Chronic L-DOPA Administration is not Toxic to the Remaining Dopaminergic Nigrostriatal Neurons, but Instead May Promote Their Functional Recovery, in Rats With Partial 6-OHDA or FeCl₃ Nigrostriatal Lesions

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Abstract: In this study, we have examined the effects of chronic L-3,4-dihydroxyphenylalanine (L-DOPA) administration on the remaining dopaminergic neurons in rats with 6-hydroxydopamine (6-OHDA) or buffered FeCl₃ partial lesions to the nigrostriatal tract. L-DOPA administration increased the turnover of dopamine in the striatum. L-DOPA administration for 1 week produced an increase in the level of striatal RTI-121 binding, a specific marker for dopamine uptake sites on the dopaminergic nerve terminals in the striatum. However, longer periods of L-DOPA treatment decreased the level of RTI-121 binding in the striatum. In the partial 6-OHDA lesion model, L-DOPA treatment had a time-dependent effect on the number of neurons demonstrating a dopaminergic phenotype i.e., neurons that are tyrosine hydroyxylase (TH)-immunopositive, on the lesioned side of the brain. In the first few weeks of treatment, L-DOPA decreased the number of TH-positive neurons but with long-term treatment, i.e., 24 weeks, L-DOPA increased the number of neurons demonstrating a dopaminergic phenotype. Even in the buffered FeCl₃ infusion model, where the levels of iron were increased, L-DOPA treatment did not have any detrimental effects on the number of TH-positive neurons on the lesioned side of the brain. Consequently, chronic L-DOPA treatment does not have any detrimental effects to the remaining dopaminergic neurons in rats with partial lesions to the nigrostriatal tract; indeed in the 6-OHDA lesion model, long-term L-DOPA may increase the number of neurons, demonstrating a dopaminergic phenotype.

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Key words: Parkinson’s disease; L-DOPA; neuroprotection; oxidative stress; neurodegeneration

Parkinson’s disease (PD) is a progressive neurodegenerative disorder, characterised by a primary loss of dopaminergic neurons in the substantia nigra (SN) zona compacta, resulting in a reduction in striatal dopamine content. For over 30 years the symptomatic relief of PD has come from L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine. At first L-DOPA is effective in most PD patients but with long-term administration, the therapeutic efficacy of L-DOPA diminishes and the patients experience disturbing side effects, for example “on-off” effects and dyskinesias.¹² In studies using in vitro conditions, L-DOPA has been demonstrated to have pro-oxidant properties, stimulating the formation of free radicals³⁴, and has been shown to be toxic to neuronal cells in culture.⁵⁶ From our own in vitro studies using physiologically relevant concentrations, L-DOPA showed some antioxidant properties combined with potentially cytotoxic pro-oxidant characteristics. For example, L-DOPA inhibited iron-dependent lipid peroxidation and was able to scavenge peroxyl or hydroxyl radicals leading to the formation of toxic semi-

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; 6-OHDA, 6-hydroxydopamine; DAT, dopamine transporter; DOPAC, dihydroxyphenylacetic acid; GBR-12935, 1-[2-(diphenyl-methoxy) ethyl]-4-(3-phenylpropyl) piperazine dihydrochloride; HPLC, high pressure liquid chromatography; HVA, homovanillic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; PBS, phosphate buffer; PD, Parkinson’s disease; RTI-121, [¹²⁵I]-3β-[4(trimethylstannyl) phenyl]-tropane-2β-carboxylic acid isopropyl ester; SN, substantia nigra; TH, tyrosine hydroyxylase.

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of reduced glutathione and/or inactivation of glutathione dependent enzymes. More importantly, we observed that L-DOPA stimulates extensive oxidative DNA damage, the actions of which were greatly magnified by the presence of trace metals such as iron. L-DOPA can also undergo auto-oxidation, producing not only the neurotoxin, 6-hydroxydopamine (6-OHDA), but also toxic quinones and several reactive oxygen species, for example hydroxyl radicals.

However, L-DOPA has not been shown to be toxic in all in vitro conditions. In foetal rat midbrain neuronal cultures Mena et al. demonstrated that the toxic effects of L-DOPA were significantly reduced if the cultures were pre-treated with glia conditioned media. Mena et al. suggested that glia secrete soluble neurotrophic factors that protect them against the toxic action of L-DOPA. Hence, in vivo the presence of glia may modify the toxic actions of L-DOPA.

