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Levodopa in Parkinson's Disease

Neurotoxicity Issue Laid to Rest?

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Abstract

Orally administered levodopa remains the most effective symptomatic treatment for Parkinson's disease. The introduction of levodopa therapy is often delayed, however, because of the fear that it might be toxic for the remaining dopaminergic neurons, and thus accelerate the deterioration of the patient's condition.

Evidence for levodopa toxicity comes mainly from *in vitro* studies which have demonstrated that levodopa can damage dopaminergic neurons by a mechanism that probably involves oxidative stress. It is widely accepted, however, that levodopa is not toxic for healthy animals and humans who do not have Parkinson's disease. It has been argued that the lesioned mesostriatal dopaminergic system could be more vulnerable to levodopa-induced toxicity, because the brain extracellular concentrations attained by levodopa are higher when the dopaminergic system is damaged, and remaining dopaminergic neurons experience a process of compensatory hyperactivity.

Evidence for *in vivo* levodopa toxicity in animal models of Parkinson's disease is scarce and contradictory. A comprehensive recent study failed to find any evidence of levodopa toxicity in rats with either moderate or severe lesions of the mesostriatal dopaminergic system.

Concerning the hypothesis of toxicity, some recent reports have shown that levodopa can have trophic effects on dopaminergic neurons *in vitro*, and our own work has shown that long term levodopa therapy promotes recovery of striatal dopaminergic markers in rats with moderate nigrostriatal lesions. Given that neither epidemiological nor clinical studies have ever provided evidence to support that long term levodopa administration can accelerate the progression of Parkinson's disease, we believe that levodopa therapy should not be delayed on the basis of an unconfirmed hypothesis.

The discoveries by Carlsson et al.[1] showing that levodopa can reverse akinesia induced by catecholamine depletion in mice, and by Ehringer and Hornykiewicz and co-workers (see reference 2 for review)[2] demonstrating for the first time striatal dopamine deficiency in Parkinson's disease, provided the background for the first clinical trials with 3,4-dihydroxyphenylalanine (dopa). Initial trials led to inconsistent results,[3] but some years later Cotzias et al.[4] reported the impressive antiparkinsonian effects of long term oral *D,L*-dopa, establishing the basis for the modern pharmacotherapy of Parkinson's disease. The improved efficacy of the combination of the *L*-isomer of dopa (levodopa) with a peripheral decarboxylase inhibitor and the major adverse effects of the treatment, including involuntary movements, motor fluctuations and mental symptoms, were described shortly thereafter.[5-8]

Levodopa therapy radically changed the life history of patients with Parkinson's disease to such an extent that a large scale, controlled, prospective study comparing levodopa *vs* placebo has never been conducted. The drug reverted the incapacitating parkinsonian signs and symptoms, but soon concern was raised about the convenience of beginning treatment early in the course of the disease, because it was found that the therapeutic effect of levodopa declines in efficacy with time and severe adverse effects were seen to emerge after several years of treatment.[9,10] At that time, neurologists disagreed on whether the adverse effects were related to the duration of levodopa treatment (and so, were due to drug-induced brain changes), or to progression of the disease (leading to a reduced efficacy and an altered response to treatment).[11-16] Evidence indicating that oxidative stress could play a role in the aetiology of Parkinson's disease was emerging, and ultimately led to the suggestion that long term exposure to levodopa could accelerate disease progression.[17-20]

Autoxidation of levodopa and dopamine can lead to the formation of hydrogen peroxide, highly reactive free radicals and quinones (fig. 1). These reactive species and other molecules with potential toxic properties can also result from the enzymatic metabolism of levodopa (fig. 1). In this article we review experimental evidence showing that levodopa is only toxic to neurons under particular experimental conditions, and discuss the relevance of these results for clinical practice.

