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Neuroprotective and neurotoxic roles of levodopa (L-DOPA) in neurodegenerative disorders relating to Parkinson's disease

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Summary. Despite its being the most efficacious drug for symptom reversal in Parkinson's disease (PD), there is concern that chronic levodopa (L-DOPA) treatment may be detrimental. In this paper we review the potential for L-DOPA to 1) autoxidize from a catechol to a quinone, and 2) generate other reactive oxygen species (ROS). Overt toxicity and neuroprotective effects of L-DOPA, both in vivo and in vitro, are described in the context of whether L-DOPA may accelerate or delay progression of human Parkinson's disease.

Keywords: L-DOPA–Parkinson's disease – Reactive oxygen species

Reactive oxygen species formed by L-DOPA and DA

Generation of H_2O_2, $O_2^{-\bullet}$, $HO^\bullet$, and $^\bullet NO_2$ by L-DOPA and DA

In the normal course of metabolism of dopamine (DA) by monoamine oxidase (MAO), one molecule of peroxide (H_2O_2,) is generated for each molecule of DA.

$$DA + H_2O + O_2 \rightarrow DOPAL + NH_3 + H_2O_2$$

[DOPAL = 3,4-dihydroxyphenylacetylaldehyde]

Although potently toxic only in high concentrations, H_2O_2 readily crosses cell membranes (Mischel et al., 1997). In the presence of ferrous iron (Fe^{2+}), the more-reactive hydroxyl radical ($HO^\bullet$) is formed by the autoxidation of H_2O_2, in what is known as the Fenton reaction (Fenton, 1894). $HO^\bullet$ reacts with all biological substrates and is most likely to react with sulfhydryl groups (-SH) (Chen et al., 1997; Liu et al., 1997) and unsaturated lipids, but also with DNA, RNA, carbohydrates, and amino acids or proteins (Chen et al., 1992; Miura et al., 1992).

$$H_2O_2 + Fe^{2+} \rightarrow HO^\bullet + HO^- + Fe^{3+}$$

The Haber-Weiss reaction, in which $O_2^{-\bullet}$ combines with H_2O_2, also generates $HO^\bullet$ (Haber and Weiss, 1934). The series of different reactions tend to be self-propagating, as one ROS tends to generate another ROS.

$$2\ O_2^{-\bullet} + H_2O_2 \rightarrow HO^\bullet + HO^- + O_2$$

Non-heme iron is found in high concentration in the substantia nigra pars compacta (SNpc) and globus pallidus (see Koeppen, 1995). Dopaminergic nerves, known to degenerate in PD, are largely nigrostriatal, meaning that the nucleus of origin is in the SNpc and the target for innervation includes basal ganglia and globus pallidus (i.e., striatum in rodents) (Morris and Edwardson, 1994).

$$HO^\bullet + O_2^{-\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+} + HO^-$$

An additional reaction generates products of nitric oxide.

$$O_2^{-\bullet} + {}^\bullet NO + H^+ \rightarrow HONOO \rightarrow HO^\bullet + {}^\bullet NO_2$$

All of the many ROS formed from L-DOPA (and DA) are potentially neurotoxic.

L-DOPA oxidation and DA oxidation to quinones and semiquinones

L-DOPA and DA, as well as DOPAC, autoxidize to orthoquinones (o-quinones, o-Q):

L-DOPA + O_2 → DOPA-Q + $O_2^{-\bullet}$
DA + O_2 → DA-Q + $O_2^{-\bullet}$
DOPAC + O_2 → DOPAC-Q + $O_2^{-\bullet}$

Both DOPA-Q and DA-Q can further undergo internal rearrangement in which the aminoalkyl chain cyclizes, respectively forming dopachrome and aminochrome (Hawley et al., 1967; Heacock, 1959). This reaction can be catalyzed by O_2 (faster rate at higher pH) (Graham, 1978; Senoh et al., 1959), Fe^{2+}, cytochromes P450 (Segura-Aguilar, 1996; Segura-Aguilar et al., 1998), prostaglandin H synthase (COX-1 and -2) (Hastings et al., 1995), lactoperoxidase (Segura-Aguilar et al., 1998), lipoxygenase (Rosei et al., 1994), xanthine oxidase (Foppoli et al., 1997), tyrosinase (Korytowski et al., 1987), $ONOO^-$, NO_2• (LaVoie and Hastings, 1997, 1999) and perhaps even $O_2^{-\bullet}$ and HO• (Ito and Fujita, 1982; Nappi et al., 1995; Spenser et al., 1995). Catecholquinone formation is not merely hypothetical, as these products are precursors in the formation of neuromelanin in brain (e.g., in SNpc) and melanin in skin (Graham, 1978).

