Levodopa Toxicity and Apoptosis

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Many in vitro studies have shown that levodopa is a potent toxin which is lethal to various cultured neuronal and non-neuronal cells. The in vitro toxicity of levodopa is linked mainly to its auto-oxidation, which generates a variety of harmful free radical species including superoxide, hydrogen peroxide, and hydroxyl radicals, and also semiquinones and quinones produced via the dopa–melanin metabolic route. Such toxic effects of levodopa can be blocked by co-treatment with antioxidants, particularly thiol-containing compounds. Several studies have shown that levodopa kills cells by triggering apoptosis, an active, intrinsic cell suicide program. Exposure of cultured neurons to levodopa induced the characteristic apoptotic cascade, including cell shrinkage, membrane blebbing, and nuclear and DNA fragmentation. Although levodopa is extremely toxic in vitro, there is no evidence that it damages nigrostriatal dopaminergic neurons in vivo in experimental animals and in patients with Parkinson's disease (PD). Likewise, although there is some evidence for the occurrence of apoptosis in the parkinsonian substantia nigra, it is not known whether levodopa administration is capable of inducing or accelerating programmed cell death of residual pigmented nigral neurons in PD.

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Toward the end of the twentieth century, clinical neurologists are fortunate to have many therapeutic options at their disposal to improve symptoms and signs, motor function, and quality of life of many patients with Parkinson's disease (PD). These include pharmacologic measures such as dopamine agonists, monoamine oxidase (MAO) inhibitors, anticholinergics, and amantadines, and also ablative, stimulatory, and transplantational surgical approaches.[1,2] However, despite the tremendous progress in the field, levodopa continues to maintain its pivotal role in the treatment of PD since its introduction about 30 years ago.[3] Oral levodopa remains the most effective antiparkinsonian drug and its efficacy can be further amplified by its combined administration with peripheral dopa decarboxylase inhibitors, MAO inhibitors and, recently, also with catechol-*o*-methyltransferase (COMT) inhibitors. The latter act to decrease peripheral metabolism of levodopa, enhance its entry through the blood–brain barrier, and increase levels and durations of the dopamine generated from the exogenous levodopa in central synapses. In all likelihood, use of levodopa as the most common and effective drug will also continue in the beginning of the next millennium.

On one hand, the mechanism of action of levodopa appears rather simple. Unlike the missing neurotransmitter dopamine, orally administered levodopa can cross the blood–brain barrier. Once in the striatum it is converted to receptor-accessible dopamine by the enzyme dopa decarboxylase present in the surviving nigrostriatal nerve terminals and in nondopaminergic cell compartments.[4,5] Therefore, exogenous levodopa can replenish the markedly reduced levels of dopamine in the parkinsonian striatum and repair the arrested or suppressed nigrostriatal dopaminergic neurotransmission. However, accumulating experience derived from both clinical and basic research indicates that the interactions of long-term levodopa with PD are far from simple. Rather, they are extremely complex and, in part, still enigmatic. Therefore, although use of levodopa provides a remarkable benefit for the patient, its utilization causes a persistent concern among neurologists and their patients. There are several reasons for this constant apprehension. First, exogenous levodopa is not precisely a physiologic treatment for PD.[6] When given orally it reaches and floods the entire brain and "bombards" not only the basal ganglia and the dopaminergic receptorial system but also the entire CNS. In addition, exogenous levodopa and the dopamine it generates are handled differently from the endogenous levodopa formed from tyrosine hydroxylation.[7] Normally, conversion of tyrosine to levodopa is a rate-limiting step and is greatly dependent on the firing rate of the dopaminergic neuron. Furthermore, the dopamine formed from endogenous tyrosine and levodopa is mostly stored in vesicles, and its release and synthesis are linked to the neuronal firing rates. By contrast, synthesis and release of the dopamine formed from exog-

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enous levodopa are entirely dissociated from the dopaminergic neuronal activity. In addition, the dopamine molecules that are produced from systemically administered levodopa in surviving nigrostriatal terminals and also in nondopaminergic striatal compartments (eg, glial cells, endothelial cells of capillaries, serotonergic and nonaminergic neurons) are, in all likelihood, not stored within vesicles but spilled into the synapse as soon as they are generated, stimulating the receptors in a nonphysiologic manner and being rapidly metabolized. These may be the reasons why chronic treatment with levodopa is associated with many adverse reactions including dyskinesias, complex motor fluctuations, and psychosis.