1. *In Vitro* Evidence of Levodopa Toxicity

Critical evidence demonstrating a toxic effect of levodopa on dopaminergic neurons comes from *in vitro* studies. The pioneering work of Michel and Hefti[37] showing that exposure to dopamine reduces the number of tyrosine hydroxylase immunoreactive neurons in primary mesencephalic cultures was followed by several reports demonstrating similar effects of levodopa.[38-45] Evidence of levodopa/dopamine toxicity was also obtained from less relevant *in vitro* systems, such as cultures of catecholamine-containing cell lines and peripheral sympathetic neurons,[46-52] and even cultures of nondopaminergic neurons.[53,54] There is substantial evidence showing that levodopa toxicity *in vitro* is mediated by oxidative stress.[39-42,44-50] Finally, most studies agreed in that catecholaminergic neurons are much more vulnerable than noncatecholaminergic ones to levodopa/dopamine toxicity.[37-45]

The fact that levodopa can destroy mesencephalic dopaminergic neurons *in vitro* cannot be disputed, but a close examination of the conditions in which levodopa toxicity was verified is required before assuming that the agent could have a toxic effect *in vivo*. First, most *in vitro* studies found that levodopa is toxic for cultured mesencephalic dopaminergic neurons in concentrations above 50 μmol/L,[37,39-45] while peak plasma concentrations of levodopa in patients with Parkinson's disease were found to be around 10 to 20 μmol/L,[55-57] and less than 2 μmol/L in the cerebrospinal fluid.[56,57] Two reports, however, found slight detrimental effects of levodopa on cultured mesencephalic dopaminergic neurons at therapeutically relevant concentrations.[38,44] Secondly, it has been reported that antioxidants such as ascorbic acid (vitamin C) are almost undetectable in primary mesencephalic

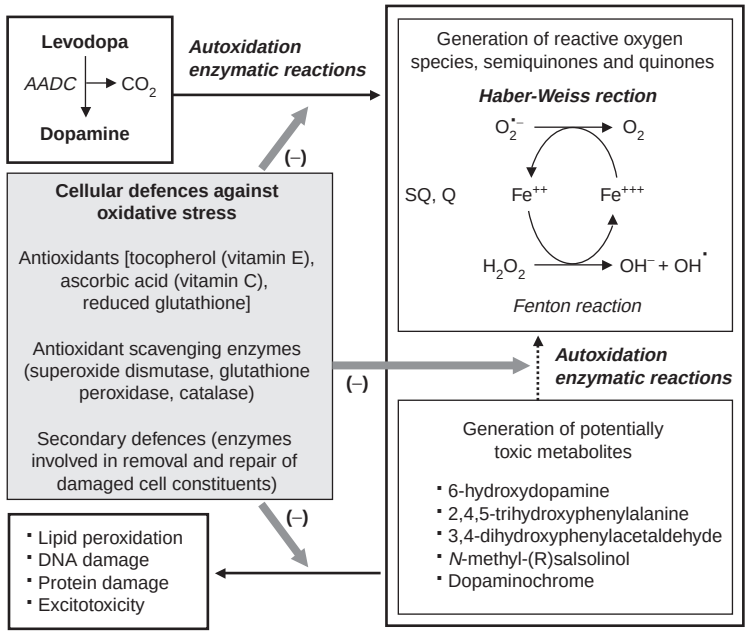

Fig. 1. Schematic representation of the major reactions that could lead to the formation of toxic compounds from oxidation of levodopa and dopamine. Autoxidation of levodopa and dopamine can lead to the generation of highly reactive chemical species such as semiquinones (SQ) and quinones (Q), superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2).[21,22] Through autoxidation and different oxidative enzymatic reactions several other molecules with potential intrinsic toxic activity can be generated. Among them are 6-hydroxydopamine,[21,22] 3,4-dihydroxyphenylacetaldehyde,[23] 2,4,5-trihydroxy-phenylalanine,[24] *N*-methyl-(R)salsolinol,[25] and dopaminochrome.[22] These molecules can in turn be also oxidised, leading to the generation of other SQ and Q, and oxygen-reactive species. The most important enzymes proposed to be involved in these reactions are monoamine oxidase,[26] tyrosine hydroxylase,[27,28] and prostaglandin H synthase.[29,30] The various reactive agents produced can damage DNA[31] and proteins,[32] induce lipid peroxidation,[33,34] or destroy cells by excitotoxicity.[24,35] In physiological conditions the cellular defences against oxidative damage[36] have enough strength to maintain the integrity of the mesotelencephalic dopaminergic system. **AADC** = aromatic L-amino acid decarboxylase.