There is controversy over whether L-DOPA administration in vivo leads to the production of free radicals. In normal rats, Spencer-Smith et al. and Obata et al. demonstrated that administration of high doses of L-DOPA, either systemically or as direct infusion into the striatum, increased the production of hydroxyl radicals in the SN. Whereas, a recent study by Camp et al., using salicylate spin trapping combined with in vivo microdialysis, demonstrated that daily administration of L-DOPA for 16 days did not enhance hydroxyl radical in the nigrostriatal dopamine system of rats with a unilateral 6-OHDA lesion. However, in 6-OHDA lesioned rats, chronic administration of L-DOPA produced increased lipid peroxidation. Such studies have lead to speculation that L-DOPA may be toxic for the remaining dopaminergic neurones in PD, thus accelerating the progression of the disease and increasing the risk of side effects.

In vivo data supporting chronic L-DOPA toxicity is far from clear. In normal rats and mice, and in non-parkinsonian patients receiving L-DOPA therapy, L-DOPA appears to have no detrimental effects on the number of TH-positive dopaminergic neurones in the SN. However, L-DOPA may react differently in the disease state where the surviving dopaminergic neurones are exposed to the pathological and biochemical factors associated with PD, for example, the accumulation of iron in the SN, or increased dopamine turnover in surviving neurones.

Since L-DOPA is widely used in PD, it is important to ascertain whether L-DOPA treatment is toxic to the remaining dopaminergic neurones in vivo. Hence, in this study we have examined the effect of chronic L-DOPA administration (periods up to 6 months) on the remaining dopaminergic neurones in rats with either 6-OHDA or buffered FeCl₃ partial lesions to the nigrostriatal tract. Assessment of the effect of drug treatment on the integrity of the nigrostriatal tract was carried out by quantifying the number of TH-positive cells in the SN. The effect of L-DOPA therapy on the concentrations and turnover of the striatal monoamines, dopamine and 5-hydroxytryptamine (5-HT) were also assessed by high pressure liquid chromatography (HPLC) analysis along with the autoradiographic assessment of dopamine uptake sites ([¹²⁵I] RTI-121 binding) in the striatum.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats with starting weights of 225 ± 25 g were housed in groups of 3, with free access to food and water, under controlled temperature (21°C ± 1°C) and a 12-hour light/dark cycle (light on 08.00 a.m.). All scientific procedures were carried out with approval of the Home Office, U.K. Two separate groups of animals were used, one for the study on TH immunohistochemistry plus [¹²⁵I] RTI-121 binding, and the second for the analysis of dopamine, 5-HT, and their metabolites in the striatum.

**Stereotactic Surgery**

**6-OHDA lesions**

Unilateral lesions of the left nigrostriatal pathway were produced by stereotaxic injection of 5 μg or 8 μg of 6-OHDA hydrobromide (dissolved in 4 μl 0.9% saline with 0.1% w/v ascorbic acid; Sigma, U.K.) under anaesthesia (small animal Immobilon®, Etorphine/Methotrimeprazine, C-Vet, U.K.) into the medial forebrain bundle (A −2.2, L +1.5 with reference to bregma, V −8.0 with reference to dura and with the tooth bar 5.0 mm above the interaural line). Postoperative care included individual caging and mashed diet.

**Buffered FeCl₃ infusions**

Unilateral iron infusions into the left SN were produced by stereotaxic injection of 4 μl of a buffered 5 mM ferric chloride (pH 7.4; prepared according to Sengstock et al.) under anaesthesia as above (A −3.0, L +2.5 with reference to bregma, V −8.5 with reference to dura and with the tooth bar 5.0 mm above the interaural line).