Another cause for anxiety is the possibility that treatment with levodopa may be toxic to the remaining dopaminergic neurons (and perhaps to other nondopaminergic neurons and glia).[8] Most importantly, there is concern that sustained levodopa administration might change the predetermined natural history of PD and accelerate its progression by increasing the rate of nigrostriatal degeneration. If levodopa is indeed toxic to the surviving dopaminergic neurons in parkinsonian patients, it would be a wise and conservative approach not to start treatment with levodopa immediately in newly diagnosed cases but rather to postpone it until the more advanced stages of the illness.[9–12]

In Vitro Toxicity of Levodopa

Many studies have shown that levodopa is a very powerful toxin that readily kills various cultured neuronal and non-neuronal cells.[13–15] In vitro toxicity of levodopa may be linked to auto-oxidation,[16] a process capable of generating a variety of toxic reactive oxygen species including hydrogen peroxide and the superoxide and hydroxyl free radicals. The presence of transition metals may enhance these toxic reactions.[14,17] Levodopa can also undergo auto-oxidation with the synthesis of semiquinone and quinone metabolic intermediaries and the formation of dopa–melanin pigments.[18] These species are also toxic and may themselves be lethal to cells in vitro. Blockade of in vitro toxicity of levodopa by combined treatment with antioxidants (particularly the thiol-containing compounds) supports the crucial role of levodopa-derived reactive oxygen species.[19–22] Levodopa and its damaging metabolites may kill cells by causing lipid peroxidation and membrane disruption.[23] It may poison the mitochondrial respiratory apparatus, mainly, but not exclusively, complex I and IV.[24–26] Levodopa has also been shown to be genotoxic and to cause nuclear DNA damage.[27,28] Dopamine itself is also extremely toxic,[14,29] being equipotent to levodopa in its lethal effects in tissue culture.[14] It has been suggested that levodopa toxicity is due, at least in part, to its conversion to dopamine.[30]

Levodopa and Apoptosis

There are two major modes of cell death, necrosis and apoptosis.[31–33] Necrosis is characterized by the nonselective death of many cell groups, associated with loss of membrane integrity, ballooning of the cell and its lysis without vesicle formation, and swelling and disintegration of intracellular organelles. The lysed cells are phagocytized by migrating macrophages, with significant local inflammatory reaction. By contrast, apoptosis is a form of cell death that is associated with activation of genetically encoded active cell suicide programs. It can involve the death of single individual cells, sometimes by purely physiologic stimuli. Morphologic characteristics include membrane blebbing, initially without loss of membrane integrity, aggregation of chromatin, cell condensation and shrinkage, loss of contacts with neighboring cells, and formation of membrane-bound vesicles termed apoptotic bodies, which are later phagocytized by macrophages with no local inflammatory response. As a rule, intracellular organelles remain intact. Cell nuclei become pyknotic, and there is fragmentation of nuclear DNA at internucleosomal sites. Apoptosis, or programmed cell death, plays a major physiologic role in the differentiation and organization of the CNS during its normal development. The developing brain loses about 50% of its cells before it achieves maturity. This neuronal loss is mediated by activation of apoptosis in neurons that may be predestined to die. More recently, there has been interest in the possibility that apoptosis is involved in the pathogenesis of degenerative human neurologic disorders, particularly those associated with aging.