cultures.[58] Ascorbic acid at concentrations of 200 μmol/L provides almost total protection from levodopa toxicity (200 μmol/L) *in vitro*.[39-42,49] The concentration of ascorbic acid in the human cerebrospinal fluid is approximately 130 μmol/L[59] and it is even higher in the brain extracellular fluid.[60] Besides, the brain content of ascorbic acid seems not to be reduced in Parkinson's disease.[61] Thirdly, most *in vitro* studies claiming to show toxic effects of levodopa used systems lacking glial cells. Mena et al. reported recently that the presence of glial cells[43] or of soluble factors produced by glial cells[45,62] protected cultured mesencephalic dopaminergic neurons from levodopa toxicity. That 'environmental' circumstances could be crucial for the levodopa-induced toxic actions to happen is further supported by studies of the effects of long term levodopa regimes on embryonic dopaminergic neurons transplanted within the striatum of rats with nigrostriatal lesions. One of these studies[38] found that levodopa impaired the morphological development of grafted dopaminergic neurons but not their survival. Two other more recent studies failed to find any detrimental effect of long term levodopa administration.[63,64] Finally, it should not be forgotten that most *in vitro* systems consist of cultured embryonic cells, which could be particularly susceptible to the toxic effects of drugs and physiological signals regulating survival. In striking contrast with other reports,

Mena et al.[62] found that dopaminergic neurons obtained from postnatal rat mesencephalon are not only resistant to levodopa toxicity, but also that levodopa had a trophic effect on them, promoting survival and neurite outgrowth.

In summary, it has been clearly demonstrated that a high concentration of levodopa is toxic for embryonic mesencephalic dopaminergic neurons cultured in particularly disadvantageous conditions (e.g. absence of glia and very low concentrations of endogenous antioxidants). Levodopa toxicity could be, however, completely prevented by soluble factors generated by glial cells or by the addition of antioxidants at physiological concentrations. Indeed, in most favourable conditions (e.g. postnatal cultured neurons, presence of glia and physiological levels of antioxidants) levodopa might have a trophic effect on dopaminergic neurons.

2. Levodopa is Not Toxic for the Healthy Mesostriatal Dopaminergic System

Shortly after levodopa administration became the standard treatment for Parkinson's disease, Cotzias et al.[65] reported that the life-span of healthy mice adapted to a diet containing large amounts of levodopa is extended. This work was followed by histopathological studies showing that long term regimens containing levodopa associated with decarboxylase inhibitors did not reduce the number of dopaminergic neurons in the substantia nigra and ventral tegmental area (ventral tegmental area) of healthy rats and mice.[66-68] Anecdotal evidence is also available on the effects of long term regimens of levodopa given to patients mistakenly diagnosed as having Parkinson's disease. The mesencephalon of 2 of these patients could be analysed, and did not show any loss of dopaminergic neurons.[69,70] Thus, there is no evidence suggesting that long term systemic administration of levodopa, even at very high doses, is toxic for 'healthy' dopaminergic neurons.

A striking recent finding was that a single intrastriatal injection of a high dose of dopamine can produce selective damage of tyrosine hydroxylase immunoreactive fibres in healthy rats.[71,72] This toxic effect seems to be accompanied by the formation of reactive oxygen species and metabolites derived from dopamine oxidation.[72] Another study demonstrated dose-dependent mortality after intraventricular administration of high doses of dopamine to rats, but failed to find any evidence of oxidative stress.[73] On the other hand, a study that examined the effects of long term systemic levodopa administration on dopaminergic fibres of healthy rats failed to reveal any reduction of the expression of dopaminergic markers in the striatum and nucleus accumbens.[74] The toxic effects observed after intracranial injections could be the result of an extremely high brain extracellular concentration of dopamine which is unlikely to be attained during oral levodopa treatment.

Spencer-Smith et al.[75] reported an increased production of hydroxyl radicals in the substantia nigra of healthy rats after an acute intraperitoneal injection of levodopa. Furthermore, Przedborski et al.[76] found that long term systemic levodopa administration can inhibit respiratory chain activity in healthy rats by a mechanism that involves oxidative stress. The reported effect was more intense at the striatum than the nigra, and was reversible. Since levodopa is not toxic for nigral neurons of healthy rats, these studies provided evidence supporting the idea that the defences against oxidative stress can manage the increased free radical production induced by levodopa, at least under physiological conditions. In Parkinson's disease the substantia nigra shows respiratory chain dysfunction,[77,78] and a severe depletion of glutathione.[79-81] The authors[75,76] suggested that if these alterations were related to the aetiology of the disease (that is, are not secondary to levodopa therapy), the addition of levodopa could further increase oxidative stress, impair mitochondrial function, and precipitate neuronal death.

