

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

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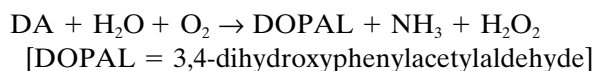
Summary. Despite its being the most efficacious drug for symptom reversal in Parkinson's disease (PD), there is concern that chronic levodopa (L-DOPA) treatment may be detrimental. In this paper we review the potential for L-DOPA to 1) autoxidize from a catechol to a quinone, and 2) generate other reactive 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 , $O_2^{\cdot -}$, HO^{\cdot} , and $\cdot NO$ 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.



Although potentially 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^{\cdot}) is formed by the autoxidation of H_2O_2 , in what is known as the Fenton reaction (Fenton, 1894). HO^{\cdot} reacts with all biological substrates and is most likely to react with sulfhydryl groups ($-SH$) (Chen et al., 1997; Liu et al., 1997) and

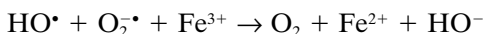
unsaturated lipids, but also with DNA, RNA, carbohydrates, and amino acids or proteins (Chen et al., 1992; Miura et al., 1992).



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



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).



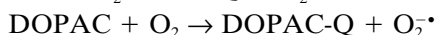
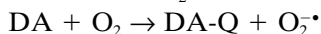
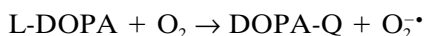
An additional reaction generates products of nitric oxide.



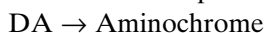
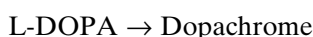
All of the many ROS formed from L-DOPA (and DA) are potentially neurotoxic.

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):



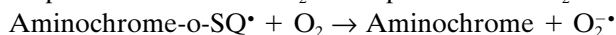
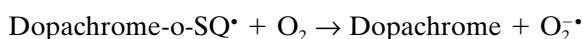
Both DOPA-Q and DA-Q can further undergo internal rearrangement in which the aminoalkyl chain cyclizes, respectively forming dopachrome and aminochrome (Hawley et al., 1967; Heacock, 1959). This reaction can be catalyzed by O_2 (faster rate at higher pH) (Graham, 1978; Senoh et al., 1959), Fe^{2+} , 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 $\text{O}_2^{\bullet -}$ and HO^{\bullet} (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).



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.



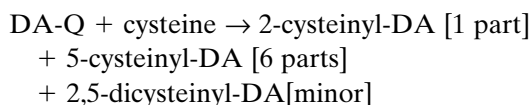
SQs formed in the reactions shown can interact with O_2 , to reform their quinones along with $\text{O}_2^{\bullet -}$.



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).



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^{\bullet} , thus promoting formation of $\text{O}_2^{\bullet -}$ and consequent self-perpetuation of HO^{\bullet} 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^{\bullet} . 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 $\cdot\text{OH}$, 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) ($67\ \mu\text{g}$ 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 $\cdot\text{OH}$ 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 $\cdot\text{OH}$ 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 $\cdot\text{OH}$ 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 $\cdot\text{OH}$ 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 $\cdot\text{OH}$ 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 cytotoxicity 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, $\cdot\text{OH}$ 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 $\cdot\text{OH}$ formation appears to relate to L-DOPA metabolism to DA, since carbidopa prevented L-DOPA (0.1 mM ; $1 \mu\text{M min}^{-1}$)-induced $\cdot\text{OH}$ 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 (Anglade 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 phenoxo 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 (IC_{50} , $450 \mu\text{M}$) and DA (IC_{50} , $8.5 \mu\text{M}$) inhibit peroxidation of ox brain phospholipids (Spencer et al., 1996). In rats with a unilateral 6-OHDA lesion, DOPA did not increase $\cdot\text{OH}$ 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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References

- Anglade P, Vyas S, Javoy-Agid F (1997) Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol Histopathol* 12: 25–31

