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Desferrioxamine and vitamin E protect against iron and MPTP-induced neurodegeneration in mice

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Summary. To elucidate the neuroprotective effects of the iron chelator desferrioxamine (DFO) and the antioxidant vitamin E on excessive iron-induced free radical damage, a chronic iron-loaded mice model was established. The relationship between striatal iron content, oxidized to reduced glutathione ratio, hydroxyl radical (·OH) levels and dopamine concentrations were observed in DFO or vitamin E pretreated iron-loaded/1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated C57BL/6 mice. The results demonstrated that both DFO and vitamin E inhibit the iron accumulation and thus reverses the increase in oxidized glutathione (GSSG), oxidized to reduced glutathione ratios, ·OH and lipid peroxidation levels. The striatal dopamine concentration was elevated to normal value. Our data suggested that: (1) iron may induce neuronal damage and thus excessive iron in the brain may contribute to the neuronal loss in PD; (2) iron chelators and antioxidants may serve as potential therapeutic agents in retarding the progression of neurodegeneration.

Keywords: Iron, MPTP, DFO, vitamin E, neurodegeneration

Introduction

The aetiology of the neurodegeneration in Parkinson's disease (PD) remains obscure. However, an increasing body of both clinical and experimental evidence suggests that iron-induced oxidative stress may contribute to cellular stress underlying the pathogenesis of neurodegeneration in PD. Iron is selectively increased in the substantia nigra (SN), particularly in the substantia nigra pars compacta (SNc) in the parkinsonian brain (Sofic et al., 1991; Dexter et al., 1989; Riederer et al., 1989; Youdim et al., 1993; Zecca et al., 1994; for reviews, see Gerlach et al., 1994). The role of iron in PD has recently attracted attention following the finding that a regionally selective increase in iron is associated with dopamine neuron death in both PD patients and in animal models (Ben-Shachar and Youdim, 1991a; Sengstock et al., 1992, 1993, 1994). As an active free radical reaction catalyst, iron may be involved in many

processes of free radical formation and initiate the chain reaction of lipid peroxidation (LPO) in membranes leading to cell death (for review, see Götz et al., 1994). A direct relationship exists between local iron concentrations and oxidant-induced damage (Halliwell and Gutteridge, 1984).

Recent studies on nigral iron infusion in the rat model suggested that the intranigral infusion of iron can provide several long-term progression changes, including a progressive decrease in striatal dopamine (DA) and homovanillic (HVA), atrophy of the SN, increase in apomorphine-induced rotational behavior and elevation in lipid peroxidation within the SN (Sengstock et al., 1992, 1993, 1994). This suggests that peroxidative damage may be involved in the neurodegeneration resulting from intranigral iron infusion. Thus, recent researches have been focussed on neuroprotective therapies which may prevent or slow down the progression of neuronal degeneration. On the basis of the above premise, it is reasonable to propose that iron chelators and antioxidants may act therapeutically and retard neurodegeneration in PD. Some studies have found that desferrioxamine, a powerful iron chelator, can inhibit iron-dependent free radical reactions, and it has already been successfully shown to diminish oxidant damage in animal models (Halliwell, 1987, 1989). Moreover, the finding that DFO can protect rats against 6-hydroxydopamine (6-OHDA)-induced reductions in striatal dopamine and dopamine-related spontaneous locomotor activity (Ben-Shachar et al., 1991b, 1992) promoted further research on the protective role of DFO against iron-induced free radical damage. Vitamin E, as a major lipid soluble antioxidant in biological systems, may trap peroxyl radicals and interrupt the lipid peroxidation chain reactions (Packer and Landvik, 1990). Alpha-tocopherol (vitamin E) has been reported protecting partially against the toxicity of 6-OHDA-induced depletion in striatal DA in rats (Cadet et al., 1989). In addition, a chronic vitamin E deficiency increased the susceptibility of mice to MPTP toxicity (Odunze et al., 1990) and produced a selective reduction in the number of dopaminergic nerve cell bodies in the SN of rats (Dexter et al., 1994). Patients deficient in vitamin E showed a reduction in striatal ^{18}F-dopa uptake on positron emission tomography (PET), similar to that seen in PD (Dexter et al., 1994). Furthermore, high doses of α-tocopherol and ascorbate given early in PD delays the need for L-DOPA by 2 to 3 years (Fahn, 1992).