Dopamine can exert the characteristic biochemical and morphologic features of apoptosis in several neuronal (eg, chick sympathetic neurons, mouse cerebellar granule cells, and cortical neurons) and non-neuronal (eg, rat PC-12 cells and mouse thymocytes, splenocytes, and lymphocytes) cell cultures.[34–36] Such dopamine-induced programmed cell death could be inhibited by co-treatment with thiol-containing antioxidants[37] and by vector-driven expression of the apoptosis-blocking proto-oncogene bcl-2.[38] On the basis of these in vitro findings, we suggested that inadvertent activation of intrinsic, genetically controlled inert cell suicide programs in nigral neurons by "unrestrained" dopamine and its toxic oxidation products may lead to their degeneration by an apoptotic mode in PD. Several ultrastructural morphologic studies have already demonstrated that dopaminergic neurons are indeed undergoing an active process of apoptosis in the parkinsonian substantia nigra.[39–42] There is sufficient evidence that the nigra in PD is under conditions of excess oxidative stress.[43–45] This may be due, in part, to auto- and enzymatic oxidation of dopamine, the presence of the transition metal iron, and

synthesis of neuromelanin, which generates semiquinones and quinones en route.

In the parkinsonian nigra and striatum, at least part of the exogenous levodopa is taken up by dendrites and nerve terminals, respectively, of the surviving dopaminergic neurons and is converted therein to dopamine.[4,5] In theory, toxic exogenous levodopa and the dopamine subsequently formed could contribute to the activation of dormant death programs of nigral neurons and lead to their enhanced death by apoptosis. Levodopa is also an extremely potent inducer of apoptosis in cultured postmitotic chick sympathetic neurons.[46] At a concentration range of 0.01 to 0.3 M, exposure to levodopa led to the characteristic apoptotic cascade including cell shrinkage, marked membrane blebbing, and nuclear fragmentation shown, among others, by flow cytometry and by fluorescence and scanning electron microscopy. Similar findings were observed using a variety of other neuronal and nonneuronal cells. Levodopa-induced apoptosis was inhibited by the antioxidants dithiothreitol, *N*-acetylcysteine and reduced glutathione, indicating that it may be mediated by auto-oxidation products of levodopa. Similar findings were reported in catecholaminergic PC-12 cells.[47]

As yet, there is no evidence indicating that systemic levodopa administration is responsible for the apoptotic neurons observed in the parkinsonian nigra.[39,40] The number of neurons identified as undergoing apoptosis in postmortem nigral tissues obtained from patients with PD is rather small.[39–42] It would have been expected that an assault of a systemically administered toxic agent such as levodopa would result in a much larger number of apoptotic nigral neurons and definitely larger than that caused only by the as yet unknown endogenous etiology of the illness. One explanation is that neurons succumbing to apoptosis are very rapidly phagocytized by macrophages and disappear without a trace of local inflammation or other damage. Another related possibility is that, at the time of autopsy, illness is well advanced and most of the levodopa-triggered apoptotic neurons have already disappeared. A third, most plausible option (see below) is that levodopa treatment is not toxic to and does not evoke apoptosis of nigral neurons in the in vivo situation, including patients with PD.

Is Levodopa Toxic In Vivo in Animals and Patients?

