

Levodopa

Anthony J. Santiago and Stewart A. Factor

Albany Medical Center, Albany, New York, U.S.A.

INTRODUCTION AND HISTORY

Parkinson's disease (PD), like other neurodegenerative disorders, is clinically heterogeneous (1). Age of onset, the relative prominence of certain signs and symptoms, course and rate of progression, and the responsiveness to therapy are variable but still assist in differentiating it from atypical forms of parkinsonism (2). Mainly described by its cardinal motor manifestations (bradykinesia/akinesia, rigidity, resting tremor, and postural instability), progression is inevitable, as there is a continuous loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNpc) (3).

Before 1918, treatment was primarily supportive (4). However, the encephalitis epidemic of 1917–1926 and the emergence of the postencephalitic form of parkinsonism led to a more aggressive pursuit of effective therapies. The pursuit initially focused on the development of an effective vaccine, and then necessarily toward symptomatic therapy (5,6). As we cannot, at this time, halt the progression of PD, symptomatic relief remains

the only available proven approach to care. While this is at times inadequate, the available symptomatic therapies for PD are far more effective than those available for any other neurodegenerative disease (3).

A number of natural remedies have been tried to treat the symptoms of PD over the last century and a half. Charcot, in the latter half of the nineteenth century, described the use of potato plant extracts, such as Bulgarian belladonna and atropine, to treat PD. These were initially received with great promise, but fell short of expectations. In the decade following the emergence of postencephalitic parkinsonism, many studies were published comparing the various plant extracts, evaluating the effectiveness of certain agents for specific symptoms; for example, stramonium was felt to be effective for rigidity and hyoscine for tremor (4).

By the early 1950s, synthetic drugs became available to treat the symptoms of PD. Trihexyphenidyl hydrochloride, a synthetic anticholinergic, was highly touted for its effectiveness for relieving rigidity, tremor, akinesia, and oculogyric crisis. It was heralded as more effective than the plant extracts and better tolerated than other early synthetic preparations (4,7,8). While still used today, its limitations in treating all the symptoms of PD were recognized even then.

Levodopa (LD) has become the cornerstone of symptomatic therapy. It is a metabolic precursor of the neurotransmitter dopamine (see below). D/L Dopa was first synthesized in 1911 (9). Guggenheim, in 1913, isolated LD from the broad bean plant (10). Its use in PD only emerged after the important works of various researchers in the late 1950s and early 1960s that demonstrated that dopamine depletion was characteristic of PD. Carrlson in 1957 and 1958 (11,12) demonstrated in animal models that the akinetic effects of reserpine (an agent known to deplete dopamine) could be reversed by LD. In addition, Carrlson reported that the striatum was a site of dopamine concentration (11,12). Hornykiewicz in 1960 showed that the striatum of parkinsonian brains were depleted of dopamine and 2 years later that intravenous doses of LD (50 mg) had anti-parkinsonian effects (13). However, studies in the early and mid-1960s showed variable results, and, in fact, treatment with LD was almost abandoned. It was the seminal work of Cotzias, who examined the role of high-dose oral LD in modifying parkinsonism, that dramatically changed the landscape of PD treatment (14,15). LD was ultimately approved by the U.S. Food and Drug Administration (FDA) for use in PD in 1970, 60 years after its discovery and more than 10 years after the realization that dopamine depletion was the key abnormality in PD (16). In 1973, the combined use of a peripheral aromatic amino acid decarboxylase inhibitor (AADI) with LD was reported. Its use resulted in a decrease in peripheral metabolism of LD to dopamine and fewer peripheral side effects such as hypotension and nausea

(17). Controlled-release formulations were tested in the 1980s to treat fluctuations (see below), and one was approved in the United States in 1991 (3).