It should be point out that iron is not a specific toxin for dopaminergic neurones; rather, iron can be toxic to all types of neuronal and glial cell depending on its local concentration and form (Arendash et al., 1994). Treatment with ferrocene to rats for 4 weeks showed up to 50% increase in iron content in various brain regions including, SN, cerebellum and cerebral cortex (Ward et al., 1995). A new animal model of cerebral infraction was developed by magnetic embolization with carbonyl iron particles (Akai et al., 1995). Carbonyl iron diet can also cause changes in several other organs. Administration of ferrocene can also result in an increase of iron contents in heart, liver, spleen and pancreas (Ward et al., 1995). An additive increase in hepatocellular injury, promotion liver fibrogenesis and cirrhosis was reported when ethanol is administrated to iron-loaded rats (Tector et al., 1995; Tsukamoto et al.,

1995). Studies also demonstrate a direct correlation between the concentration of free iron and the induction of LPO and the associated functional abnormalites in rat liver mitochondrial in mild dietary iron overload (Ceccarelli et al., 1995).

In summary, there are considerable experimental studies and clinical observations suggesting the treatment of PD may be entering a "neuroprotective era", therefore, promoting a need for effective and safe neuroprotective agents against iron toxicity. However, to the best our knowledge, the influence of systemic injection of DFO on brain iron content and the possibly protective effect of DFO and vitamin E on neurons against excessive brain iron deposition and MPTP-induced oxidative damage has not been studied. This is the focus of the present study.

Materials and methods

Reagents

Carbonyl iron, dopamine (DA), 3,4-dihydroxylphenylacetic acid (DOPAC), salicylate (SA), 2,5-/2,3-dihydroxylbenzoic acid (2,5-/2,3-DHBA), N-ethylmaleimide (NEM), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate (NADPH), 1,1,3,3-tetraethoxypropane (TEP), sodium octyl sulfate (SOS), bovine serum albumin (BSA) were obtained from Sigma (MO, USA). MPTP was a gift from the Shanghai Pharmalogical Institute, the Academy of Medical Sciences of China.

Animals and treatments

Weanling C57BL/6 mice of both sexes (21 ± 3 days old) were purchased from the Laboratory Animal Center of Tianjin Hemological Institute, the Academy of Medical Sciences of China. They were divided into three groups fed with either (1) a regular laboratory diet; (2) a high iron diet (carbonyl iron, 25 g iron/kg diet) or (3) a high iron plus high vitamin E diet (7 g/kg diet). From day 21, some animals in group 2 were injected (i.m.) with DFO (250 mg/kg body weight) daily for 10 days, while continuing to receive the high iron diet. On the 30th day, a sample of each of the normal and high iron diet mice, all animals receiving DFO, and group 3 animals were injected (i.p.) with MPTP (30 mg/kg body weight). The four treatment groups were designated MPTP, iron + MPTP, iron + MPTP + DFO and iron + MPTP + vitamin E groups, respectively. Remaining animals fed with a high iron diet or a normal diet were injected with an equal volume of 0.9% saline (i.e. iron-loaded and control group, respectively). All mice were given salicylate i.p. at 90th minute following the MPTP or saline injection and were decapitated at 120th minute. Brains were quickly removed for used immediately or stored at −70°C.