**L-DOPA and Benserazide Treatment**

Drug treatments were commenced 7 days after surgery. L-DOPA plus benserazide (F. Hoffmann-La Roche Ltd., Switzerland) were administered in the drinking wa-
ter at target doses of 200 mg/kg per 24 hours and 50 mg/kg per 24 hours, respectively. Groups of animals (n = 6 per group) were treated with L-DOPA plus benserazide or vehicle for 1, 2, 6, 12, or 24 weeks. For animals receiving L-DOPA plus benserazide, water containing these drugs was their only source of fluid. Drug intake was assessed by monitoring the volume of fluid consumed and the body weight of the animals three times per week. Drug concentrations in the drinking water were altered accordingly, and fresh drug solutions were prepared three times per week. L-DOPA and benserazide were dissolved in a minimum volume of 2 M HCl and made up to the desired volume with sterile distilled water, final pH of the solution was 5.00–5.25. To reduce the oxidation of the L-DOPA, a small amount of ascorbic acid (0.05% w/v) was also added and the bottles were protected from the light. Rats drink in a cyclical nature; hence, administering the drug in the drinking water mimics closely the multiple doses of L-DOPA taken daily by PD patients, and also avoids the undue stress of multiple daily injections to the animals. This regime has been previously found to produce plasma levels of L-DOPA of 0.895 ± 0.3 μg/ml (mean ± S.E.M) at the end of the dark cycle. Control animals received the drug vehicle only.

Tissue Preparation

At each time point in the study, animals were killed by cervical dislocation. The brains removed and cut coronally at the level of the infundibular stem (bregma −4.16 mm) forming a hind brain block and a forebrain block. In the first group of animals, the hindbrain blocks containing the SN were placed in 4% paraformaldehyde in 0.1 M phosphate buffer (PBS), pH 7.4 for 7 days and subsequently cryoprotected in a 30% sucrose solution. The fixed tissue was then frozen in isopentane at −70°C and consequently cryoprotected in a 30% sucrose solution. The forebrain blocks containing the SN were placed in 4% paraformaldehyde in 0.1 M phosphate buffer (PBS), pH 7.4 containing 0.05% sodium azide. The forebrain blocks were then apposed to FUJI BAS-MP 2040S imaging plates along with calibration standards ([125I]-autoradiographic microscales 1.25–640 nCi/mg, Amersham, U.K.) for 90 minutes in a lead encased chamber. Imaging plates were then read on a FUJI BAS-1500 phosphorimager (Fuji, Japan). [125I] RTI-121 binding was quantified using AIS software (Imaging Research Inc., Canada) at four rostral to caudal levels through the striatum. Levels in the striatum were defined according to the Paxinos and Watson22 rat brain stereotaxic atlas, using the following interaural and bregma coordinates for each level: level 1, 10.7, 0.7; level 2, 9.7, 0.7; level 3, 8.7, −0.3; and level 4, 8.2, −0.8. Binding studies were only carried out on the 5 μg 6-OHDA and buffered FeCl₃ infusion lesioned animals.

[125I] RTI-121 Radioligand Binding

Binding assays were performed according to the protocol of Hirata et al.21 but with minor modifications. Tissue sections were incubated for 60 minutes at room temperature with 15 pM [125I]-3-[4(trimethylstannyl) phenyl]-tropene-2-carboxylic acid isopropyl ester, RTI-121 (Dupont NEN, U.K.) using a binding buffer consisting of 137.0 mM NaCl, 2.7 mM KCl, 10.14 mM Na₂HPO₄, 1.76 mM KH₂PO₄ and 10 mM NaI. Specific binding was determined in the presence of 10 μM L-[2-(diphenylmethoxy) ethyl]-4-(3-phenylpropyl) piperazine dihydrochloride (GBR-12935, Sigma) and represented >94% of total binding. After 60 minutes, tissue sections were then washed twice with fresh buffer for 20 minutes each at room temperature and then rinsed in ice-cold distilled water and then allowed to dry. Sections were then apposed to FUJI BAS-MP 2040S imaging plates along with calibration standards ([125I]-autoradiographic microscales 1.25–640 nCi/mg, Amersham, U.K.) for 90 minutes in a lead encased chamber. Imaging plates were then read on a FUJI BAS-1500 phosphorimager (Fuji, Japan). [125I] RTI-121 binding was quantified using AIS software (Imaging Research Inc., Canada) at four rostral to caudal levels through the striatum. Levels in the striatum were defined according to the Paxinos and Watson22 rat brain stereotaxic atlas, using the following interaural and bregma coordinates for each level: level 1, 10.7, 1.7; level 2, 9.7, 0.7; level 3, 8.7, −0.3; and level 4, 8.2, −0.8. Binding studies were only carried out on the 5 μg 6-OHDA and buffered FeCl₃ infusion lesioned animals.