3. Studies in Animal Models of Parkinson's Disease

As summarised in section 2, it has not been possible to demonstrate a toxic effect of long term

levodopa therapy on the dopaminergic neurons of healthy rats and mice, nor in humans who did not have Parkinson's disease. It has been argued that an intact set of dopaminergic neurons may be able to rapidly reduce extracellular levodopa/dopamine concentrations after systemic levodopa administration,[82] thus protecting healthy animals and humans from their toxic effects. In patients with Parkinson's disease not only are dopaminergic neurons reduced in number, but they are also under the influence of a still unknown insult, and could then be more vulnerable to levodopa toxicity.

Attempts to evaluate levodopa toxicity in animal models of parkinsonism followed two different approaches (table I). In the first approach, levodopa was administered simultaneously with a toxin known for its ability to selectively destroy dopaminergic neurons. Two reports claimed that levodopa reduced the toxic effects of methylphenyltetrahydropiridine (MPTP) and 6-hydroxydopamine (6-OHDA) in rodents[83,84] while another found the opposite, that is an enhancement of 6-OHDA toxic effects by levodopa (table I).[85] The reasons for the disagreement between studies are not clear since they did not differ greatly in methodology. The authors of the first 2 studies attributed the protective effect of levodopa to its ability to reduce the uptake of toxins into dopaminergic neurons. This experimental approach has the advantage of evaluating the effects of levodopa on neurons involved in a process of cell death, but some major differences with the condition of patients under treatment must be pointed out: (i) in these experiments dopaminergic neuronal death was induced in a step-like fashion with high doses of toxins; it seems unlikely that dopaminergic neurons in the human parkinsonian brain are ever exposed to such an extreme toxic insult; and (ii) levodopa administration was performed for just a few days around the time of the lesion, giving dopaminergic neurons insufficient time to develop compensatory adaptations against oxidative stress. This kind of adaptation probably occurs in the parkinsonian brain after long term exposure to levodopa.[92]

Other researchers chose to first generate the animal model with a selective neurotoxin and begin levodopa administration once the effect of the toxin ended. These experiments required long term levodopa administration. Dopaminergic neurons surviving the insult would be exposed to higher extracellular concentrations of levodopa/dopamine[82] and could also be more vulnerable as a consequence of the compensatory hyperactivity in which they were engaged.[93,94] In one of these studies, Fukuda et al.[86] induced the degeneration of mesencephalic dopaminergic neurons by systemic administration of MPTP and subsequently treated mice for 70 days with levodopa or vehicle. The number of tyrosine hydroxylase immunoreactive neurons in the substantia nigra and ventral tegmental area was not different between animals receiving levodopa or vehicle. The authors discussed their findings as suggestive of levodopa toxicity, however, because they found a reduced number of haematoxylin-stained cells in the ventral mesencephalon of levodopa-treated mice. It is surprising that dopaminergic neurons were normal in number while nondopaminergic cells were reduced, since the latter are supposed to be much less vulnerable to levodopa toxicity (see section 1).

Ogawa et al.[87] claimed that long term systemic levodopa increased lipid peroxidation in 6-OHDA-lesioned mice, but the study lacked satisfactory controls, and lipid peroxidation is not an index of irreversible neuronal damage. The most frequently cited work on *in vivo* levodopa toxicity was carried out by Blunt et al.[88] The authors induced a severe loss of dopaminergic neurons with 6-OHDA and then treated the rats with levodopa plus carbidopa or vehicle during 6 months. Long term levodopa had no effect on the scarce remaining tyrosine hydroxylase immunoreactive neurons in the substantia nigra, but reduced their number in the ventral tegmental area. The authors interpreted these findings cautiously, suggesting that either a toxic effect of levodopa leading to dopaminergic cell loss or a reduced expression of tyrosine hydroxylase in otherwise intact mesostriatal neurons could account for their results.