Methods

- *Determination of total iron concentration in striata:* total iron concentration in striata were determined by atomic absorption and emission spectroscopy, using a method similar to that reported by Uitti et al. (1989).
- *Determination of GSSG and total glutathione (GSSG + GSH):* following decapitation, frontal cortices were rapidly excised over ice and the tissues homogenized in 10 volumes of 0.4 mol/L cold perchloric acid (PCA) containing 0.1 mmol/L diethylenetriamine-pentaacetic acid and centrifugated at 10,000 g for 5 minutes at 4°C. Supernatants were analyzed immediately for GSH and GSSG concentrations by an enzymatic recycling procedure, as described by Tietze (1969) and modified by Cooper

et al. (1980), based on the activity of glutathione reductase. GSSG was mesured by first removing of GSH with NEM. A further aliquot of supernatant, without the addition of NEM and diluted with 10 volumes of buffer, was used for assaying total glutathione. A standard curve was constructed using GSSG solutions of known concentrations.

- *Determination of DA, DOPAC and hydroxyl radical (2,5- and 2,3-DHBA):* DA, DOPAC and 2,3-, 2,5-DHBA were assayed simultaneously by reverse-phase high performance liquid chromatography with electrochemical detection (HPLC-ECD), according to the technique of Kim et al. (1987) and Floyd et al. (1984). SA concentrations were analyzed in the same sample by UV detection at 295 nm. The levels of ·OH were expressed as the ratios of DHBA to salicylate (DHBA/SA) (Althaus et al., 1993). The HPLC apparatus was from Waters Association utilizing ODS columns (250 × 4.6 mm, C_{18} reverse-phase, 10 μm), +0.8 V oxidizing detector voltage with a sensitivity of 10. nA F.S. The mobile phase was composed of 30 mmol/L citrate and 30 mmol/L acetate pH3.6, 0.2 mmol/L SOS and 5~10% methanol and eluted at 1.0 ml/min. All HPLC analysis were performed using an external standard method.
  Immediately following decapitation, striatal and brainstem (including nigra) tissues were excised, weighed and homogenized by sonication in 10% trichloroacetic acid (TCA). The homogenate was centrifugated at 13,000 g for 20 minutes at 4°C. Supernatants were retained, and 20 μl aliquots were 20 minutes at 4°C. Supernatants were retained, and 20 μl aliquots were injected into HPLC system. All samples were assayed within 24 hours of decapitation.
- *Determination of LPO level:* LPO was assessed by measurement of malondialdehyde (MDA) concentration using the thiobarbituric acid test with dual-beam spectrophotometric technique as described by Uchiyama (1978). A standard curve was constructed employing TEP (0.10~0.25 nmol/L) and results were expressed as nmol MDA/mg protein.
- *Determination of protein:* protein content in brain homogenate samples were determined by the method of Lowry (1951) using BSA as a standard.
- *Statistics:* All values are represented as means ± SD. Statistical significance was examined using an unpaired two-tailed Student's t-test and values less than $p < 0.05$ were accepted as significant.

Results

Total iron concentrations in striatum

A significant increase in total iron concentration was observed in striata of the iron-loaded group, compared with the control and MPTP-treated groups. However, pretreatment with DFO, or supplementation with high doses of vitamin E, to iron-loaded/MPTP-treated mice reduced striatal total iron concentrations to normal level (Fig. 1).

Changes of total glutathione and GSSG concentrations and of GSSG/(GSSG + GSH) ratios

There was a significant reduction of total glutathione ($p < 0.001$) and an increase of GSSG ($p < 0.001$) in iron-loaded and MPTP-injected mice compared with control animals. Thus, higher GSSG/(GSSG + GSH) ratios were observed in these two groups. However, total glutathione and GSSG concentrations and GSSG/(GSSG + GSH) ratios in the iron-loaded/MPTP-treated mice were not significantly different from those in animals receiving iron or MPTP alone. Although the decrease of GSH was not improved by administration of DFO or vitamin E, the increase of GSSG concentrations were retarded