HPLC Analysis

Monoamines were extracted essentially as described by Sullivan et al.23 and in brief, striata were individually weighed and homogenized in 0.5 ml of ice-cold buffer (50 mM trichloroacetic acid, 0.5 mM, disodium ethylenediaminetetraacetic acid [EDTA], 0.5 pmol/μL 3,4-dihydroxybenzylamine hydrobromide as an internal standard) for 30 seconds using ultrasonicator tissue disrupter (Soniprep, Sanyo, U.K.). After keeping on ice for 10

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minutes for extraction, the samples were centrifuged (Heraeus, U.K.) at 12,000 g for 10 minutes, at 4°C. Supernatants were filtered (0.45 μm, Whatman, U.K.) and loaded onto an autosampler (Gina 50, Gynkotek, U.K.) kept at 5°C on-line with HPLC system. Samples were analyzed for dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) using phosphate buffer (0.1 mM KH₂PO₄, 0.1 mM EDTA, 1 mM octyl sodium sulfonate, 10% methanol V/V, pH 2.75 adjusted with orthophosphoric acid; flow rate 0.9 ml/minute), Altex 3 μm ODS column (4.6 mm × 7.5 cm, Beckman, U.K.), analytical cell (model 5011, ESA Analytical, U.K.) and Coulouchem-II detector with electrode one set at −0.20 V and electrode two at +0.34 V with respect to palladium reference electrode (ESA Analytical) as described by Datla and Curzon. Chromatographic data were collected and analyzed by computer-based software (Chromeloen, Gynkotek, UK). Both control and L-DOPA-treated groups from lesioned and unlesioned sides were analyzed on the same day.

Statistical Analysis

Over all effects of lesioning, region, L-DOPA treatment and time were analysed using MANOVA. Individual mean differences were compared by unpaired Student’s t-test between subjects and paired t-test within subjects.

RESULTS

6-OHDA Lesions

Substantia nigra TH-positive cells

5 μg 6-OHDA. The total number of TH-positive cells in the substantia nigra on the unlesioned side of the brain in vehicle-treated group were comparable over the duration of the study but there were regional differences within the substantia nigra (Fig. 1A–E). 6-OHDA lesioning significantly reduced the number of TH-positive cells, and the reduction in the number was progressive with time. Importantly, within the substantia nigra, there were regional differences in the sensitivity to 6-OHDA. The maximum cell loss was observed in level A, which is closest to the injection site (Fig. 1A) when compared to the distal regions (Fig. 1E; effect of region P < 0.005). Similarly, the progression of the lesion with time was observed to a greater degree in the SN levels closest to the site of injection (Fig 1A–E).

On the lesioned side of the brain, L-DOPA treatment had a significant time-dependent effect on the number of neurons expressing TH. Initially, L-DOPA treatment caused a reduction in the number of neurons demonstrating TH immunoreactivity; this reached significance after 6 and 12 weeks of L-DOPA treatment compared to vehicle-treated animals. However, prolonged (24 weeks) L-DOPA treatment caused a marked increase in the number of TH +ve neurons in the lesioned side of the brain compared to lesioned animals that had received vehicle treatment (Fig. 1A–F). This functional recovery after 24 weeks of L-DOPA treatment was more pronounced in the SN levels closest to the site of lesioning (Fig. 1A).

8 μg 6-OHDA. The higher dose of 6-OHDA caused a more pronounced loss of TH +ve cells in the SN (~69%) and similarly the lesion was progressive with time (Fig 2, which represents the mean cell counts in the SN). These animals were studied for 6 weeks only, since after 6 weeks the lesion was almost complete. Similarly, there was also a time-dependent response to the L-DOPA treatment. After 2 weeks of L-DOPA therapy, there was a significantly higher number of TH +ve neurons in the lesioned SN compared to lesioned animals that received drug vehicle. However, after 6 weeks of L-DOPA therapy, the number of TH +ve neurons was equal on the lesioned side of the brain in both the L-DOPA-treated and vehicle-treated animals.

Striatal RTI binding sites (5 μg dose only). On the unlesioned side of the brain, in the vehicle-treated animals, the striatal density of RTI-121 binding significantly changed with time, rising to a peak 2 weeks into the study but then gradually decreased with time (Fig. 3, open columns). Similarly, the RTI-121 binding density varied within the four striatal regions tested with time (data not shown). After 1 week of L-DOPA treatment, there was a small but significant increase in RTI-121 binding density on the unlesioned side of the brain, but the density tended to decrease with time to a level below that seen in the vehicle-treated group (Fig. 3, hatched columns). Lesioning with 6-OHDA produced a small (13%) but significant reduction in the RTI-121 binding density, but this did not change with time or L-DOPA treatment (data not shown).

