for striatal MPP^+ content) or one-way ANOVA followed by a Bonferroni Multiple-Comparisons Procedure as a post-hoc analysis.

3. Results

3.1. Local carboxyfullerene versus MPP^+-induced neurotoxicity in rat brain

To evaluate the neuroprotective effect of carboxyfullerene on MPP^+-induced neurodegeneration, MPP^+ was locally infused in the substantia nigra of anesthetized rats. Four hours after the infusion, cytochrome c was increased in the cytosolic extract of the infused substantia nigra, whereas cytochrome c oxidase (subunit IV) was absent (Fig. 1). The absence of cytochrome c oxidase in the cytosolic extract indicated that the cytosolic extract was free of mitochondrial contamination. Furthermore, 5 days after the infusion, the fluorescent end products of lipid peroxidation were increased in the infused substantia nigra (Fig. 2). At the same time, the striatal dopamine content was depleted to 43% of the intact control. While intranigral infusion of carboxyfullerene alone did not damage the nigrostriatal dopaminergic system, simultaneous infusion of carboxyfullerene and MPP^+ attenuated MPP^+-induced elevations in cytosolic cytochrome c (Fig. 1) and lipid peroxidation in the infused

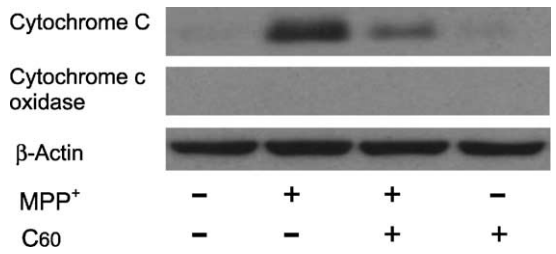

Fig. 1. Effects of carboxyfullerene on MPP^+-induced elevation in cytochrome c in cytosolic extract of the infused substantia nigra. A representative result of cytochrome c in the cytosolic fraction of substantia nigra was demonstrated by Western blot 4 h after an intranigral infusion of MPP^+ ± carboxyfullerene (C_{60}). Cytochrome c oxidase was not detected indicating the purity of the cytosolic fraction. Fifty micrograms of protein were loaded in each lane in all experiment. Similar results were obtained in triplicates.

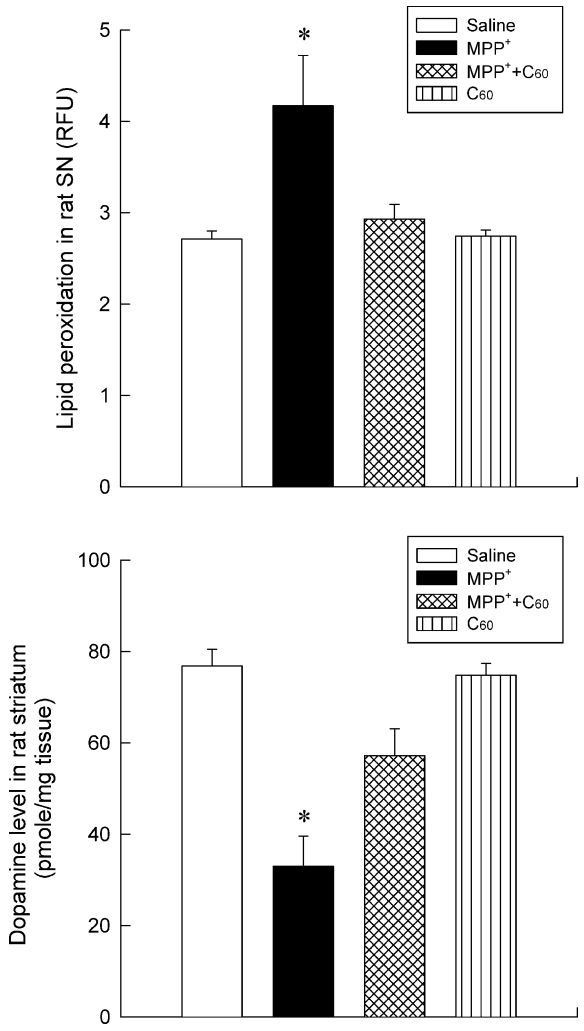

Fig. 2. Effects of carboxyfullerene on MPP^+-induced oxidative injuries in the nigrostriatal dopaminergic system. Five days after an intranigral infusion of MPP^+ (3 μg/μl per injection site) ± carboxyfullerene (C_{60}, 10 μg/μl per injection site), striatal dopamine content was determined using HPLC–EC detection. The LP in the microdissected substantia nigra was measured and reported as relative fluorescence units (RFU). Values are the mean ± S.E.M. (n = 6–12). $^*P < 0.05$ in the MPP^+ group compared to the intact controls and $MPP^+ + C_{60}$ group by an ordinary one-way ANOVA followed by a Bonferroni test as a post-hoc analysis.

substantia nigra (Fig. 2). Furthermore, carboxyfullerene diminished the MPP^+-induced depletion in striatal dopamine content (Fig. 2).