Table I. Summary of the experimental work evaluating the putative toxic effect of levodopa on dopaminergic neurons in animal models of Parkinson's disease

Reference	Experimental design	Method of evaluation of dopaminergic neuronal survival	Main results
Melamed et al.[83]	Mice injected with MPTP and given levodopa (50 mg/kg IP) plus carbidopa (100 mg/kg IP) or vehicle plus carbidopa for 6 days, beginning 2 days before MPTP	Mice killed 30 days after MPTP. Determination of the dopamine content in striatal homogenates	MPTP-induced striatal dopamine depletion was markedly attenuated by levodopa
Ogawa et al.[84]	Mice treated with levodopa plus carbidopa (75 mg/kg and 7.5 mg/kg IP) or vehicle plus carbidopa for 7 days, and injected ICV with 6-OHDA 1h after the last injection of levodopa	Mice killed 7 days after 6-OHDA. Determination of the dopamine content in striatal homogenates	6-OHDA-induced striatal dopamine depletion was markedly attenuated by levodopa
Naudon et al.[85]	Mice treated once with levodopa plus benserazide (200 mg/kg and 25 mg/kg IP) or vehicle plus benserazide, and injected ICV with 6-OHDA 1h later	Mice killed 14 days after 6-OHDA. Determination of the dopamine content in striatal homogenates	6-OHDA-induced striatal dopamine depletion was enhanced by levodopa
Fukuda et al.[86]	Mice lesioned with MPTP. Levodopa plus carbidopa (60 mg/kg and 6 mg/kg IP) or vehicle started 2 days after the last injection of MPTP and maintained for 70 days	Mice perfused 2 days after the last injection of levodopa. Cell counts of TH immunolabelled and haematoxylin stained cells in the SN and VTA	No effect of levodopa on TH immunolabelled neurons, but reduced number of nondopaminergic cells in levodopa-treated mice
Ogawa et al.[87]	Mice lesioned with 6-OHDA. Levodopa (200 mg/kg IP without decarboxilase inhibitors) started 4 wks after the lesion and maintained for 4 wks. Lack of satisfactory controls	Determination of thiobarbituric acid reacting substances as an index of lipid peroxidation	Mice with 6-OHDA lesions treated with levodopa showed higher levels of thiobarbituric acid reacting substances than an independent group of mice with 6-OHDA lesions but killed 4 wks before and which did not receive vehicle
Blunt et al.[88]	Severe lesion induced by injection of 6-OHDA in the MFB of rats. Levodopa plus carbidopa (200 mg/kg and 25 mg/kg PO) or vehicle started 45 days after the lesion and given for 6 months	Cell counts of TH immunolabelled cells in the SN and VTA	Few dopaminergic cells survived to 6-OHDA in the SN. No effect of levodopa in the SN, but reduced number of dopaminergic neurons in the VTA of levodopa-treated rats
Blunt et al.[63]	Severe lesion induced by injection of 6-OHDA in the MFB of rats. Fetal mesencephalic grafts or sham grafts performed 7 wks after the lesion. Levodopa plus carbidopa (200 mg/kg and 25 mg/kg PO) or vehicle started 24h after the graft and given for 5 wks	Cell counts of TH immunolabelled cells in the SN and VTA, and of grafted cells in the striatum. Autoradiographic detection of ^{3}H-mazindol binding sites in the striatum	Levodopa had no detrimental effect on survival of remaining mesencephalic TH immunoreactive neurons, nor on grafted embryonic mesencephalic neurons. Levodopa increased ^{3}H-mazindol binding in both the intact and lesioned striatum
Murer et al.,[74] Dziewczapolski et al.[89]	Moderate or severe lesions obtained with injections of different doses of 6-OHDA in the MFB of rats. Levodopa plus carbidopa (170 mg/kg and 17 mg/kg PO) or vehicle started 3 months after the lesion and given for 6 months. The experiment was replicated	Rats killed 7 days after last administration of levodopa. Cell counts of TH immunolabelled cells in the SN and VTA. Evaluation of several striatal dopaminergic markers (TH, membrane dopamine transporter, VMAT2) by immunoautoradiography	Cell counts in the SN and VTA were similar in rats treated with levodopa or vehicle. No toxic effect of levodopa on striatal markers in severely-lesioned rats. Increased expression of TH, membrane dopamine transporter and VMAT2 in the striatum of moderately lesioned rats treated with levodopa

Table I. Contd

Reference	Experimental design	Method of evaluation of dopaminergic neuronal survival	Main results
Rioux et al.[90]	Severe lesions induced in monkeys by MPTP administration during 2 wks. Levodopa plus carbidopa (dose titrated to each monkey) or vehicle started 4 days after the last injection of MPTP, and continued for 9 wks	Monkeys killed 40h after the last dose of levodopa. Autoradiographic detection of ^{3}H-mazindol binding sites in the caudate and putamen. Determination of the dopamine content in striatal homogenates	No significant difference in striatal dopamine content between animals treated with levodopa or vehicle. Significant increase of ^{3}H-mazindol binding in the dorsal caudate and putamen of levodopa treated monkeys relative to control animals
Yahr et al.[91]	A unique report comparing patients with Parkinson's disease who did or did not receive levodopa	Qualitative observation of pigmented neurons in mesencephalic tissue sections	No histopathological difference between treated and untreated patients