L-DOPA → Dopachrome
DA → Aminochrome

Catechol semiquinones are formed by several processes. Catechol o-quinone interaction with cytochrome P450 involves a one-electron reduction to form a semiquinone (SQ) radical (Baez et al., 1995; Segura-Aguilar et al., 1998), which by undergoing redox cycling (i.e., self-propagating reactions) are far more cytotoxic than quinones.

Dopachrome + NADPH → Dopachrome-o-SQ•
Aminochrome + NADPH → Aminochrome-o-SQ•

SQs formed in the reactions shown can interact with O_2, to reform their quinones along with $O_2^{-\bullet}$.

Dopachrome-o-SQ• + O_2 → Dopachrome + $O_2^{-\bullet}$
Aminochrome-o-SQ• + O_2 → Aminochrome + $O_2^{-\bullet}$

The preceding four reactions, by self-propagating and reforming "substrates", tend to continue until NADPH is fully consumed, or until O_2 is depleted (Segura-Aguilar et al., 1998).

Catechol oxidation of sulfhydryl moieties and crosslinking of proteins

The electronegative nature of ortho-oxygens on the catechol ring, and the electron-deficient and unstable aromatic ring confer a partial positive charge to positions 2 and 5 of the ring, facilitating nucleophilic attack by sulfhydryls (Kato et al., 1986; Monks et al., 1992), which are present largely in GSH, cysteine or in proteins. Sulfhydryls tend to covalently bond to the catechol ring at positions 2 and 5 (binding at position 5 : position 2 = 6 : 1 ratio) (Ito et al., 1988; Kato et al., 1986).

DA-Q + cysteine → 2-cysteinyl-DA [1 part]
+ 5-cysteinyl-DA [6 parts]
+ 2,5-dicysteinyl-DA[minor]

Catecholquinone binding to cysteinyl groups in proteins leads to loss of protein function (Hastings and Zigmond, 1994; Stadtman, 1992), while binding to GSH leads to cellular depletion of GSH, resulting in inadequate cytoplasmic antioxidant load. As mitochondrial proteins are rich in sulfhydryl moieties, it is not surprising that oxidized catechols inhibit mitochondrial Complex I (Ben-Shachar et al., 1995; Morikawa et al., 1996; Przedborski et al., 1993), promote opening of the mitochondrial permeability transition pore, uncouple oxidative phosphorylation (increased state 4 respiration), and lead to mitochondrial swelling (Berman and Hastings, 1999).

As cysteinyl-DA (or any other cysteinyl-catechol) is more-readily oxidized to the o-quinoidal form than the parent catechol (Monks et al., 1992; Shen et al., 1996), this process accounts for crosslinking in protein, with additional loss of function and eventual appearance of protein aggregates such as Lewy bodies in Parkinson's disease (Tran and Miller, 1999).

Catechol-induced hydroxylation of guanine bases of DNA

Catecholestrogens, through redox cycling between Q and SQ forms, generate HO•, thus promoting formation of $O_2^{-\bullet}$ and consequent self-perpetuation of HO• radicals (as discussed above), particularly in the presence of metal ions like Fe^{2+} (Liehr et al., 1986). Non-catechol estrogens do not generate HO•. When incubated with cells, catecholestrogens induce 8-hydroxylation of guanine bases of DNA (Han and Liehr, 1995). This effect is notable with 4-

hydroxyestradiol but not 2-hydroxyestradiol, both catechols (Han and Liehr, 1994a, 1994b).

In addition to these direct effects on DNA, DA and L-DOPA inhibit DNA polymerase, ribonucleotide reductase and thymidylate synthase – enzymes involved in DNA repair (Wick, 1989). Ultimately, DA and L-DOPA cause strand breaks and base modification of DNA (Graham, 1978).