Striatal monoamine and their metabolite levels

5 μg 6-OHDA. In the unlesioned striatum, basal dopamine, DOPAC, HVA levels, and dopamine turnover in the vehicle-treated rats remained constant over the study period, whereas 5-HT and 5-HIAA levels decreased with time (data not shown). L-DOPA treatment increased DOPAC, HVA levels, and dopamine turnover significantly within first week, and remained increased throughout the 24-week treatment period (see Fig. 4). The effect of L-DOPA treatment on striatal dopamine levels was variable, with only significant increases in dopamine levels.
FeCl₃ was unaffected by L-DOPA treatment despite the extent of the lesion being not progressive with time. TH-positive neurons, Substantia nigra TH-positive cells, showed a significant reduction in the number of neurons, with increased iron levels (see Fig. 5). Movement Disorders, Vol. 16, No. 3, 2001

8 µg 6-OHDA. Lesioning with the 8 µg dose of 6-OHDA produced a more pronounced reduction in striatal dopamine levels when examined at weeks 1 and 2; however, levels of dopamine had returned to almost control levels by the end of 6 weeks (data not shown). This was despite a marked reduction in the number of TH-positive neurons in the SN. Likewise, striatal levels of HVA and DOPAC on the lesioned side of the brain were markedly reduced or undetectable at weeks 1 and 2 of the study, but tended to return to control levels after week 6 of the study (data not shown). In contrast, striatal dopamine levels on the lesioned side of the brain in the rats subsequently treated with L-DOPA were significantly decreased throughout the 6-week study period. Similarly, on the lesioned side of the brain in the L-DOPA-treated animals, the striatal levels of HVA and DOPAC were either undetectable or significantly reduced during the study period. Due to the undetectable striatal levels of either HVA or DOPAC in many of the lesioned animals, it was not possible to calculate dopamine turnover in the lesioned striata; however, dopamine turnover in the unlesioned striatum of rats treated with L-DOPA was increased (data not shown). Lesioning with higher dose of 6-OHDA only produced transient changes in striatal 5-HT and 5-HIAA levels during the first 2 weeks of the study (data not shown).

Buffered FeCl₃ Infusion

Substantia nigra TH-positive cells.

Infusion of buffered FeCl₃ produced a small but significant reduction in the number of TH-positive neurons, but the extent of the lesion was not progressive with time after lesioning (see Fig. 5). This small lesion with FeCl₃ was unaffected by L-DOPA treatment despite the presence of increased iron levels (see Fig. 5).

Striatal RTI-121 binding sites.

Despite the small loss of TH-positive cells in the SN after buffered FeCl₃ infusion, no changes in the striatal RTI-121 binding sites were observed. Also, over the 24-week treatment period, L-DOPA treatment had no effect on RTI-121 binding on the infused side compared to vehicle-treated animals (data not shown).

Striatal monoamine and their metabolite levels.

Infusion of buffered FeCl₃ in the SN did not alter striatal dopamine or 5-HT levels, nor levels of their metabolites or turnover (data not shown). L-DOPA treatment, however, increased DOPAC, HVA, and dopamine turnover in the control side, similar to 6-OHDA study, but remained unchanged on the infusion side. Similarly, 5-HT levels decreased and 5-HT turnover increased with L-DOPA as in 6-OHDA study, but infusion of the buffered FeCl₃ did not change 5-HT or its metabolism (data not shown).

DISCUSSION

The results from this study correlate with a recent study by Murer et al.²⁵ where they demonstrated that after a single administration period of 6 months, L-DOPA produced differential effects that were dependent on the size of the lesion created by 6-OHDA in rats. The authors utilised immunolabelling for TH, dopamine transporter (DAT), and the vesicular monoamine transporter (VMAT2) as markers of the integrity of dopaminergic nerve terminals in the striatum, and cell counts and TH density as a marker in the SN. In sham-lesioned animals or animals with a very severe 6-OHDA lesion, L-DOPA had no effect when compared to vehicle-treated animals. Whereas, in animals with only a partial 6-OHDA lesion to the SN, all three markers of dopaminergic terminal integrity (TH, DAT, and VMAT2) in the striatum and TH density in the SN were increased in lesioned animals subsequently treated with L-DOPA for 6 months, compared to vehicle-treated animals. No shorter periods of L-DOPA administration were tested.