- Baez S, Linderson Y, Segura-Aguilar J (1995) Superoxide dismutase and catalase enhance autooxidation during one-electron reduction of aminochrome by NADPH-cytochrome P-450 reductase. *Biochem Mol Med* 54: 12–18
- Ben-Shachar D, Zuk R, Glinka Y (1995) Dopamine neurotoxicity: Inhibition of mitochondrial respiration. *J Neurochem* 64: 718–723
- Berlett BS, Stadman (1997) Protein oxidation in aging, disease and oxidative stress. *J Biol Chem* 272: 20313–20316
- Berman SB, Hastings TG (1999) Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: Implications for Parkinson's disease. *J Neurochem* 73: 1127–1137
- Camp DM, Loeffler DA, LeWitt PA (2000) L-DOPA does not enhance hydroxyl radical formation in the nigrostriatal dopamine system of rats with a unilateral 6-hydroxydopamine lesion. *J Neurochem* 74: 1229–1240
- Chen CS, Chao HT, Pan RL, Wei YH (1997) Hydroxyl radical-induced decline in motility and increase in lipid peroxidation and DNA modification in human sperm. *Biochem Mol Biol Intl* 43: 291–303
- Chen JW, Zang L, Lian X, Hwang F (1992) Effect of hydroxyl radical on Na(+)-K(+)-ATPase activity on the brain microsomal membranes. *Cell Biol Intl Rep* 16: 927–936
- Colado MI, O'Shea E, Granados R, Esteban B, Martin AB, Green AR (1999) Studies on the role of dopamine in the degeneration of 5-HT nerve endings in the brain of Dark Agouti rats following MDMA or "ecstasy" administration. *Br J Pharmacol* 126: 911–924
- Datla KP, Blunt SB, Dexter DT (2001) 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 FeCl(3) nigrostriatal lesions. *Mov Disord* 16: 424–434
- de la Monte SM, Sohn YK, Ganju N, Wands JR (1998) p53- and CD95-associated apoptosis in neurodegenerative diseases. *Lab Invest* 78: 401–411
- Ebadi M, Govitrapong P, Sharma S, Muralikrishnan D, Shavali S, Pellett L, Schafer R, Albano C, Eken J (2001) Ubiquinone (coenzyme q(10)) and mitochondria in oxidative stress of parkinson's disease. *Biol Signals Recept* 10: 224–253
- Fenton HJH (1894) Oxidation of tartaric acid in the presence of iron. *J Chem Soc* 65: 899–910
- Filloux F, Townsend JJ (1993) Pre- and post-synaptic neurotoxic effects of dopamine demonstrated by intrastratial injection. *Exp Neurol* 119: 79–88
- Foppoli C, Coccia R, Cini C, Rosei MA (1997) Catecholamine oxidation by xanthine oxidase. *Biochim Biophys Acta* 1334: 200–206
- Fornstedt B, Brun A, Rosengren E, Carlsson A (1989) The apparent autooxidation rate of catechols in dopamine-rich regions of human brain increases with the degree of depigmentation of substantia nigra. *J Neural Transm [-PD Sect.]* 1: 279–295
- Fornstedt B, Pileblad E, Carlsson A (1990) In vivo autooxidation of dopamine in guinea pig striatum increases with age. *J Neurochem* 55: 655–659
- Graham DG (1978) Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol Pharmacol* 14: 633–643
- Gwinn-Hardy K, Evidente VG, Waters C, Muentner MD, Hardy J (1999) L-dopa slows the progression of familial parkinsonism. *Lancet* 353: 1850–1851
- Haber F, Weiss J (1934) The catalytic decomposition of H₂O₂ by iron salts. *Proc Royal Soc London (Biol)* 147: 332–351
- Han X, Liehr JG (1994a) DNA single strand breaks in kidneys of Syrian hamsters treated with steroidal estrogens. Hormone-induced free radical damage preceding renal malignancy. *Carcinogenesis* 15: 997–1000