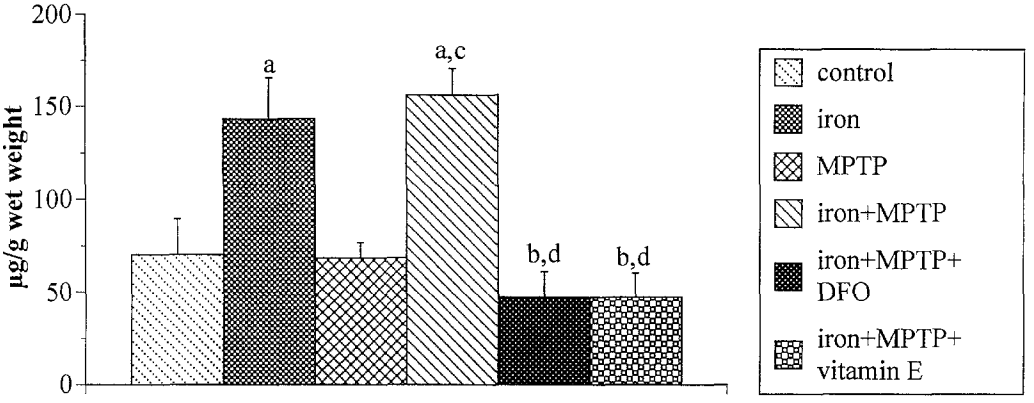

Fig. 1. Effect of DFO and vitamin E on striatal iron concentration of C57 BL/6 mice. Data are presented as mean ± SD (µg/g wet weight) of five to six animals in each group. Statistical significance was examined using Student's t-test (unpaired two-tailed). a, b, c, and d indicate significant differences ($p < 0.05$) compared with control, high iron diet fed, MPTP-injected and iron-loaded/MPTP-treated groups, respectively

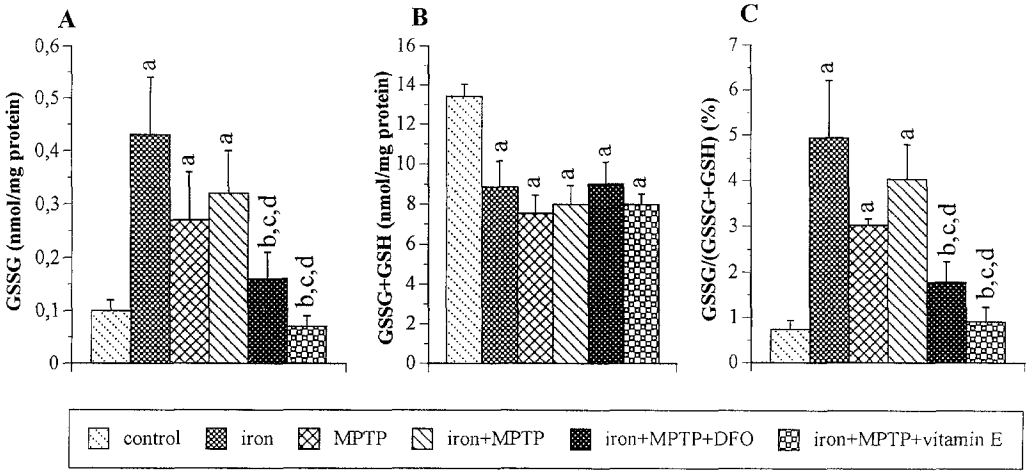

Fig. 2. Effect of DFO and vitamin E on **A** GSSG concentrations, **B** total glutathione (GSSG + GSH) concentrations and **C** GSSG/(GSSG + GSH) ratios in C57 BL/6 mice. Data are presented as mean ± SD (n = 5 for each group). a, b, c, and d indicate significant differences ($p < 0.05$) compared with control, iron-loaded, MPTP-treated and iron-loaded/MPTP-treated groups, respectively

completely and the ratio of GSSG/(GSSG + GSH) was reduced to normal values (Fig. 2).