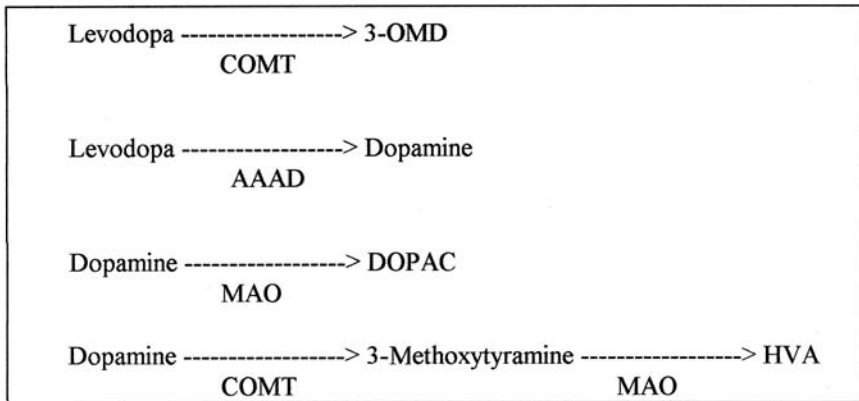
Still regarded as the most potent symptomatic therapy for PD, LD has its drawbacks. Late complications such as motor fluctuations and dyskinesias are associated with chronic administration. Neuropsychiatric disturbances can be frequent and serious adverse effects. Questions have arisen regarding its potential toxicity to nigrostriatal neurons as well as a possible association with melanoma. The question of when to initiate therapy with LD is still ongoing. This chapter will review the pharmacology of LD, its role in the emergence and progression of motor complications, its possible toxicity, whether tolerance develops, if it can be of assistance in diagnosing PD, and its effect on mortality of PD.

PHARMACOLOGY

Dopamine depletion, particularly in the striatum, is the neurochemical hallmark responsible for the motor features of PD. However, dopamine cannot be utilized as a treatment because it does not cross the blood-brain barrier (BBB), and its use is associated with several side effects. On the other hand, LD, an aromatic amino acid and precursor to dopamine, readily crosses the BBB. When it is administered orally, it is converted to dopamine in the extracerebral tissues via decarboxylation. To lessen the peripheral effects of dopamine and increase the brain bioavailability of LD, it is often co-administered with AADIs like carbidopa or benserazide. AADIs do not cross the BBB and therefore will not affect conversion to dopamine in the brain. Their use reduces the amount of LD required to attain an adequate response by approximately 75% and increases its plasma half-life from 50 to 90 minutes.

Two major enzymatic pathways for LD exist leading to the formation of 3-O-methyldopa (3-OMD), both peripherally and centrally (Fig. 1). Dopamine is subsequently converted to 3,4-dioxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the central nervous system (CNS).

Transport of LD across the gut mucosa and BBB involves an energy-dependent carrier-mediated system. Large neutral amino acids (LNAA) compete for transport at the same sites. When oral LD was administered with a high-protein meal, there was an overall reduction in its plasma level. When IV LD was administered with a high-protein meal, the anticipated clinical response was diminished, indicating an effect at the BBB as well (18). Upon entering the CNS, LD is converted to dopamine in dopaminergic neurons and probably other cells containing dopa decarboxylase.



COMT = Catechol-O-methyltransferase
 MAO = Monoamine oxidase

FIGURE 1 Levodopa metabolism. 3-OMD = 3-O-methyldopa, AAAD = aromatic amino acid decarboxylase, DOPAC = 3,4-dioxyphenylacetic acid, HVA = homovanillic acid.

MOTOR FLUCTUATIONS AND DYSKINESIAS: DEFINITIONS

It is established that a loss of 50–60% of nigrostriatal neurons or a reduction in striatal dopamine concentrations of approximately 80% is required to cause clinical symptoms (19). The surviving neurons can initially compensate but subsequently, with continued disease progression, fail. The loss of the ability to store and release dopamine appropriately results in less reliable responses to LD (20). Glial cells can also convert LD to dopamine, but they lack the machinery for appropriate regulation (21). In PD, the loss of nigrostriatal innervation is associated with putaminal D2 receptor upregulation with a subsequent decline, possibly below baseline, which may be related to both disease and treatment (22).

These presynaptic and postsynaptic changes are important not only for responsiveness to LD but also the occurrence of motor fluctuations (wearing-off, dyskinesias, unpredictable responses). Historical literature suggests that the rate is approximately 50% for motor fluctuations and dyskinesias after 5 years of disease duration and as high as 90% for patients with onset of PD under age 40 (23). Ahlskog and Muenter compared more recent literature to older studies and found that the rate is probably 35–40% after 4–6 years of disease duration (24). These figures vary depending on the study, and these variances may relate to definitions and measuring tools.

The response to LD treatment is complex, and understanding it requires many considerations. Muenter and Tyce defined the long-duration

response (LDR) as the gradual motor improvement seen after repeated dosing and subsequent decline over days upon LD withdrawal. This effect remains present even after long-term chronic therapy (20). The short-duration response (SDR) is defined as that which parallels the plasma concentrations of LD. It seems to be present to some extent from the beginning of therapy (22). Nutt and colleagues showed that after a 3-day withdrawal of LD, a patient receiving a single dose would have a full SDR only, without a LDR. It may be that the LDR leads to the remarkable early responsiveness to LD and its loss results in the subsequent dependency on the SDR for symptomatic relief (25,26). A negative or inhibitory response has also been described; it is a worsening of motor function occurring prior to the SDR. It can last minutes to hours and has been termed a “super-off” (27). These three responses are imposed on a diurnal pattern of motor function (better performance in the morning with subsequent decline throughout the day) and on top of the continued endogenous dopamine activity (28,29). Nutt and colleagues propose that the residual endogenous dopamine activity as well as the LDR essentially determine the off-time (25).