- Han X, Liehr JG (1994b) 8-Hydroxylation of guanine bases in kidney and liver DNA of hamsters treated with estradiol: Role of free radicals in estrogen-induced carcinogenesis. *Cancer Res* 54: 5515–5517
- Han X, Liehr JG (1995) Microsome-mediated 8-hydroxylation of guanine bases of DNA by steroid estrogens: Correlation of DNA damage by free radicals with metabolic activation to quinones. *Carcinogenesis* 16: 2571–2574
- Hastings TG (1995) Enzymatic oxidation of dopamine: the role of prostaglandin H synthase. *J Neurochem* 64: 919–924
- Hastings TG, Zigmond MJ (1994) Identification of catechol-protein conjugates in neostriatal slices incubated with [3H]dopamine: impact of ascorbic acid and glutathione. *J Neurochem* 63: 1126–1132
- Hastings TG, Lewis DA, Zigmond MJ (1996) Role of oxidation in the neurotoxic effects of intrastratial DA injections. *Proc Natl Acad Sci USA* 93: 1956–1961
- Hawley MD, Tatawawadi SV, Piekarski S, Adams RN (1967) Electrochemical studies of the oxidation pathways of catecholamines. *J Am Chem Soc* 89: 447–450
- Heacock RA (1959) The chemistry of adrenochrome and related compounds. *Chem Rev* 59: 181–327
- Hoyt KR, Reynolds IJ, Hastings TG (1997) Mechanisms of dopamine-induced cell death in cultured rat forebrain neurons: interactions with and differences from glutamate-induced cell death. *Exp Neurol* 143: 269–281
- Hunot S, Brugg B, Ricard D, Michel PP, Muriel MP, Ruberg M, Faucheux BA, Agid Y, Hirsch EC (1997) Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with Parkinson's disease. *Proc Natl Acad Sci USA* 94: 7531–7536
- Ilic TV, Jovanovic M, Jovicic A, Tomovic M (1999) Oxidative stress indicators are elevated in de novo Parkinson's disease patients. *Funct Neurol* 14: 141–147
- Ito S, Fujita K (1982) Conjugation of dopa and 5-S-cysteinyl-dopa with cysteine mediated by superoxide radical. *Biochem Pharmacol* 31: 2887–2889
- Ito S, Kato T, Fujita K (1988) Covalent binding of catechols to proteins through the sulfhydryl group. *Biochem Pharmacol* 37: 1707–1710
- Jellinger K, Paulus W, Grundke-Iqbal I, Riederer P, Youdim MB (1990) Brain iron and ferritin in Parkinson's and Alzheimer's diseases. *J Neural Transm Park Dis Dement Sect* 2: 327–340
- Jenner PG, Brin MF (1998) Levodopa neurotoxicity: experimental studies versus clinical relevance. *Neurology* 50[Suppl 6], S39–S43; discussion S44–S48
- Kato T, Ito S, Fujita K (1986) Tyrosinase-catalyzed binding of 3,4-dihydroxyphenylalanine with proteins through the sulfhydryl group. *Biochim Biophys Acta* 881: 415–421
- Koeppen AH (1995) The history of iron in the brain. *J Neurol Sci* 134[Suppl]: 1–9
- Korytowski W, Sarna T, Kalyanaraman B, Sealy RC (1987) Tyrosinase-catalyzed oxidation of dopa and related catecholamines: a kinetic electron spin resonance investigation using spin-stabilization and spin label oximetry. *Biochim Biophys Acta* 924: 383–392
- Kostrzewa RM (2000) Review on apoptosis vs. necrosis of substantia nigra pars compacta in Parkinson's disease. *Neurotoxicity Res* 2: 239–250
- Kostrzewa RM, Kostrzewa JP, Brus R (2000) Dopaminergic denervation enhances susceptibility to hydroxyl radicals in rat neostriatum. *Amino Acids* 19: 183–199
- Kristal BS, Conway AD, Brown AM, Jain JC, Ulluci PA, Li SW, Burke WJ (2001) Selective dopaminergic vulnerability: 3,4-