Changes of ·OH levels

Both a high iron diet or MPTP-injection alone caused an increase of 2,5 DHBA/SA ratios in both measured regions compared with those in the control group. 2,3-DHBA concentrations were below the level of detection in the above three groups. 2,5-DHBA/SA ratios in the iron-loaded/MPTP-treated

Table 1. Effects of DFO and vitamin E on ratios of 2,5DHBA/SA and 2,3DHBA/SA in the iron-load and iron-loaded/MPTP-treated C57BL/6 mice brain

	n	Striatum		Brainstem	
		2,5DHBA/SA	2,3DHBA/SA	2,5DHBA/SA	2,3DHBA/SA
Control	5	2.50 ± 0.51	ND	3.18 ± 0.26	ND
Iron	5	4.66 ± 0.50[a]	ND	7.78 ± 2.79[a]	ND
MPTP	5	8.78 ± 1.06[a]	ND	4.12 ± 0.86	ND
Iron + MPTP	6	46.0 ± 19.1[a,b,c]	4.5 ± 0.8[a,b]	40.6 ± 7.9[a,b,]	3.93 ± 1.82[a,b,c]
Iron + MPTP + DFO	5	2.70 ± 0.15[a,c,d]	ND[d]	4.31 ± 0.56[b,d]	ND[d]
Iron + MPTP + vitamin E	5	7.76 ± 1.51[a,b,d]	ND[d]	5.70 ± 0.85[a,d]	ND[d]

Data are expressed as mean ± SD, in $\times 10^{-3}$. [a,b,c,d] $p < 0.05$ compared with control, iron-loaded, MPTP (30 mg/kg × 1) and iron-loaded/MPTP groups, respectively. *ND* not detectable

group were significantly elevated compared with all 3 control groups. Interestingly, the 2,5-DHBA/SA ratio in striatum and brainstem in the iron-loaded/MPTP-treated group was 20 and 8 times higher respectively compared with the iron-loaded mice. 2,3-DHBA levels were also increased in these two brain regions. However, pre-treating iron-loaded/MPTP-treated mice with DFO and vitamin E led to a significant attenuation of 2,5-DHBA/SA ratios in both the cerebral areas, and reduced 2,3-DHBA concentrations to a level below the analytical limit (Table 1).

Changes of DA and DOPAC concentrations

DA levels in the striatum of iron-loaded mice were unchanged, however, DOPAC concentrations were significantly decreased. Both DA and DOPAC concentrations were significantly reduced in the brainstem of iron-loaded mice. A single injection of MPTP (30 mg/kg) caused DA and DOPAC concentrations in both brain areas to be significantly reduced, particularly in the iron-

Table 2. Effects of DFO and vitamin E on concentrations of DA and DOPAC in the iron-loaded and iron-loaded/MPTP-treated C57BL/6 mice brain

	n	Striatum		Brainstem	
		DA	DOPAC	DA	DOPAC
Control	8	537.1 ± 68.5	172.3 ± 53.0	19.6 ± 0.5	18.5 ± 3.1
Iron	5	469.3 ± 50.6	88.1 ± 27.4	ND	ND
MPTP	5	279.1 ± 16.0[a]	47.9 ± 10.3[a]	6.4 ± 2.0[a]	4.7 ± 1.9[a]
Iron + MPTP	6	31.4 ± 5.1[a,b,c]	14.2 ± 1.7[a,b,c]	ND[c]	9.0 ± 2.1[a,c]
Iron + MPTP + DFO	5	677.2 ± 63.8[b,c,d]	31.8 ± 8.7[a,b,d]	8.6 ± 1.0[a,b,d]	ND[a,c,d]
Iron + MPTP + vitamin E	5	616.2 ± 82.8[b,c,d]	61.6 ± 6.0[a,d]	ND[a,c]	ND[a,c,d]