Catechol-induced lipid peroxidation

In picomolar and low μM concentrations, catechol estrogens oxidize low density lipoproteins particularly in the presence of metal ions, forming lipid peroxides. In high μM concentrations, an antioxidant effect is produced (Markides and Liehr, 1998; Wang and Liehr, 1995). Thus, catechols have both toxic potential and neuroprotective potential.

Neurotoxic potential of L-DOPA and DA

Experimental evidence relating to ROS production and DA denervation

In assessing dihydroxybenzoic acid (DHBA) as the spin-trap product of salicylic acid and $^{\bullet}$OH, we recently found that the basal level of 2,3-DHBA was increased 4 to 5-fold while 2,5-DHBA was increased 2.5-fold in adult rat neostriatum which had been largely DA- denervated (99% reduction in endogenous DA content) following neonatal treatment with 6-hydroxydopamine (6-OHDA) (67 μg in each lateral ventricle at 3 d after birth; desipramine pretreatment, 20 mg/kg i.p., 1 hr) (Kostrzewa et al., 2000).

Experimental evidence relating to L-DOPA- (DA-) induced ROS prevention or production

In rats with a fully DA-innervated neostriatum, acute treatment with levodopa (L-DOPA) (60 mg/kg i.p.; carbidopa pretreatment, 12.5 mg/kg i.p., 30 min) significantly reduced neostriatal content of 2,3-DHBA by 60% and 2,5-DHBA by 95%. When given to those rats that had been largely DA-denervated, L-DOPA did not alter 2,3-DHBA but reduced 2,5-DHBA of neostriatum by 50%. Because 6-hydroxydopa (6-OHDOPA) – a known generator of reactive oxygen species – had a similar effect, this neuroprotective nature of L-DOPA appears to be attributable to the scavenging of $^{\bullet}$OH by L-DOPA as it bathed the neostriatal neuropil, (Kostrzewa et al., 2000). In support of these findings, others find that higher dose L-DOPA (100–500 mg/kg) does not increase 2,3-DHBA content of neostriatum (Camp et al., 2000; Ishida et al., 2000). One cautionary note, however, is that L-DOPA may generate $^{\bullet}$OH if carbidopa or another dopa decarboxylase inhibitor is not present (Obata and Yamanaka, 1996).

With repeated L-DOPA treatments (50 or 200 mg/kg/d i.p. $\times$16 d), the $^{\bullet}$OH content was found to be increased transiently in DA-denervated neostriatum, but not in intact neostriatum. There was no long-term effect of L-DOPA treatment on neostriatal $^{\bullet}$OH content (Camp et al., 2000; Ishida et al., 2000). And Colado et al. (1999) found that L-DOPA (25 mg/kg; benserazide pretreatment, 6.25 mg/kg) did not alter $^{\bullet}$OH content in a microdialysate of hippocampus.

In vitro studies relating to cytotoxicity of L-DOPA and DA

DA and L-DOPA were first found to be toxic to melanoma cells, which have a high level of tyrosinase and melanin (Wick, 1979; Wick, 1980). The toxicity of DA and L-DOPA have since been found in practically any other type of cell: dopaminergic neurons (Michel and Hefti, 1990), sympathetic neurons (Ziv et al., 1994), PC12 cells (Offen et al., 1997; Walkinshaw and Waters, 1995), melanoma (Wick et al., 1977), striatal neurons (Luo, 1998; McLaughlin et al., 1998), neuroblastoma (Graham, 1978), SN neuroblastoma hybrid cells (MES 23.5 or MES) (Zhang et al., 1998) and cortical cells (Hoyt et al., 1997), cerebellar granule cells (Shirvan et al., 1997), and thymocytes (Offen et al., 1995). Metabolic products of L-DOPA (e.g., DOPAL) may account for the cytotoxicity (Kristal et al., 2001).

The most prominent suspect mechanisms underlying L-DOPA- and DA-induced cytotoxity include in vitro autoxidation of catechols, leading to protein-protein crosslinking, analogous to products in Lewy bodies (Montine et al., 1995) in cells; and hydroxylation of guanine bases in DNA, damage to DNA reparative enzymes, and DNA damage (see above discussion).

DA D_2 receptor agonists (e.g., bromocriptine and 2-N-phenethyl-N-propyl-amino-5-hydroxytetralin) inhibited levodopa-induced toxicity to rat embryonic ventral mesencephalon; and the effect was attenuated in a D_2 receptor antisense oligonucleotide (Takashima et al., 1999).