Several patterns of motor fluctuations have been described. They progress from simple predictable patterns early on to more complicated unpredictable ones and, as expected, become more difficult to treat. The earliest type is the end of dose wearing-off effect. With this pattern, the antiparkinsonian effect of LD wears off toward the end of dose in a predictable fashion. This has also been referred to as end-of-dose failure. This is followed by complicated wearing-off where the duration of response of LD becomes more variable so that the timing of wearing off becomes less predictable. At this point, patients begin to experience delayed-on (a delay in onset of effect of LD) and dose failures (otherwise known as no-on). The random on-off effect is when LD response varies in an unpredictable manner unrelated to timing of the dose. This often happens suddenly like a light switch being turned on and off. Dyskinesias can also occur in various patterns. Most common are peak dose dyskinesias. With this, choreic movements occur when plasma LD levels are at their peak. Usually the patient has an on time with no dyskinesias, but as they reach peak effect they develop the involuntary movements. Diphasic dyskinesias are when choreic or dystonic movements occur at the beginning and end of dose. The legs seem to be more involved. Some patients have dyskinesias for the entire time they are on (square wave dyskinesias). Dyskinesias may occur in the off state as well, and this is usually in the guise of dystonia. The patterns include early morning dystonia and off period dystonia. Finally, patients may fluctuate abruptly from severe immobility to severe dyskinesia known as yo-yoing.

CLINICAL TRIALS OF LEVODOPA

Early Trials

The initial studies of LD as first line therapy for PD were carried out in the late 1960s to early 1970s. These studies were quite different from modern ones in that the patients had varying durations of disease, some quite advanced, and standard measures such as the Unified Parkinson's Disease Rating Scale (UPDRS) were not yet devised. The results, however, were dramatic. The breakthrough report was that by Cotzias et al. (14). After conflicting findings and consideration of abandoning dopa therapy, Cotzias was able to demonstrate the definitive effectiveness of LD (as opposed to the D/L-Dopa). These investigators examined 28 patients in an open-label manner with intermittent replacement with placebo and utilized LD without a dopa decarboxylase inhibitor. The duration of disease ranged from 1 to 30 years (mean 10 years), and they used a 0–4 scale. All patients responded with 20 of them having a marked to dramatic improvement, and some returned to work. All motor features improved. Some patients developed fluctuations and dyskinesias quickly, and it was suggested that these problems related to duration of disease. Many studies followed that supported these findings (30,31). Sweet and McDowell (32) studied 100 patients treated for up to 5 years in an open-label fashion. Forty-seven of them completed the whole 5 years. The patients improved remarkably by 6 months (60% of patients were more than 50% improved), and despite worsening over the next 4.5 years the Cornell weighted scale scores remained significantly better than that seen at baseline. All the signs of PD improved, and some patients were able to resume working. The severity of the parkinsonian features at initiation of therapy had little bearing on the ultimate response. This remarkable result was observed despite the fact that more than half the patients being treated suffered from concomitant dementia. It became clear that LD was not a cure for PD (as previously hoped) as it did not stop progression of disease and was associated with several late complications. In particular, motor fluctuations and dyskinesias were observed from the beginning with LD therapy. Five-year estimates based on these early studies were approximately 50% for both (31,32). Three issues regarding early LD therapy may have impacted on these figures. First, patients with advanced disease were included, and they certainly were more susceptible to the onset of late complications. Second, dopa decarboxylase inhibitors were not used in a majority of patients since they were not widely available. It was later shown that these inhibitors decreased the frequency of fluctuations. Finally, patients were treated with the maximum tolerated dose. This use of high doses may have increased the likelihood of dyskinesias and fluctuations. Some studies have indicated that lower doses of LD bring about a similar

response but fewer complications (33), while others were not in agreement (34). Nevertheless, clinicians tend to use the lowest possible effective dose.