Data are expressed as mean ± SD, in pmol/mg protein. [a,b,c,d] $p < 0.05$ compared with control, iron-loaded, MPTP-treated and iron-loaded/MPTP-treated groups, respectively. *ND* not detectable

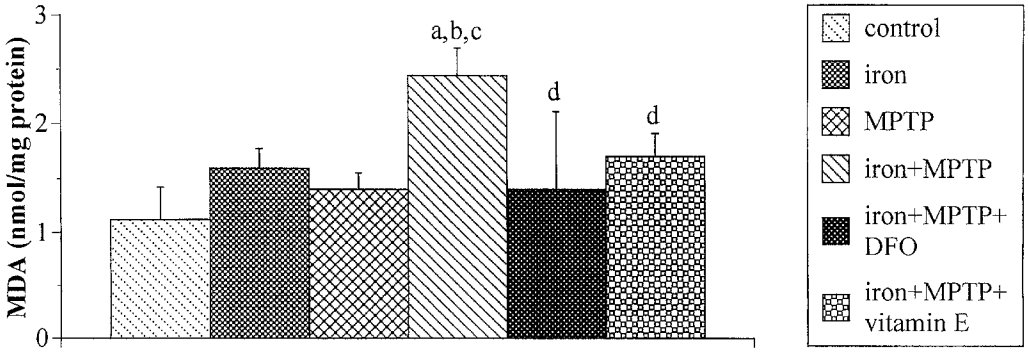

Fig. 3. Effect of DFO and vitamin E on MDA levels in C57 BL/6 mice brain. Data are presented as averages of six to eight mice (mean ± SD, nmol MDA/mg protein). a, b, c and d indicate significant differences ($p < 0.05$) compared with control, iron-loaded, MPTP-treated and iron-loaded/MPTP-treated groups, respectively

loaded group. The DA concentration in the iron-loaded/MPTP-treated group was approximately 5% to 10% of control, iron-loaded and MPTP-treated groups, respectively. Nevertheless, administration of DFO or vitamin E to iron-loaded/MPTP-treated mice, resersed the decrease in striatal DA, while striatal DOPAC concentration was also increased, although this effect was not as marked as that seen in DA. In the brainstem, compared with iron-loaded/MPTP-treated group, DFO injection markedly enhanced striatal DA concentrations. Administration of vitamin E, however, did not cause DA or DOPAC concentrations to increase. The DOPAC concentration was even further decreased in both DFO and vitamin E pre-treated groups (Table 2).

Lipid peroxidation

MDA formation was unchanged in the iron-loaded group. However, an injection of MPTP to iron-loaded mice increased the generation of MDA significantly ($p < 0.001$). Nevertheless, administration of DFO and vitamin E to iron-loaded/MPTP-treated group also reduced the concentration of MDA to normal levels (Fig. 3).

Discussion

Free radical formation and oxidative stress have been suggested to be involved in the neurodegeneration of the substantia nigra in PD. Strategies designed to interfere with reaction aimed at slowing down, or stopping, the progressive neurodegenerative course of this disease may have therapeutic value. A number of neuroprotective strategies have been considered; these include free radical scavengers, monoamine oxidase-B inhibitors, iron chelators and glutamatic antagonists. As iron chelators can retard neurodegeneration (Ben-Shachar et al., 1992) and free radical scavengers are capable of breaking the chain reaction of free radical formation directly, they

are thought to be promising approaches for retarding dopamine neuron degeneration in PD.