In vivo evidence of L-DOPA- and DA-neurotoxicity

When L-DOPA was added to the microdialysate, •OH content of the effluent from rat SN increased in a concentration-dependent manner, and this was increased further by inhibition of mitochondrial complex I activity (Smith et al., 1994). The mechanism of L-DOPA-induced •OH formation appears to relate to L-DOPA metabolism to DA, since carbidopa prevented L-DOPA (0.1 mM; 1 μM min^{-1})-induced •OH formation in striatum (Obata and Yamanaka, 1996).

When DA is injected into the striatum, cysteinyl-DA complexes were found (Hastings et al., 1996) and cell death was prominent (Filloux and Townsend, 1993). DOPA/DA-induced apoptotic vs. necrotic cell death is discussed elsewhere (Kostrzewa, 2000).

Evidence of oxidative stress in Parkinsonians

Evidence of oxidative damage of SN in Parkinsonians is inferred by the following findings.

1. The SN of Parkinsonians has a higher content of malondialdehyde (Ilic et al., 1999), GSH (Perry et al., 1982), and an elevation in the GSSG/GSH ratio (Sian et al., 1994).
2. Mitochondria are damaged and oxidative phosphorylation is impaired (Ebadi et al., 2001; Shoffner et al., 1992).
3. Iron metabolism is impaired (Jellinger et al., 1990; Riederer et al., 1989; Youdim et al., 1993).
4. The level of cysteinyl-catechols is increased in the SN during aging (Fornstedt et al., 1989, 1990) and more-so in Parkinsonians (Fornstedt et al., 1989; Spenser et al., 1998). Protein crosslinking is also increased (Berlett and Stadman, 1997), signified by presence of Lewy bodies (Leroy et al., 1998).
5. An increased level of 8-hydroxy-2-hydroxyguanine deoxyguanosine is found in the SN of Parkinsonians (Sanchez-Ramos et al., 1994).
6. Antibodies for catechol-modified proteins were found in the serum of Parkinsonians (Rowe et al., 1998).
7. A reduction in CSF levels of superoxide dismutase and glutathione reductase was found in de novo Parkinsonians (Ilic et al., 1999).

In postmortem specimens of human brain, the ratio of 5-S-cysteinyl-DA/DA, 5-S-cysteinyl-DOPAC/DOPAC and 5-S-cysteinyl-DOPA/DOPA were higher in the SN when this region was depigmented and degenerated. Parallel elevations in these ratios were found in the caudate (Fornstedt et al., 1989). An accelerated rate of apoptosis in the SN of Parkinsonians has been reported (Anglande et al., 1997; de la Monte et al., 1998; Hunot et al., 1997; Mochizuki et al., 1996; Tatton et al., 1998).

Antioxidant effects of catechols

The ease at which catechols are oxidized, immediately brings to mind the potential for catechols to act as antioxidants. By becoming oxidized, in place of some other cellular element, catechols can be viewed as cell protectors (i.e., mimicking vitamin E, which acts identically). Catechols are excellent free radical scavengers. In "sequestering" a radical, the phenolic ring is converted to a phenyoxy radical, which is somewhat more stable if the catechol is alkyl substituted (Markides and Liehr, 1998), as by action of catechol-O-methyltransferase – thus forming 3-methoxydopamine. Both L-DOPA (IC50, 450 μM) and DA (IC50, 8.5 μM) inhibit peroxidation of ox brain phospholipids (Spencer et al., 1996). In rats with a unilateral 6-OHDA lesion, DOPA did not increase •OH content of the nigrostriatal dopaminergic system (Camp et al., 2000).

Epidemiological evidence for and against L-DOPA toxicity

Although the verdict is still out, the weight of evidence seems to indicate that chronic L-DOPA therapy is not toxic, but perhaps neuroprotective (Datla et al., 2001). There is no clear demonstration of L-DOPA producing apoptotic death of SNpc dopaminergic neurons of human Parkinsonians (Melamed et al., 1998). L-DOPA may even slow progression of familial PD (Gwinn-Hardy et al., 1999; Murer et al., 1998). Clinical trials are under way to determine long-term L-DOPA effects on survival of SNpc neurons in parkinsonians. (Jenner and Brin, 1998).

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