- dihydroxyphenylacetaldehyde targets mitochondria. *Free Radic Biol Med* 30: 924–931
- LaVoie MJ, Hastings TG (1997) Peroxynitrite potentiates the oxidation of dopamine in vitro: implications for MPTP- and methamphetamine-induced toxicity. *Soc Neurosci Abstr* 23: 1371
- LaVoie MJ, Hastings TG (1999) Peroxynitrite and nitrite induce oxidation of dopamine: implications for nitric oxide in dopaminergic cell loss. *J Neurochem* 73: 2546–2554
- Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chemova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH (1998) The ubiquitin pathway in Parkinson's disease. *Nature* 395: 451–452
- Liehr JG, Ulubelen AA, Strobel HW (1986) Cytochrome P450-mediated redox cycling of estrogens. *J Biol Chem* 261: 16865–16870
- Liu TZ, Lin TF, Chiu DT, Tsai KJ, Stern A (1997) Palladium or platinum exacerbates hydroxyl radical mediated DNA damage. *Free Radic Biol Med* 23: 155–161
- Luo Y, Umegaki H, Wang X, Abe R, Szatmary I (1998) Dopamine induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. *J Biol Chem* 273: 3756–3764
- Markides C, Liehr JG (1998) Concentration-dependence of pro-oxidant and antioxidant properties of catechol estrogens. *Arch Biochem Biophys* 360: 105–112
- Mattammal MB, Strong R, Lakshmi VM, Chung HD, Stephanson AH (1995) Prostaglandin H synthase-mediated metabolism of dopamine: implications for Parkinson's disease. *J Neurochem* 64: 1645–1654
- McLaughlin BA, Nelson D, Erecinska M, Chesselet (1998) Toxicity of dopamine to striatal neurons in vitro and potentiation of cell death by a mitochondrial inhibitor. *J Neurochem* 70: 2406–2415
- Melamed E, Offen D, Shirvan A, Djaldetti R, Barzilai A, Ziv I (1998) Levodopa toxicity and apoptosis. *Ann Neurol* 44: S149–154
- Michel PP, Hefti F (1990) Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. *J Neurosci Res* 26: 428–435
- Mischel RE, Kim YS, Sheldom RA, Ferriero DM (1997) Hydrogen peroxide is selectively toxic to immature murine neurons in vitro. *Neurosci Lett* 231: 17–20
- Miura T, Muraoka S, Ogiso T (1992) Oxidative damage to bovine serum albumin induced by hydroxyl radical generating systems of oxanthine oxidase + EDTA-Fe³⁺ and ascorbate + EDTA-Fe³⁺. *Chem Biol Interact* 85: 243–254
- Moichizuki H, Goto K, Mori H, Mizuno Y (1996) Histology detection of apoptosis in Parkinson's disease. *J Neurol Sci* 137: 120–123
- Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG (1992) Quinone chemistry and toxicity. *Toxicol Appl Pharmacol* 112: 2–16
- Montine TJ, Farris DB, Graham DG (1995) Covalent crosslinking of neurofilament proteins by oxidized catechols as a potential mechanism of Lewy body formation. *J Neuropathol Exp Neurol* 54: 311–319
- Morikawa N, Nakagawa-Hattori Y, Mizuno Y (1996) Effect of dopamine, dimethoxyphenylethylamine, papaverine, and related compounds on mitochondrial respiration and Complex I activity. *J Neurochem* 66: 1174–1181
- Morris CM, Edwardson JA (1994) Iron histochemistry of the substantia nigra in Parkinson's disease. *Neurodegeneration* 3: 277–282
- Murer MG, Dziewczapolski G, Menalled LB, Garcia MC, Agid Y, Gershanik O, Raisman-Vozari R (1998) Chronic levodopa is not toxic for remaining dopamine neurons, but instead promotes their recovery, in rats with moderate nigrostriatal lesions. *Ann Neurol* 43: 561–575
- Nappi AJ, Vass E, Prota G, Memoli S (1995) The effect of hydroxyl radical attack on dopa, dopamine, 6-hydroxydopa, and 6-hydroxydopamine. *Pigment Cell Res* 8: 283–293
- Obata T, Yamanaka Y (1996) Protective effect of carbidopa on hydroxyl radical generation in the rat striatum by dopamine. *Neurosci Lett* 221: 13–16
- Offen D, Ziv I, Gorodin A, Barzilai A, Malik Z, Melamed E (1995) Dopamine-induced programmed cell death in mouse thymocytes. *Biochim Biophys Acta* 1268: 171–177
- Offen D, Ziv I, Barzilai A, Gorodin S, Glater E, Hochman A, Melamed E (1997) Dopamine-melanin induces apoptosis in PC12 cells: possible implications for the etiology of Parkinson's disease. *Neurochem Int* 31: 207–216
- Perry TL, Godin DV, Hansen S (1982) Parkinson's disease: a disorder due to nigral glutathione deficiency? *Neurosci Lett* 33: 305–310
- Prinsze C, Dubbelman TM, Van Stevenick J (1990) Protein damage, induced by small amounts of photodynamically generated singlet oxygen or hydroxyl radicals. *Biochim Biophys Acta* 1038: 152–157
- Przedborski S, Jackson-Lewis V, Muthane U, Jiang H, Ferreira M, Naini AB, Fahn S (1993) Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. *Ann Neurol* 34: 715–723
- Rosei MA, Blarzino C, Foppoli C, Mosca L, Coccia R (1994) Lipoxigenase-catalyzed oxidation of catecholamines. *Biochem Biophys Res Commun* 200: 344–350
- Riederer P, Sofic E, Rausch WD, Schmidt B, Reynolds GP, Jellinger K, Youdim MB (1989) Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. *J Neurochem* 52: 515–520
- Sanchez-Ramos JR, Overvik E, Ames BN (1994) A marker of oxyradical-mediated DNA damage (8-hydroxy-2-deoxyguanosine) is increased in nigrostriatum of Parkinson's disease brain. *Neurodegeneration* 3: 197–204
- Segura-Aguilar J (1996) Peroxidase activity of liver microsomal vitamin D 25-hydroxylase catalyzes 25-hydroxylation of vitamin D₃ and oxidation of dopamine to aminochrome. *Biochem Mol Med* 58: 122–129
- Segura-Aguilar J, Metodiewa D, Welch C (1998) Metabolic activation of dopamine o-quinones to o-semiquinones by NADPH cytochrome P450 reductase may play an important role in oxidative stress and apoptotic effects. *Biochim Biophys Acta* 381: 1–6
- Senoh S, Creveling CR, Udenfriend S, Witkop B (1959) Chemical, enzymatic and metabolic studies on the mechanism of oxidation of dopamine. *J Am Chem Soc* 81: 6231–6240
- Shen XM, Xia B, Wrona MZ, Dryhurst G (1996) Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteinyl conjugates of dopamine: possible metabolites of relevance to Parkinson's disease. *Chem Res Toxicol* 9: 1117–1126
- Shirvan A, Ziv I, Barzilai A, Djaldetti R, Zilkh-Falb R, Michlin T, Melamed E (1997) Induction of mitosis-related genes during dopamine-triggered apoptosis in sympathetic neurons. *J Neural Transm [Suppl]* 50: 67–78
- Shoffner JM, Watts RL, Juncos JL, Torroni A, Wallace DC (1992) Mitochondrial oxidative phosphorylation defects in Parkinson's disease. *Ann Neurol* 32: 226–227
- Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F, Jenner P, Marsden CD (1994) Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann Neurol* 36: 333–334
- Smith TS, Parker WD Jr, Bennett JP Jr (1994) L-Dopa increases nigral production of hydroxyl radicals in vivo: potential L-dopa toxicity? *Neuroreport* 5: 1009–1011