Striatal section with immunoreactive fibers manifested a reduction in TH immunoreactive fibers 5 days after local infusion of MPP^+ in the substantia nigra (Fig. 3A). Intranigral infusion of carboxyfullerene and MPP^+ mixture diminished the MPP^+-induced reduction in TH density of the striatum ipsilateral to the infused substantia nigra compared to the intact side of the same brain (Fig. 3B).

3.2. Systemic carboxyfullerene versus MPTP-induced neurotoxicity in mice

Treatment with a daily dose of MPTP (10 mg/kg) or carboxyfullerene (10 mg/kg, i.p.) for 4 consecutive days had no effect on striatal dopamine level in mice 24 h after the last injection. However, simultaneous administration of carboxyfullerene and MPTP significantly depleted dopamine content in mouse striatum (Fig. 4). Furthermore, systemic administration of a daily dose of 30 mg/kg MPTP for 4 days reduced the striatal dopamine content to 50% of the control group. Co-administration of carboxyfullerene (10 mg/kg, i.p.) further augmented the MPTP-induced depletion of striatal dopamine content in mouse striatum and no mortality was observed (Fig. 4). When the dose of systemic carboxyfullerene increased to 30 mg/kg, which alone did not cause any changes in the nigrostriatal dopaminergic system, intraperitoneal administration of carboxyfullerene resulted in 100% mortality in mice receiving 30 mg/kg MPTP injection (Table 1).

3.3. Effects of carboxyfullerene on striatal MPP^+ level and hepatic P450 level

The effect of carboxyfullerene on the striatal MPP^+ level was investigated in mice daily treated with PBS (as control), carboxyfullerene (10 mg/kg), MPTP (20 mg/kg) or carboxyfullerene + MPTP for 4 days. While the striatal MPP^+ level was not detectable in PBS and carboxyfullerene-treated mice, the striatal MPP^+ level averaged 2.1 ± 0.1 pmoles/mg tissue in MPTP-treated mice (n = 5) versus 2.5 ± 0.1 pmoles/mg tissue (n = 5) in MPTP and carboxyfullerene co-treated mice ($P < 0.05$ by Student's t-test). At the same time, the effect of carboxyfullerene on the cytochrome P450 was determined using liver microsomal preparation. Systemic administration of carboxyfullerene or MPTP decreased cytochrome P450 content 24 h after the last injection. Simultaneous administration of carboxyfullerene augmented MPTP-induced reduction in cytochrome P450 content (Table 2).

Table 1
Effects of systemic carboxyfullerene (C_{60}, 30 mg/kg, i.p.) on the mortality of mice receiving MPTP injection (30 mg/kg, i.p.)

	Vehicle	MPTP	MPTP + C60	C60
Number of survived/total mice tested	6/6	9/9	0/9	7/7
Striatal dopamine level (μmole/mg protein)	1.20 ± 0.04	0.52 ± 0.04[a]	ND	1.31 ± 0.05

Values are the mean ± S.E.M.

[a] $P < 0.05$ in the MPTP-treated group, compared to the vehicle controls, and C_{60} group by one-way ANOVA followed by a Bonferroni test as a post-hoc analysis.

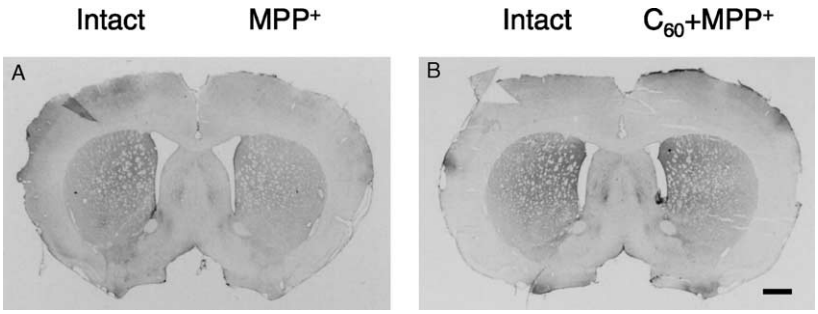

Fig. 3. Striatal sections processed with TH antibody according to the ABC technique. MPP^+ (3 μg/μl per injection site) ± carboxyfullerene (10 μg/μl per injection site) was locally infused in rat substantia nigra. (A) and (B) show a magnified view of striatal sections from a brain locally infused with MPP^+ (A) or MPP^+ plus carboxyfullerene mixture (B) in the substantia nigra on the right brain hemisphere. Note the enhancement of TH-like staining in the striatum of carboxyfullerene co-infused animal (B) as compared to the MPP^+-infused animal (A). Scale bar: 1 mm.