There is no doubt that levodopa is a very potent toxin in vitro and that it can cause neuronal cell death by both necrosis and apoptosis. Surprisingly, however, most in vivo studies using long-term administration of large levodopa doses to experimental animals and clinical investigations in patients did not demonstrate damage to nigrostriatal neurons or acceleration of the illness, respectively. For example, mice fed very large doses of levodopa daily for 18 months showed no evidence of damage to nigral dopaminergic neurons.[48] Similar negative findings have been reported elsewhere.[49] Because these studies were carried out in normal animals with intact nigrostriatal projections, it was still possible that if such neurons were rendered vulnerable they would be damaged by levodopa toxicity. Therefore, acute levodopa treatment can potentiate the toxic effects induced by intracerebroventricular injections of 6-hydroxydopamine in mice.[50] Long-term treatment with levodopa induced additional damage to rat ventral tegmental (but not nigrostriatal) dopaminergic neurons surviving prior exposure to 6-hydroxydopamine.[51] Closely similar findings were reported in mice.[52] By contrast, it should be noted that these findings were not confirmed in a similarly conducted recent study in which levodopa was given for 6 months.[53] Likewise, we have shown no damage to dopaminergic neurons by long-term daily injections of levodopa, although the animals were made vulnerable by combined systemic administration of MPTP.[54] Chronic levodopa administration was toxic to and destroyed fetal mesencephalic dopaminergic neurons that had been grafted into striatum in rats.[55] By contrast, no damage could be demonstrated to fetal dopaminergic neurons transplanted into rat corpora striata denervated by 6-hydroxydopamine lesions after chronic (27 weeks) levodopa treatment.[56] Likewise, repeated levodopa injections to pregnant mice did not damage fetal nigrostriatal neurons and did not impair their postnatal development.[57] For obvious reasons, clinical studies in humans to show or disprove levodopa toxicity are lacking and are difficult to carry out, mainly because of the current absence of comparative data. In addition, PD continues to progress under chronic levodopa therapy. However, because levodopa exerts a clinical benefit, it is difficult to determine whether the disease progresses at its predetermined pace or whether it is further accelerated. Nevertheless, most if not all studies indicate that chronic levodopa treatment is not toxic to human nigrostriatal dopaminergic neurons. Thus, there was no evidence of nigral damage in a nonparkinsonian patient treated with large oral doses of levodopa for several years.[58] Similarly negative findings were reported in five patients given sustained oral levodopa treatment for several (up to 26) years.[59] In addition, a postmortem examination of a patient with PD who had undergone a fetal nigral transplantation showed excellent survival and axonal outgrowth of dopaminergic neurons within the graft despite uninterrupted and continuous levodopa administration postoperatively.[60] Other workers examined the problem clinically from follow-up of their patients and concluded that chronic levodopa does not aggravate the natural history of PD.[61]

Possible Explanations for the Discrepancy Between Marked Levodopa Toxicity In Vitro and Its Absence In Vivo

In general, revelations obtained from investigations in cell cultures do not necessarily reflect the situation in the whole living organism. In cultures, neurons are dispersed and are therefore more exposed to attack by levodopa present in the medium than cells in compact tissues. More importantly, cells in culture may lack adequate defense mechanisms that naturally occur in the target tissue in vivo, such as enzymes that scavenge toxic free radical species (superoxide dismutase, catalase, glutathione peroxidase), antioxidants (eg, reduced glutathione), and trophic nerve growth factors. There may also be other intracellular safeguards, such as the soluble protein calretinin.[62] One of the most potent factors inhibiting inadvertent apoptosis is the proto-oncogene bcl-2.[63,64] PC-12 cells overexpressing bcl-2 were immune to dopamine and also to levodopa toxicity in vitro.[38,65] It is possible that ample and even upregulated activity of the bcl-2 family of antiapoptotic proto-oncogenes in the brain and, particularly, in the parkinsonian substantia nigra provides the necessary blockade against the chronic onslaught of systemic levodopa, the generated dopamine, and reactive oxidation species.[66]

Another crucial component of the protective apparatus present in vivo are glial cells, which are remarkably resistant to the toxicity of dopamine, H_2O_2, MPTP, and also levodopa.[67] Lethal effects of the latter agents toward neuronal cells are drastically suppressed and often completely blocked by the addition of glial cells to the culture medium (Offen et al., manuscript in preparation). It is likely that glial cells normally present in the substantia nigra act to neutralize toxic effects of environmental toxins as well as those of endogenous noxious compounds such as dopamine, iron, free radicals, semiquinones, and quinones, and also of exogenous levodopa. One (but not the only) feature that renders glia immune to and also protective against oxidative toxins is their high intracellular content of the potent natural antioxidant glutathione. A few studies looking for signs of neuronal apoptosis in the parkinsonian nigra unexpectedly reported a relatively high number of glial cells showing the morphologic characteristics of programmed cell death.[39,42] A plausible explanation for this intriguing phenomenon would be that, because they as a major defense line against local toxins and also exogenous levodopa, the glial cells bear the brunt of their attack and may die apoptotically at their post shielding and saving their protégé neurons. In addition, it is also possible that concentrations of levodopa in the media of tissue culture experiments are much higher than those reached in the CNS of experimental animals and parkinsonians in vivo after systemic drug administration.[68,69] However, it should be noted that levodopa was given chronically for extended periods in animals and certainly in patients with PD. Surprisingly, levodopa may even be protective under certain circumstances.[70] Preconditioning of neurons by exposure to sublethal concentrations of levodopa renders the cells resistant to repeated exposure to lethal doses of the drug (Offen et al., manuscript in preparation). Such levodopa-pretreated cells show higher concentrations of reduced glutathione.[71] Peripheral blood lymphocytes obtained from parkinsonian patients treated chronically with levodopa show much higher resistance than those obtained from control subjects when exposed to a variety of toxic agents including dopamine, H_2O_2, and levodopa. Therefore, it is possible that when levodopa is given chronically to patients, cells in peripheral tissues and neurons in the brain, in general, and in the nigra, in particular, are preconditioned, develop or strengthen defense mechanisms such as content of glutathione, free radical scavenging enzymes and trophic factors, become immune to the toxic effects of systemic levodopa, and survive. Also, chronic levodopa treatment may not be associated at all with enhanced oxidative stress, at least in plasma.[72]