Desferrioxamine, as a powerful iron chelator, is widely and effectively used in the preventation and treatment of oxidative damage caused by iron overload in systemic organs and a number of animal models (Wolfe et al., 1985; Krause et al., 1986; Halliwell and Gutteridge, 1986; Cohen, 1990; Andreoli and Cohen, 1989; Sharma et al., 1990). However, DFO was thought to have an extremely low access across the blood-brain barrier (BBB). Therefore, in most of the studies on the diseases of central nervous system DFO was administrated by intracerebroventricular injection, which is clinically impractical. However, several investigators have demonstrated that DFO can enter the brain (Ikeda et al., 1989; Keberle, 1964). Intraperitoneally injection of DFO to ferrocene-loaded rats significantly reduced the brain iron content after only 2 weeks of administration, it has clearly shown that DFO are able to cross the BBB (Ward et al., 1995). DFO produced a significant reduction in iron uptake by the brain in the 15-day old rats (Crowe and Morgan, 1994). Even without the data of brain iron content from 21-day iron-supplemented mice, however, from the result of an increase in iron concentration after one month's iron-administration, we do believe that the iron content in 21-day iron-fed mice must be elevated, only to the extent different from mice fed iron-loaded diet for 30 days. This was because most of the iron transport into the brain occurred during prenatal and the first 3 weeks of postnatal life (Taylor and Morgan, 1990). In the present study, the results that intramuscular injection of DFO significantly lower iron concentration in brain suggests that DFO does retard brain iron accumulation. DFO can cross the BBB to reach the regions of iron deposition. This penetration of DFO into brain in our study maybe due either to the ability of DFO to cross BBB itself, or to the immatural BBB of weanling mice. Nevertheless, the resulting decrease of cerebral iron concentrations appeared to be favorable for the neurons.

Vitamin E is an effective chain-breaking agent and antioxidant, it acts to inhibit lipid peroxidation by binding with peroxyl radicals directly, and thus forming stable tocopheroxyl (Packer and Landvik, 1990). It intercalates into, and stabilizes the cell membrane (Erin et al., 1984). Dietary supplementation of vitamin E can increase its concentration in tissues and brain (Vatassery et al., 1988). Previous studies have demonstrated that vitamin E can prevent, or attenuate, free radical-induced neuropathological changes in models of spinal trauma (Saunders et al., 1987) and stroke (Mizoi et al., 1986). A diet deficient in vitamin E result in a more abnormal in electroencephalography of rats during ischemia and reperfusion (Lin et al., 1995). Brain iron concentration of mice in simultanously fed with high dose of vitamin E and iron, in our study, was markedly diminished compared with that of iron loading alone. This demonstrates that the cell membrane stability effect of vitamin E may achieve an inhibition to iron crossing cerebral membrane system.

According to our results, pre-injection of DFO, or supplementation of a high dosage of vitamin E, inhibited iron accumulation in brain entirely, thus resulting in a significant protection against the synergistic neurotoxic effects