ICV = intracerebroventricular; **IP** = intraperitoneal; **MFB** = medial forebrain bundle; **MPTP** = methylphenyltetrahydropiridine; **6-OHDA** = 6-hydroxydopamine; **PO** = orally; **SN** = substantia nigra; **TH** = tyrosine hydroxylase; **VMAT2** = vesicular monoamine transporter type 2; **VTA** = ventral tegmental area.

The same group performed another study on the effect of levodopa plus carbidopa on survival of grafted dopaminergic neurons to the striatum of 6-OHDA-lesioned rats.[63] Since 'sham grafted' animals were included in the experiment, the effect of levodopa on host dopaminergic neurons and fibres could also be analysed by the authors. No detrimental effect was found on host dopaminergic cells bodies and fibres, nor on grafted neurons. Indeed, the authors reported an increased density of ^{3}H-mazindol striatal binding sites in animals treated with levodopa (mazindol is a marker for the dopamine transporter). More recent studies from our laboratories further analysed the effect of a 6-month oral levodopa treatment in rats with 6-OHDA lesions.[74,89] Two groups of animals were used, with moderate (60 to 70% nigral cell loss) or severe (>90% nigral cell loss) lesions, in an attempt to model 2 different stages in the evolution of Parkinson's disease. Several dopaminergic markers were evaluated at nigral and striatal levels, including tyrosine hydroxylase, the dopamine transporter and the vesicular monoamine transporter. The expression of the latter 2 markers is less likely than that of tyrosine hydroxylase to be modified by pharmacological treatments,[95] and could consequently be considered better indexes of the integrity of dopaminergic neurons. Cell counts and surface area measurements at the nigral level revealed no detrimental effect of long term levodopa administration. Furthermore, no evidence of enhanced damage of striatal dopaminergic fibres was observed in severely lesioned rats treated with levodopa. Strikingly, moderately lesioned rats receiving levodopa showed a partial recovery of all 3 dopaminergic markers at the striatal level and a more dense tyrosine hydroxylase immunoreactive striatal fibre network than vehicle-treated control rats. A similar finding was recently reported by Rioux et al.,[90] who found an enhanced striatal binding of ^{3}H-mazindol in MPTP lesioned monkeys treated with levodopa compared with those animals receiving vehicle.

In summary, no conclusive evidence for levodopa toxicity has yet been provided by studies performed in animal models of Parkinson's disease. The lack of levodopa toxicity is supported by anecdotal human studies. Burns et al.[96] reported a lack of progression of human MPTP parkinsonism during long term treatment with levodopa for 7 years. Finally, the unique histopathological observations of mesencephalic tissue sections from patients with Parkinson's disease who either had or had not been treated with levodopa, led Yahr et al.[91] to conclude that 'histopathologically, treated and untreated patients appeared quite similar'.

4. Clues from Epidemiological and Clinical Studies

A relevant piece of information regarding drug toxicity is the mortality of the population under treatment. Several epidemiological studies claimed that long term levodopa therapy increases life expectancy in patients with Parkinson's disease.[97-103] In fact, some reports suggested that the beneficial effect on life expectancy is higher when levodopa administration is initiated earlier.[104-106] However, a recent critical study reviewing published data on mortality from parkinsonian patients[107] claimed that an increase in patient's life expectancy occurred during the first decade following the generalisation of levodopa utilisation, but that the effect of the drug on survival has been diminishing in magnitude in subsequent decades. The author hypothesised that the greater effect on life expectancy observed early after the popularisation of levodopa utilisation reflected the delayed decease of a selected group of frail parkinsonian patients who died 5 to 10 years later. This interpretation implies that levodopa improves survival only when it is introduced late in the course of the disease, by delaying death of severely incapacitated patients. The findings of Uitti et al.,[103] who found that risk of death following initiation of levodopa was significantly reduced regardless of the time elapsed from disease onset, do not support that conclusion. Furthermore, Rajput et al.[106] reported recently that survival in Parkinson's disease is reduced if introduction of levodopa is delayed until postural instability develops. Most recent studies accept, however, that life expectancy of patients with Parkinson's disease is still inferior to that of the general population.[103,107-111] A recent report of the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP) study[112] showed, however, that life expectancy of groups of carefully selected patients receiving long term levodopa treatment [with or without selegiline (deprenyl)] were not different from those of the general population.