When dopaminergic neurons in the parkinsonian nigra die beyond a certain critical point (probably over 60% loss), the remaining neurons become hyperactive and fire more rapidly than neurons in an intact nigra.[73] It was suggested that because of the increased "wear and tear" associated with such hyperactivity, progression of PD may become accelerated. Although there is no clinical proof for a more accelerated course as the disease advances, exogenous levodopa, via the central production of unstored dopamine freely spilled into synapses, would be expected to stimulate autoreceptors present on striatal nerve endings and on nigral dendrites of surviving hyperactive nigrostriatal neurons and to suppress their firing rates.

In conclusion, levodopa is a powerful toxin in vitro but most of the currently available investigations fail to show that it damages nigral dopaminergic neurons in vivo. However, although these data are reassuring and encouraging, they are still inconclusive because it cannot yet be completely ruled out that nigral dopaminergic neurons may be particularly susceptible to the toxic effects of systemic levodopa. For that reasons, it would be a logical measure to remain on the safe side, exercise caution, delay initiation of levodopa for as long as possible, and use other drugs, such as potent dopamine agonists, in this interim period.[9–12]

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References

1. Marsden CD. Parkinson's disease. Lancet 1990;335:908–952
2. Agid Y. Parkinson's disease: pathophysiology. Lancet 1991;337: 1321–1324