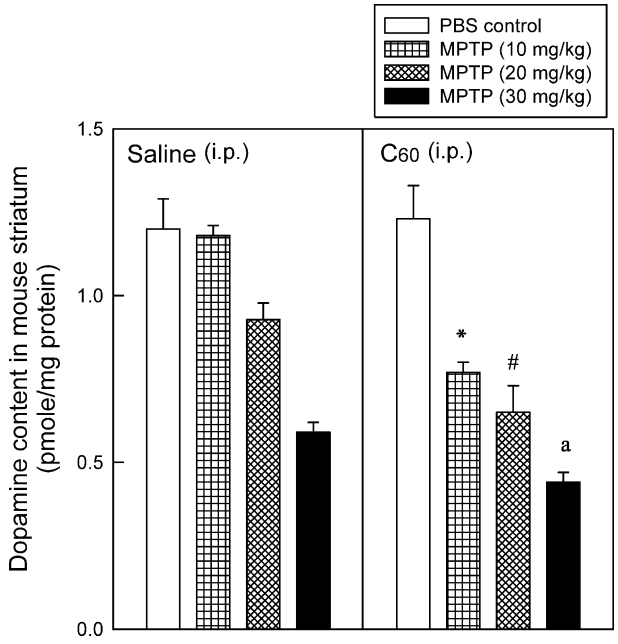

Fig. 4. Effects of systemic carboxyfullerene on MPTP-induced oxidative injuries in the nigrostriatal dopaminergic system in mice. Twenty-four hours after a daily dose of MPTP or carboxyfullerene (C_{60}, 10 mg/kg, i.p.) for 4 consecutive days, striatal dopamine content was depleted 24 h after the last systemic administration of MPTP. Values are the mean ± S.E.M. ($n = 6$–12). * $P < 0.05$ in the MPTP (10 mg/kg) of C_{60} group compared to that of saline group; (#) $P < 0.05$ in the MPTP (20 mg/kg) of C_{60} group compared to that of saline group; (a) $P < 0.05$ in the MPTP (30 mg/kg) of C_{60} group compared to that of saline group by an ordinary one-way ANOVA followed by a Bonferroni test as a post-hoc analysis.

4. Discussion

In the present study, carboxyfullerene differentially modulated the neurotoxicity induced by systemic MPTP or local MPP^+. Intranigral infusion of carboxyfullerene not only suppressed MPP^+-induced elevations in cytosolic cytochrome c and lipid peroxidation in the infused substantia nigra but also attenuated depletions in dopamine content and TH-positive axons in the ipsilateral striatum of anesthetized rats, indicating that carboxyfullerene is neuroprotective. In contrast, systemic injection of carboxyfullerene did not prevent the neurotoxicity induced by intraperitoneal injection of MPTP in mice. Systemic carboxyfullerene actually potentiated MPTP-induced depletion of dopamine content and resulted in death. Our data suggested that carboxyfullerene possesses an antioxidative property. Nevertheless, the potentiation by systemic carboxyfullerene of MPTP-induced neurotoxicity might limit the use of carboxyfullerene as a neuroprotective agent in biological systems.

Several strategies have been proposed to prevent biological organisms from oxidative injury, including supplementation with antioxidants and up-regulation of endogenous antioxidative defense systems by neurotrophic factors (Ebadi et al., 1996; Hou et al., 1997; Simom and Standaert, 1999). Due to its unusual structure that possesses a very large electronegative center, carboxyfullerene, a water-miscible fullerene derivative, has been suggested as a therapeutic antioxidant (Lin et al., 1999, 2002; Tsao et al., 1999). Indeed, systemic administration of carboxyfullerene has been reported to decrease mortality of transgenic mice carrying

Table 2
Effects of systemic carboxyfullerene (C_{60}, 10 mg/kg) and MPTP (20 mg/kg) on the cytochrome P450-dependent monooxygenase in mouse liver