Reduced mortality associated to levodopa therapy is commonly attributed to the reduced disability of patients receiving treatment, and not to a levodopa-dependent reduced rate of disease progression (neuroprotective effect). In fact, it has been pointed out that levodopa could increase life expectancy because of symptomatic effects mediated by postsynaptic actions but could simultaneously enhance the loss of dopaminergic neurons.[93,94] If this were the case, one could expect a relationship between the duration of treatment (and therefore the cumulative levodopa dose) and the degree of disability and emergence of irreversible adverse effects. Early epidemiological studies suggested that patients treated longer with levodopa showed a higher degree of disability,[10] and that the prevalence of dyskinesias and fluctuations in disability increases with duration of levodopa treatment.[97,113-115] More recent works suggest, however, that neither the progression of the disease, nor the occurrence of adverse effects, were dependent on the time of starting levodopa therapy, but on the natural rate of disease progression and severity of the disease at treatment onset.[12,116-120] Together these reports support the idea that dyskinesia and response fluctuations appear as a consequence of disease progression and/or pharmacokinetic and pharmacodynamic changes associated with long term exposure to levodopa, and not because of a toxic action of the treatment on the remaining dopaminergic neurons. This conclusion is supported by other facts: (i) motor fluctuations appear shortly after the beginning of levodopa therapy in human and monkey MPTP severe parkinsonism;[121-123] (ii) withdrawal of levodopa (drug holiday) reinstated responsiveness to treatment and reduced motor complications for periods of up to 2 years, supporting the idea that these changes are not irreversible;[124,125] (iii) motor complications are not prevented by administrating levodopa with drugs supposed to prevent oxidative stress, such as tocopherol (vitamin E) and selegiline;[120] and (iv) a whole range of motor complications are now recognised to occur after long term monotherapy of 'nonprimed' patients and MPTP-lesioned monkeys with dopamine receptor agonists.[126-132]

Dementia has not been reported to occur in Parkinson's disease before the utilisation of levodopa, and it has also been suggested to result from levodopa toxicity.[93,94] This fact seems unlikely, however, since: (i) nondopaminergic cells are much less vulnerable than dopaminergic ones to levodopa toxicity (see section 1); and (ii) the risk of developing dementia is not related to the duration of therapy.[15,117] Different aetiologies could underlie dementia in Parkinson's disease, including Alzheimer's disease, Lewy body dementia, and multi-infarct dementia.[133] Failure to detect dementia in the prelevodopa era could be explained by the reduced life expectancy of patients (dementia is more common in aged parkinsonian patients) and the confounding effect of their motor disability. The existence of an elevated incidence of dementia in the present population of parkinsonian patients can, conversely, be related to the failure of levodopa therapy to reduce mortality to rates similar to those of the general population. Mortality is higher in parkinsonian patients with dementia than in those without cognitive impairment.[108,109] Thus, levodopa could be promoting survival of patients until the emergence of incapacitating signs that do not respond to dopamine replacement therapy.[134]

There are other ways, apart from analysing mortality and adverse effects, to study a putative toxic effect of levodopa on parkinsonian patients. A study comparing the clinical scores in a drug-free state between patients treated long term with levodopa or placebo could be expected to provide relevant information on the effect of levodopa on Parkinson's disease progression. Gwinn-Hardy et al.[135] performed a retrospective analysis of a family affected by an autosomal dominant form of parkinsonism, including individuals who were treated long term with levodopa and others who were not, and found that levodopa slowed the progression of parkinsonism, A large scale, prospective study of this kind in sporadic Parkinson's disease, had not been undertaken until recently, and the results of such a study, the 'Earlier vs Later L-DOPA (ELLDOPA)' trial, are not yet available.[136]