3. Cotzias GC, Van Woert MH, Schiffer LM. Aromatic amino acids and modification of parkinsonism. N Engl J Med 1967; 276:374–379
4. Melamed E, Hefti F, Wurtman RH. Non-aminergic striatal neurons convert exogenous L-dopa to dopamine in parkinsonism. Ann Neurol 1980;8:528–536
5. Hefti F, Melamed E. L-dopa's mechanism of action in Parkinson's disease. Trends Neurosci 1980;3:229–231
6. Melamed E. Mechanism of action of exogenous L-dopa: Is it a physiological therapy for Parkinson's disease? In: Jankovic J, Tolosa E, eds. Advances in Parkinson's disease and movement disorders. Baltimore: Urban and Schwarzenberg, 1988:87–97
7. Melamed E. Biochemical and functional differences between dopamine formed from endogenous tyrosine and exogenous L-dopa in nigro-striatal dopaminergic neurons. Neurochem Int 1992;20(Suppl):115–117
8. Fahn S. Is levodopa toxic? Neurology 1996;47(Suppl):S184–195
9. Fahn S, Bressman SB. Should levodopa therapy for parkinsonism be started early or late? Evidence against early treatment. Can J Neurol Sci 1984;11:200–206
10. Rajput AH, Stern W, Laverty WH. Chronic low dose levodopa therapy in Parkinson's disease: an argument for delaying L-dopa therapy. Neurology 1984;34:991–996
11. Melamed E. Initiation of levodopa therapy in parkinsonian patients should be delayed until the advanced stages of the disease. Arch Neurol 1986;43:402–405
12. Quinn NP. A case against early levodopa treatment of Parkinson's disease. Clin Neuropharmacol 1994;17:S43–49
13. Wick MM, Byers L, Frei E. L-dopa: selective toxicity for melanoma cells in vitro. Science 1977;197:468–469
14. Tanaka M, Sotomatsu A, Kanai H, Hirai S. Dopa and dopamine cause cultured neuronal death in the presence of iron. J Neurol Sci 1991;101:198–203
15. Mena MA, Pardo B, Casarejos MJ, Fahn S, de Yebenes JG. Neurotoxicity of levodopa on catecholamine rich neurons. Mov Disord 1992;7:23–31
16. Graham DJ, Tiffany SM, Bell WR, Gutknecht WF. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine and related compounds towards C1300 neuroblastoma cells in vitro. Mol Pharmacol 1978;14:644–653
17. Newcomer TA, Rosenberg PA, Aizenman E. Iron-mediated oxidation of 3,4-dihydroxyphenylalanine to an excitotoxin. J Neurochem 1995;64:1742–1748
18. Graham AG. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. Mol Pharmacol 1978;14:633–643
19. Pardo B, Mena MA, Fahn S, de Yebenes JG. Ascorbic acid protects against levodopa-induced neurotoxicity on a catecholamine-rich human neuroblastoma cell line. Mov Disord 1993;8:278–284
20. Mena MA, Pardo B, Paino CL, de Yebenes JG. Levodopa toxicity in fetal rat midbrain neurons in culture: modulation by ascorbic acid. Neuroreport 1993;4:438–440
21. Basma AN, Morris EJ, Nicklas WJ, Geller HM. L-dopa cytotoxicity to PC-12 cells in culture is via its auto-oxidation. J Neurochem 1995;64:825–832
22. Pardo B, Mena MA, Casarejos MJ, Paino CL, de Yebenes JG. Toxic effects of L-dopa in mesencephalic cell cultures: protection with anti-oxidants. Brain Res 1995;682:133–143
23. Li CL, Werner P, Cohen G. Lipid peroxidation in brain: interactions of L-dopa/dopamine with ascorbate and iron. Neurodegeneration 1995;4:147–153
24. Przedborski S, Jackson-Lewis V, Muthane U, et al. Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. Ann Neurol 1993;34:715–723