In addition, we have demonstrated that the effects of L-DOPA in the 6-OHDA partial lesion model are time-dependent and that functional recovery was seen only after long-term L-DOPA. We also observed such effects in male rats, similar to the study of Murer et al.²⁵ who used female rats that are less susceptible to the toxic effects of 6-OHDA²⁶ and differ in their susceptibility during the estrous cycle (Datla et al., unpublished data). In contrast to the study by Murer et al.²⁵ who demonstrated an increase in dopamine transporter immunoactivity, we did not find any increase in the density of the dopamine transporter when assessed by RTI-121 binding.
FIG. 1. Effects of L-3,4-dihydroxyphenylalanine (L-DOPA) treatment on the number of tyrosine hydroxylase (TH)-positive cells in the substantia nigra of unilateral 5 μg 6-hydroxydopamine (6-OHDA) lesioned rats. Values represent mean ± S.E.M. of number of TH-positive cells. n = 6 per group. Vehicle treated groups are given as control side (white bars) and lesioned side (narrow cross-hatched bars), while L-DOPA-treated groups are given as control side (wide cross-hatched bars) and lesioned side (dotted bars). Substantia nigra was again subdivided into five regions A to E. A represents a region closer to injection site, while E was further away. TH cells in whole of substantia nigra are given in F. MANOVA analysis of data from A to E showed a progression of the lesion with time in the untreated animals ($F_{(4,24)} = 10.86, P < 0.001$); and a progression with time in the L-DOPA-treated animals ($F_{(4,24)} = 8.40, P < 0.001$) between subjects and within subjects effects of region ($F_{(4,96)} = 4.19, P < 0.005$); lesion ($F_{(1,24)} = 254.91, P < 0.001$); lesion by region ($F_{(4,96)} = 6.14, P < 0.005$), and L-DOPA by lesion by region by time ($F_{(16,96)} = 1.73, P = 0.054$). In the mean TH-positive cell counts for the whole SN (panel F), effect of lesion ($F_{(1,45)} = 288.1, P < 0.001$) within subjects and time ($F_{(4,45)} = 4.40, P < 0.005$), time by L-DOPA ($F_{(4,45)} = 3.43, P < 0.02$) between subjects were seen after MANOVA. *, **, ***$P < 0.05$, $P < 0.01$, and $P < 0.005$ vs. unlesioned after paired Student’s $t$-test, respectively. a, b, c$P < 0.05$, $P < 0.01$, and $P < 0.005$ vs. unlesioned vehicle group, d, e, f$P < 0.05$, $P < 0.01$, and $P < 0.005$ vs. lesioned vehicle group after unpaired Student’s $t$-test, respectively.
FIG. 1. Continued.
in the striatum after 6 months of L-DOPA treatment. This may be due to differences in the extent of the partial lesion in the two studies or to differences in the techniques used to detect levels of the dopamine transporter. In the study, we only observed a 15% reduction in RTI-121 binding despite a marked reduction in the number of TH-positive neurons in the substantia nigra, whereas, in the study by Murer et al., who utilised immunoradiolabeling, the partial lesion with 6-OHDA produced an approximate 60% reduction in the dopamine transporter.

In the past, we have demonstrated that in vitro L-DOPA can stimulate extensive oxidative DNA damage, an effect magnified by the presence of iron. However, in the buffered FeCl₃ lesion model, L-DOPA had
no detrimental effects. Likewise, in this model, L-DOPA failed to stimulate any functional recovery after 24 weeks of treatment, as seen in the partial 6-OHDA model. This may relate to the size of the lesion, as more cells were traumatised and killed in the 6-OHDA model, or it may relate to the effects of the toxins themselves. 6-OHDA may produce relatively specific lesions to dopaminergic neurons, whilst iron may also affect other nondopaminergic neurons and glial cells. Also, FeCl$_3$ may produce acute local necrosis, whereas 6-OHDA may cause an ongoing neurodegenerative process in the dopaminergic nigrostriatal neurons. In the past, researchers have demonstrated that glia can protect against the toxic effects of L-DOPA in tissue culture. However, in this model where FeCl$_3$ may also be toxic to glia, L-DOPA treatment still failed to be toxic to the remaining dopaminergic neurons in the SN.