5. Has Levodopa Beneficial Effects Beyond its Symptomatic Action?

Recent studies suggested that under certain conditions levodopa might have beneficial rather than detrimental effects on the mesostriatal dopaminergic system (table II). Levodopa could protect dopaminergic neurons from toxins by reducing their transmembrane transport.[83,84] Furthermore, levodopa can have antioxidant properties *in vitro*, depending on its concentration, the presence of other antioxidants and transition metals.[33,34] It was shown that exposure of mesencephalic cultures to levodopa increases culture glutathione content and makes dopaminergic neurons more resistant to oxidative stress.[39,42] Glutathione was seen to be severely depleted from the parkinsonian substantia nigra.[79-81] If levodopa enhances glutathione levels *in vivo* after oral administration,[137] the true glutathione content of the parkinsonian substantia nigra could be even lower than was previously reported. Mena and co-workers[62] found that levodopa promoted survival and neurite outgrowth of mesencephalic dopaminergic neurons obtained from neonates and co-cultured with cortical astrocytes. An analogous observation was done some years before by De Vitry et al.,[138] who found that repeated exposure to low concentrations of dopamine increases the number tyrosine hydroxylase immunoreactive neurons in embryonic brain stem cultures. These observations are consistent with reports demonstrating an antiapoptotic effect of dopamine on developing retinal tissue,[139] and that exposure to D2-class dopamine receptor agonists increases neurite complexity in cultured mesencephalic dopaminergic neu-

Table II. Putative beneficial actions of levodopa beyond its symptomatic effect mediated by activation of striatal dopamine receptors

- Intrinsic antioxidant properties
- Induction of defenses against oxidative stress
- Competition for the dopamine transporter leading to reduced uptake of toxins
- Induction of enzymes involved in catecholamine synthesis
- Promotion of survival of dopaminergic neurons
- Promotion of neurite growth and target reinnervation

rons.[140] Finally, our laboratories reported recently[74] that a 6-month oral treatment with levodopa increased the expression of several markers of dopaminergic nerve terminals at the striatal level in rats with moderate nigrostriatal lesions. The dopaminergic neuropile was more dense in the striatum of animals receiving levodopa than in those treated with vehicle. Despite morphological and neurochemical partial recovery, no improvement in behavioural indices of parkinsonism were found. Increased expression of striatal dopaminergic markers in parkinsonian animals under long term levodopa therapy has also been reported by others (table I).[63,90]

After a decade of experiments designed to demonstrate that levodopa could be toxic for dopaminergic neurons a series of reports appeared suggesting that it might have beneficial effects beyond those mediated by activation of striatal dopamine receptors. Further work is necessary to determine if these previously unrecognised effects of levodopa contribute in any way to its therapeutic action in Parkinson's disease.

6. Conclusion

The following can be concluded from the above discussion.

- Levodopa can damage embryonic mesencephalic dopaminergic neurons cultured in the absence of glia and antioxidants. In contrast, when glia and antioxidants are present, levodopa has been shown to promote neuronal survival and neurite outgrowth.
- Long term administration of levodopa is not toxic for the dopaminergic system of healthy animals and humans, and increases the life expectancy of mice.
- Despite some conflictive reports, the bulk of the evidence indicates that long term administration of levodopa is not toxic for the remaining dopaminergic system in animal models of Parkinson's disease. In certain conditions levodopa can promote the recovery of dopaminergic markers in the striatum of animals with lesions of the mesostriatal dopaminergic system.
- Epidemiological studies support the premise that levodopa increases life expectancy of patients with Parkinson's disease. It is our opinion that no epidemiological or clinical evidence has yet been provided supporting the premise that levodopa can accelerate the loss of remaining dopaminergic neurons in Parkinson's disease. The ELLDOPA study will surely provide valuable information about the effect of levodopa on Parkinson's disease progression.[136] Pharmacokinetic and pharmacodynamic modifications resulting in adverse effects must be expected following long term exposure to levodopa, but they must not be taken as evidence of irreversible neuronal damage.

Taking into account the available evidence, we think that the introduction of levodopa therapy in patients with Parkinson's disease should not be delayed because of the fear that it might be toxic for remaining dopaminergic neurons. Of course, other factors aside from toxicity must influence the decision to initiate treatment either with levodopa, other drugs, or a combination of drugs.[136] Among these factors we can mention the patient's age,[141,142] the incidence of motor adverse effects for the different drugs,[143-145] and their ability to reduce disability and increase survival.[111,112,145] The fact that levodopa increases patients' life expectancy and can produce long lasting reductions in patients' disability scores, underlies the need for further study of its biological effects.

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