25. Przedborski S, Jackson-Lewis V, Fahn S. Antiparkinsonian therapies and brain mitochondrial complex I activity. Mov Disord 1995;10:312–317
26. Pardo B, Mena MA, de Yebenes JG. L-dopa inhibits complex IV of the electron transport chain in catecholamine-rich human neuroblastoma NB69 cells. J Neurochem 1995;64:576–582
27. Spencer JP, Jenner A, Arumoa OI, Marsden CD, Halliwell B. Intense oxidative DNA damage promoted by L-dopa and its metabolites: implications for neurodegenerative disease. FEBS Lett 1994;353:246–250
28. Wick MM. Levodopa/dopamine analogs as inhibitors of DNA synthesis in human melanoma cells. J Invest Dermatol 1989; 92:329S–331S
29. Michel PP, Hefti F. Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. J Neurosci Res 1990;26:428–435
30. Alexander T, Sortwell CE, Sladek CD, Roth RH, Steece-Collier K. Comparison of neurotoxicity following repeated administration of L-dopa, D-dopa and dopamine to embryonic mesencephalic dopamine neurons in cultures derived from Fischer 344 and Sprague-Dawley donors. Cell Transplant 1997;6:309–315
31. Boobis AR, Fowthrop DJ, Davies DS. Mechanisms of cell death. Trends Pharmacol Sci 1989;10:275–280
32. Martin SJ. Apoptosis: suicide, execution or murder? Trends Cell Biol 1993;3:141–144
33. Cohen JJ. Apoptosis. Immunol Today 1993;14:126–130
34. Ziv I, Melamed E, Nardi N, et al. Dopamine induces apoptosis-like cell death in cultured chick sympathetic neurons—a possible novel pathogentic mechanism in Parkinson's disease. Neurosci Lett 1994;170:136–140
35. Offen D, Ziv I, Gordin S, Malik Z, Barzilai A, Melamed E. Dopamine-induced programmed cell death in mouse thymocytes. Biochim Biophys Acta 1995;1268:171–177
36. Zilkha-Falb R, Ziv I, Nardi N, Offen D, Melamed E, Barzilai A. Monoamine-induced apoptotic neuronal cell death. Cell Mol Neurobiol 1997;7:110–118
37. Offen D, Sternin H, Ziv I, Melamed E, Hochman A. Prevention of dopamine-induced cell death by thiol antioxidants: possible implication for treatment of Parkinson's disease. Exp Neurol 1996;141:32–39
38. Ziv I, Offen D, Haviv R, et al. The proto-oncogene bcl-2 inhibits cellular toxicity of dopamine: possible implications for Parkinson's disease. Apoptosis 1997;2:149–155
39. Mochizuki H, Goto K, Mori H, Mizuno Y. Histochemical detection of apoptosis in Parkinson's disease. J Neurol Sci 1996; 137:120–123
40. Anglade P, Vyas S, Javoy-Agid F, et al. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 1997;12:25–31
41. Tompkins MM, Basgall EJ, Zamvini E, Hill WD. Apoptotic-like changes in Lewy body-associated disorders and normal aging in substantia nigral neurons. Am J Pathol 1997;150:119–131
42. Kosel S, Egensperger R, von Eitzen U, Mehraein P, Graeber MB. On the question of apoptosis in the parkinsonian substantia nigra. Acta Neuropathol (Berl) 1997;93:105–108
43. Fahn S, Cohen G. The oxidant stress hypothesis in Parkinson's disease. Evidence supporting it. Ann Neurol 1992;32:804–812
44. Olanow CW. A radical hypothesis for neurodegeneration. Trends Neurosci 1993;16:439–444
45. Jenner P, Dexter DT, Sian J, Schapira AHV, Marsden CD. Oxidative stress as a cause of nigral cell death in Parkinson's disease and incidental Lewy body disease. Ann Neurol 1992;32: S82–S87
46. Ziv I, Zilkha-Falb R, Offen D, Shirvan A, Barzilai A, Melamed E. Levodopa induces apoptosis in cultured neuronal cells—a