of excessive iron and MPTP. This was manifested by the complete reversion of the reduction of striatal DA concentration. The concentration of GSSG and ratio of GSSG/(GSSG + GSH), generally accepted as indices of oxidative stress, were markedly elevated in iron-loaded/MPTP-treated mice. In addition, the decrease of GSH, an early event of oxidative stress (Jenner, 1993), was also observed in this group of mice. When the mice were administrated DFO or vitamin E, the increased GSSG concentration fell to normal level. Although GSH concentrations were unchanged, the contents of GSSG were decreased. This indicated a decrease of hydrogen peroxide (H_2O_2) formation (Spina and Cohen, 1989). Thus, the generation of reactive hydroxyl radicals and lipid peroxidation induced by excessive iron and MPTP were inhibited. Moreover, the significant decrease of ·OH level by DFO in this study confirms that iron is the basis of this free radical damage. The same effect achieved by vitamin E implies that the free radical scavengers play a key neuroprotective role. As a marker of dopaminergic neuron injury, the loss of the striatal dopamine was inhibited when mice were administrated DFO and vitamin E. The more significant increase of DA than DOPAC concentration suggests a reduction of dopamine turnover. A similar result has also been reported by Perry et al. (1985). It is well known that both dopamine autoxidation and its oxidative deamination by monoamine oxidase (MAO) result in the formation of hydrogen peroxide. Excessive hydrogen peroxide in the presence of ferrous iron (Fe^{2+}) can proceed through the Fenton reaction and to be reduced to form ·OH, which appears to be the primary mediator of oxidative damage. In PD brain, the surviving neurons increase DA metabolism (Zigmond et al., 1990). A reduction of DA turnover by DFO and vitamin E may reduce the oxidative stress. Our study also showed that pretreatment with DFO or vitamin E caused a great reduction of lipid peroxidation levels. These experimental results provide evidence for a strong association between brain iron content, free radical formation and neuronal damage. Mechanisms that iron contribution to neurodegeneration are hypothesized to involve damage to membrane lipids and/or DNA incurred as a result of iron-catalyzed oxygen free radical formation and decomposite lipid hydroperoxides (Halliwell, 1989). Intracellular concentration of free calcium (Ca^{2+}) may than increase, resulting in activation of Ca^{2+}-stimulated proteases, lipases, and endonucleases with consequent neurodegeneration. Inhibition of excessive iron deposition by DFO and vitamin E, thus, retarded hydroxyl radical initiated these series of biochemical events leading to call death. The alternation of ·OH levels and DA contents, as presented in this paper, provides direct evidence to support the hypothesis that iron may initiate the neurodegeneration process. To the best of our knowledge, this is the first report concerning the relationship between cerebral iron level and hydroxyl radical formation following excessive iron and/or MPTP administration, and systemic treatment with a iron chelator as well as antioxidant.

As a powerful iron chelating agent, DFO has been extensively studied. Its possible antioxidative properties were also suggested in recent years. Soriani et al. (1993) suggests the possible antioxidative role may be independent of its iron chelation; previous studies have shown the antioxidative chain-breaking

ability of DFO (Hartley et al., 1990) and its possible ability to scavenge peroxyl radicals (Darley-Usmar et al., 1989) and superoxide radicals (Sinaceur et al., 1984). A recent study also suggested that DFO can inhibit the oxidative chemistry of peroxynitrite. Peroxynitrite, a strong oxidizing species formed by reacting nitric oxide (NO) combining with superoxide, can directly oxidize molecules or protonate to peroxynitrous acid, yielding an oxidant which is reactively similar to the hydroxyl radical in a transition metal-independent mechanism (Denicola et al., 1995). The notable protective effect of DFO presented in this experiment maybe a combined effect of these mechanisms. However, the chelation of iron must be the primary neuroprotective mechanism because of its critical role in inhibiting excessive iron-initiated free radical reaction.

The DATATOP study (Deprenyl and Tocopherol Antioxidative Therapy Of Parkinsonism) demonstrated that there was no therapeutic benefit of α-tocopherol alone, or any synergistic interaction between α-tocopherol and L-deprenyl in PD (Parkinson Study Group, 1989a,b, 1993). The discrepancy between these data and those of the present study may result from a difference in the ability of vitamin E to penetrate the BBB of weanling mice and that of adult PD patients, or, alternatively, from an inadequate amount of α-tocopherol accumulated in the central nervous system of the patients (Parkinson Study Group, 1993). Despite this difference, the neuroprotective role of vitamin E against oxidative stress-induced neuron damage should not be ignored.

In conclusion, the ability of an iron chelator to retard the degeneration of dopaminergic neurons implies that iron maybe a factor underlying neuronal damage and that the pathogenesis of PD maybe attributed to excessive iron accumulation in brain. Supplementation of vitamin E also inhibited iron accumulation in brain, and hindered iron and MPTP-mediated free radical damage. This strongly suggests that free radicals indeed contributed to the neurotoxicity of excessive iron and MPTP. Iron chelators and antioxidants play pivotal role in protecting neurons from this degeneration, and therefore, may offer promising neuroprotective strategies in PD therapy.

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