	PBS (5)	C60 (5)	MPTP (5)	MPTP + C60 (5)
Microsomal protein (mg/g liver)	16.9 ± 0.8	14.6 ± 1.0	18.0 ± 2.3	17.4 ± 1.0
Cytochrome P450 (nmol/mg protein)	0.55 ± 0.02	0.31 ± 0.03[a]	0.44 ± 0.04[a]	0.28 ± 0.04[a]

Values are the mean ± S.E.M. Numbers in parentheses represent sample size.
[a] $P < 0.05$ in the drug-treated groups compared to the PBS control by one-way ANOVA followed by a Bonferroni test as a post-hoc analysis.

familial amyotrophic lateral sclerosis (Dugan et al., 1997) and *E. coli*-induced meningitis (Tsao et al., 1999). Furthermore, the data from the present study on rats showed that local carboxyfullerene could inhibit oxidative injuries and cytochrome c release, indicating an inhibition by carboxyfullerene of MPP^+-induced neurotoxicity via an apoptotic mechanism. These data are consistent with our previous study in which local carboxyfullerenes has been reported to suppress iron-induced oxidative injuries in the nigrostriatal dopaminergic system (Lin et al., 1999). In addition, our recent data demonstrated that intracerebroventricular infusion of carboxyfullerene reduced cortical infarction by transient focal ischemia-reperfusion (Lin et al., 2002). Accordingly, carboxyfullerene appears to be neuroprotective.

In contrast to the neuroprotection of carboxyfullerene, our data actually demonstrated toxic effects of carboxyfullerene, i.e. systemic administration of carboxyfullerene potentiated the MPTP-induced dopamine depletion in mouse striatum and caused mortality of MPTP-treated mice. Our study further showed that simultaneous administration of carboxyfullerene and MPTP increased striatal MPP^+ level, indicating that carboxyfullerene is capable of increasing MPP^+ level in CNS. The mechanism underlying the potentiation by systemic carboxyfullerene of MPP^+ level is unknown. One of the possibilities may be due to the carboxyfullerene-induced inhibition of metabolism of MPTP. It is known that systemically administered MPTP can be metabolized peripherally (Sayre, 1989) and centrally (Markey et al., 1984). Cytochrome P450 is one of the major enzymes responsible for metabolism and/or detoxification of MPTP in the liver (Sayre, 1989). In agreement with other studies (Chen et al., 1998; Ueng et al., 1997), our data showed that carboxyfullerene decreased cytochrome P450 level. Accordingly, carboxyfullerene may inhibit the detoxification of MPTP in the periphery and more MPTP can pass through blood–brain-barrier and become MPP^+ which in turn, enhanced striatal dopamine depletion. As to the increased mortality, systemic MPTP reportedly increased MPP^+ levels in heart, brain, and lung (Fuller and Hemrick-Luecke, 1990) and several pathological changes by systemic MPTP have been demonstrated, including, perivascular edema in lung (Johannessen et al., 1986), norepinephrine depletion in heart (Fuller et al., 1989) and neurodegeneration in CNS (Sayre, 1989; Chiueh et al., 1992). While no studies have definitively indicated which target is the site of the lethal effect by systemic MPTP, carboxyfullerene-induced increase in striatal MPP^+ level indicates that MPP^+ levels may also increase in other vital organs, including heart and lung. In this case, high dose of carboxyfullerene may result in death of MPTP-treated mice via supplying lethal doses of MPTP in mice (Fuller and Hemrick-Luecke, 1990).

So far, our study suggests that systemic carboxyfullerene may be toxic in the MPTP-treated mice, however, systemic carboxyfullerene is reportedly neuroprotective in Dugan's study (Dugan et al., 1997). Differences between the present study and Dugan's study (Dugan et al., 1997) need to be clarified. In Dugan's study, only carboxyfullerene, but no other drug, was used in mice carrying the human mutant SOD gene (Dugan et al., 1997) which eliminates the possibility of carboxyfullerene interacting with other chemicals. Furthermore, in our study, carboxyfullerene appeared to be toxic only when MPTP was co-existed in mice.

In conclusion, although our previous study and the present study have shown that local application of carboxyfullerene is capable of inhibiting oxidative injuries in biological organisms, the data from the present study also demonstrated that intraperitoneal injection of carboxyfullerene actually potentiated the MPTP-induced neurotoxicity and resulted in death. These data indicate that local carboxyfullerene may be non-toxic and neuroprotective, however, systemic carboxyfullerene can be toxic in biological organisms.

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