To formulate an explanation for the effects of L-DOPA in the 6-OHDA partial lesion model, we must examine more closely what may be happening in this model. Firstly, Bowenkamp, using the partial 6-OHDA lesion model, demonstrated a 85% reduction in the number of TH-immunoreactive cells on the lesion side, whilst there was only a 50% reduction in the number of neurons.
on the lesioned side as determined by double TH and fluorogold labelling. This suggests that some neurons lose their neurotransmitter phenotype, i.e., become TH-immunonegative when exposed to 6-OHDA. Bowers-kamp subsequently demonstrated that such neurons can be rescued with a single infusion of glial cell line-derived neurotrophic factor (GDNF) into the SN. \(^{27}\) Importantly, a similar phenomenon appears to occur in the SN of PD patients, where some SN neurons contain neuromelanin but do not express TH. \(^{28}\) These studies suggest that in the animal model and in PD, some neurons may be traumatised and are non-functional as dopaminergic neurons, but can be rescued.

Secondly, there are some reports demonstrating a process of delayed cell death after 6-OHDA injection into the SN. \(^{29}\) According to such studies, 6-OHDA induces death of a large proportion of dopaminergic cell bodies during the first few days after administration, and then a small proportion of the remaining neurons suffer a process of protracted cell death. \(^{29}\) In this study, there was a small but clear progressive nature to the 5 \(\mu\)g 6-OHDA lesion and a more pronounced progression in the 8 \(\mu\)g 6-OHDA lesion with time, thus supporting the process of protracted cell death in this animal model.

L-DOPA administration produced increased turnover of dopamine throughout the study period that may lead to the formation of reactive oxygen species and toxic metabolites from dopamine auto-oxidation. Indeed, recent studies have shown that intrastratal administration of a single high dose of dopamine can produce selective damage to TH neurons, a process accompanied by the formation of reactive oxygen species and metabolites from dopamine oxidation. \(^{30}\) Consequently, short-term L-DOPA administration may lead to a moderate increase in oxidative stress in the nigrostriatal system. In dopaminergic neurons that have previously been exposed to the toxin 6-OHDA but survived, this additional oxidant stress may have adverse effects on the neurons themselves and on the levels of antioxidants. Such an additional stress may have caused the neurons to lose their dopaminergic phenotype. Further tests utilising dual labelling with specific markers for neurons (e.g., NeuN) and for dopaminergic neurons are required before this can be confirmed. Alternately, increased dopamine turnover after L-DOPA administration per se, may have had an adverse effect on TH expression.

Long-term L-DOPA administration in the 5 \(\mu\)g 6-OHDA partial lesion model caused an increase in the number of neurons expressing TH, suggesting long-term L-DOPA treatment stimulates functional recovery. This recovery could be attributable either to recovery of dopaminergic markers in injured but not dead neurons, or to delaying the process of protracted cell death. The mechanism by which L-DOPA would have this beneficial effect is not clear. One way L-DOPA could have this beneficial effect could be the production of neurotrophic factors by striatal neurons or remaining nigral dopaminergic cells. In support of this, Okazawa et al. \(^{31}\) have shown that L-DOPA promoted the expression of brain-derived neurotrophic factor mRNA in the mouse striatum. Also Carvey et al. \(^{32}\) reported that striatal extracts from PD patients who were under chronic L-DOPA therapy promoted dopaminergic neuron growth in culture. Neurotrophins have been clearly demonstrated to promote neuronal survival, neurite outgrowth, and synaptic organization in several neuronal systems including the dopaminergic system.

Alternately, the “oxidative stress” occurring as a consequence of L-DOPA therapy may not necessarily lead to neuronal damage. Indeed, Han et al. \(^{33}\) demonstrated that auto-oxidizable compounds such as L-DOPA induce a rise in the antioxidant reduced glutathione (GSH) levels in fetal mesencephalic cultures, which protected them against further toxic insult. This suggests that low level “oxidative stress” produced by L-DOPA may stimulate antioxidant production, thus protecting cells against further damage. Whether this stimulation of GSH production by L-DOPA occurs in vivo or with long-term L-DOPA treatment is not known. Finally, L-DOPA could influence the infiltration and/or reactivity of other support cells, e.g., glia, astrocytes, and oligodendrocytes, in the lesioned area. Such cells are not only important sources of neurotrophic factors but also antioxidants.

Clearly this work has important clinical implications. This study demonstrates that long-term L-DOPA administration has no detrimental effects on the remaining dopaminergic neurons in both animal models, but instead may promote some functional recovery of damaged neurons in the partial 6-OHDA lesion model.

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