- Spencer JPE, Jenner P, Halliwell B (1995) Superoxide-dependent depletion of reduced glutathione by L-dopa and dopamine: Relevance to Parkinson's disease. *Neuroreport* 6: 1480–1484
- Spencer JP, Jenner A, Butler J, Aruoma OI, Dexter DT, Jenner P, Halliwell B (1996) Evaluation of the pro-oxidant and antioxidant actions of L-DOPA and dopamine in vitro: implications for Parkinson's disease. *Free Radic Res* 24: 95–105
- Stadtman ER (1992) Protein oxidation and aging. *Science* 257: 1220–1224
- Takashima H, Tsujihata M, Kishikawa M, Freed WJ (1999) Bromocriptine protects dopaminergic neurons from levodopa-induced toxicity by stimulating D(2)receptors. *Exp Neurol* 159: 98–104
- Tatton NA, MacLean-Fraser A, Tatton WG, Perl DP, Olanow CW (1998) A fluorescent double-labeling method to detect and confirm apoptotic nuclei in Parkinson's disease. *Ann Neurol* 44: S142–S148
- Walkinshaw G, Waters, CM (1995) Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA: Implications for the treatment of Parkinson's disease. Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA. *J Clin Invest* 95: 2458–2464
- Tran PB, Miller RJ (1999) Aggregates in neurodegenerative disease: crowds and power? *Trends Neurosci* 22: 194–197
- Wang MY, Liehr JG (1995) Induction by estrogens of lipid peroxidation and lipid peroxide-derived malonaldehyde-DNA adducts in male Syrian hamsters: role of lipid peroxidation in estrogen induced kidney carcinogenesis. *Carcinogenesis* 16: 1941–1945
- Wick MM (1979) Levodopa and dopamine analogs: Melanin precursors as antitumor agents in experimental human and murine leukemia. *Cancer Treat Rep* 63: 991–997
- Wick MM (1980) Levodopa and dopamine analogs as DNA polymerase inhibitors and antitumor agents in human melanoma. *Cancer Res* 40: 1414–1418
- Wick MM (1989) Levodopa/dopamine analogs as inhibitors of DNA synthesis in human melanoma cells. *J Invest Dermatol* 92: S329–331
- Wick MM, Byers L, Frei E (1977) L-dopa: Selective toxicity for melanoma cells in vitro. *Science* 197: 468–469
- Youdim MBH, Ben-Shachar D, Riederer P (1993) The role of iron in etiopathology of Parkinson's disease. *Mov Disord* 8: 1–12
- Zhang J, Price JO, Graham DG, Montine TJ (1998) Secondary excitotoxicity contributes to dopamine-induced apoptosis of dopaminergic neuronal cultures. *Biochem Biophys Res Commun* 248: 812–816

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