possible accelerator of nigrostriatal degeneration in Parkinson's disease? Mov Disord 1997;12:17–23
47. Walkinshaw G, Waters CM. Induction of apoptosis in catecholaminergic PC-12 cells by L-dopa. Implications for the treatment of Parkinson's disease. J Clin Invest 1995;95:2458–2464
48. Hefti F, Melamed E, Bhawan J, Wurtman RJ. Long-term administration of L-dopa does not damage dopaminergic neurons in the mouse. Neurology 1981;31:1194–1195
49. Perry TL, Yong VW, Ito U, et al. Nigrostriatal dopaminergic neurons remain undamaged in rats given high doses of L-dopa and carbidopa chronically. J Neurochem 1984;43:990–993
50. Naudin B, Bonnet JJ, Costentin J. Acute L-dopa pretreatment potentiates 6-hydroxydopamine-induced toxic effects on nigrostriatal dopaminergic neurons in mice. Brain Res 1995;701: 151–157
51. Blunt SB, Jenner P, Marsden CD. Suppressive effect of L-dopa on dopamine cells remaining in the ventral tegmental area of rats previously exposed to the neurotoxin 6-hydroxydopamine. Mov Disord 1993;8:129–133
52. Ogawa N, Aganuma M, Kondo Y, Yamamoto M, Mori A. Differential effects of chronic L-dopa treatment on lipid peroxidation in the mouse brain with and without pretreatment with 6-hydroxydopamine. Neurosci Lett 1994;171:55–58
53. Dziewczapolski G, Murer G, Agid Y, Gershanik O, Raisman-Vozari R. Absence of neurotoxicity of chronic L-dopa treatment in 6-hydroxydopamine-lesioned rats. Neuroreport 1997;8:975–979
54. Melamed E, Rosenthal J. Can chronic levodopa therapy accelerate degeneration of dopaminergic neurons and progression of Parkinson's disease? In: Nagatsu T, Fisher A, Yoshida M, eds. Basic clinical and therapeutic aspects of Alzheimer's and Parkinson's diseases. New York: Plenum Press, 1990;253–256
55. Steece-Collier K, Collier TJ, Sladeck CD, Sladeck JR. Chronic levodopa impairs morphological development of grafted embryonic dopamine neurons. Exp Neurol 1990;110:201–208
56. Blunt SB, Jenner P, Marsden CD. The effect of L-dopa and carbidopa treatment on the survival of rat fetal dopamine grafts assessed by tyrosine hydroxylase immunohistochemistry and ^{3}H-mazindol autoradiography. Neuroscience 1991;43:95–110
57. Melamed E, Rosenthal J, Reches H. Prenatal administration of levodopa is not toxic to fetal dopaminergic neurons in vivo. Neurology 1992;42(Suppl 3):378
58. Quinn N, Parkes D, Janota I, Marsden CD. Preservation of the substantia nigra and locus ceruleus in a patient receiving levodopa (2 kg) plus decarboxylase inhibitor over a four year period. Mov Disord 1986;1:65–68
59. Rajput AH, Fenton ME, Birdi S, Macaulay R. Is levodopa toxic to human substantia nigra? Mov Disord 1997;12:634–638
60. Kordower JH, Freeman TB, Snow BJ, Olanow CW. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. N Engl J Med 1995;332:1118–1124
61. Blin J, Bonnet AM, Agid Y. Does levodopa aggravate Parkinson's disease? Neurology 1988;38:1410–1416
62. Isaacs KR, Wolpoe ME, Jacobowitz DM. Calretinin-immunoreactive dopaminergic neurons from embryonic rat mesencephalon are resistant to levodopa-induced neurotoxicity. Exp Neurol 1997;146:25–32
63. Reed JC. Bcl-2 and the regulation of programmed cell death. J Cell Biol 1994;124:1–6
64. Munez G, Clarke MF. The bcl-2 family of proteins: regulators of cell death and survival. Trends Cell Biol 1994;4:399–403
65. Offen D, Ziv I, Panet H, Wasserman L, Stein R, Melamed E, Barzilai A. Dopamine-induced apoptosis is inhibited in PC12 cells expressing bcl-2: evidence that the proto-oncogene may act as an antioxidant. Cell Mol Neurobiol 1997;17:285–304
66. Mogi M, Harada M, Kondo T, et al. Bcl-2 protein is increased in the brain from parkinsonian patients. Neurosci Lett 1996; 215:137–139
67. Mena MA, Casarejos MJ, Carazo A, Paino CL, de Yebenes GJ. Glia conditioned medium protects fetal rat midbrain neurons in culture from L-dopa toxicity. Neuroreport 1996;7:441–449
68. Olanow CW, Gauger BA, Cederbaum JM. Temporal relationships between plasma and cerebrospinal fluid pharmacokinetics of levodopa and clinical effect in Parkinson's disease. Ann Neurol 1991;29:556–559
69. Durso R, Evans JE, Josephs E, et al. Central levodopa metabolism in Parkinson's disease after administration of stable isotope-labeled levodopa. Ann Neurol 1997;42:300–304
70. Mytilineou C, Han SK, Cohen G. Toxic and protective effects of L-dopa on mesencephalic cell cultures. J Neurochem 1993; 61:1470–1478
71. Han SK, Mytilineou C, Cohen G. L-dopa upregulates glutathione and protects mesencephalic cultures against oxidative stress. J Neurochem 1996;66:501–510
72. Ahlskog JE, Uitti RJ, Low PA, Tyce GM, O'Brien JF, Nickander KK. Levodopa and deprenyl treatment effects on peripheral indices of oxidant stress in Parkinson's disease. Neurology 1996;46:796–801
73. Melamed E, Hefti F, Wurtman RJ. Compensatory mechanisms in nigrostriatal dopaminergic system in parkinsonism: studies in an animal model. Israel J Med Sci 1982;18:159–163