Recent Trials

Several studies have been completed in the last decade that provide more information about the effectiveness of LD therapy. They include comparisons of immediate-release and controlled-release formulations and comparisons of LD and dopamine agonists. The populations of patients are more homogeneous than in the early trials as the patients are primarily those with early disease (<5 years). Recent studies have shown more varied frequencies of late complications. The variances probably relate to the manner in which they are defined and detected. The CR First study (35) was a 5-year, randomized, double-blind study comparing controlled release and standard formulation carbidopa/levodopa in 618 LD-naive patients (mean duration of disease of 2.3 years). The primary endpoint was the time until onset of motor fluctuations. The definitions of motor fluctuations included that reported in patient completed diaries or observations of investigators in the clinic recorded on a standard questionnaire. The time until the onset of fluctuations was the earlier of two consecutive diary periods demonstrating their presence with either $\geq 10\%$ of the waking day with dyskinesias or $\geq 20\%$ in the off state. It could also be the time until onset of fluctuations based on the investigator questionnaire. This definition would indicate that they were not just testing for first onset of fluctuations but instead onset of functionally meaningful symptoms. Sixty percent of patients completed 5 years. Mean dose of LD in both groups was low (400–500 mg/day). There were no differences between the two formulations with regard to efficacy or frequency of motor fluctuations. Despite low doses there was a significant improvement of the UPDRS motor score that gradually diminished over time but was still better than the baseline score as seen in the earlier studies. However, only about 20% of patients in each group developed wearing off and dyskinesias, far lower than prior numbers. The CALM-PD study (36,37), a parallel-group, double-blind, randomized trial consisting of both clinical and imaging substudies, compared the rates of dopaminergic motor complications and dopamine neuron degeneration (primary endpoints), respectively, after initial treatment of early PD with pramipexole versus LD. The clinical 2-year data reported that 28% of patients assigned to pramipexole developed motor complications compared with 51% of patients assigned to LD ($p < 0.001$). However, the mean improvement in UPDRS score was significantly greater in the LD group compared with pramipexole (9.2 vs. 4.5; $p < 0.001$). When extended to 4 years, slightly

more than half (52%) of the patients initially assigned to the pramipexole group developed motor complications compared with 74% of the LD-treated patients ($p < 0.0001$). The mean improvement in UPDRS scores from baseline through 48 months was significantly greater in the LD group (3.6) than the pramipexole group (-0.98 ; $p < 0.01$). The imaging portion of the study (38,39) included 82 patients who underwent four sequential [123]I B-CIT single photon emission computed tomography (SPECT) scans over a 46-month period to compare the rate of nigrostriatal dopaminergic degeneration between the treatment groups. It is assumed that a reduction in striatal [123]I B-CIT uptake is a marker of dopamine neuron degeneration. The authors report a 40% relative reduction in the rate of loss of uptake when comparing pramipexole to LD. Whether this suggests a protective effect of the dopamine agonist with respect to LD or that LD may accelerate the rate of loss of uptake or that this is a pharmacological effect is not clear given the limits of the study design. A similar 5-year comparison of ropinirole and LD in 268 patients was reported in 2001 (40). Approximately half of the patients withdrew by the end of 5 years. At a mean dose of 16.5 mg/day, ropinirole monotherapy was well tolerated and could be maintained in 16% of patients. The primary endpoint was the appearance of dyskinesias as measured by item 32 on the UPDRS. They were shown to occur earlier and more frequently in patients treated with LD than ropinirole. Regardless of LD supplementation, 20% of ropinirole subjects experienced dyskinesias by the end of 5 years versus 45% of LD subjects. Prior to the addition of LD, 5% of the ropinirole group and 36% of the LD group developed dyskinesias. The differences were statistically significant. The change from baseline of the UPDRS activities of daily living (ADL) score was similar between the two groups, but there was a significant difference in favor of the LD group for the change from baseline of the UPDRS motor score, which improved by approximately four times compared to the ropinirole group. This difference in efficacy was reported in the 6-month interim report published earlier (41). LD treatment is associated with greater therapeutic benefit (both early in the course of disease as well as later), and at least half of the patients developed motor complications after several years regardless of initial treatment.

Thirty years of experience and literature have led to several conclusions regarding LD therapy in PD. It is currently the most potent symptomatic therapy for PD. We have learned quite a bit about the nuances of treatment such that our goals have changed. We now treat with the lowest effective dose, not the highest tolerated one, we avoid frequent small doses, which only add to the unpredictable responses seen, and we have developed adjunctive therapies that complement LD. In short, we have become better

at utilizing LD to treat our patients. The outcome is fewer late complications, though we do not state that these are no longer a problem.

DOES LEVODOPA CAUSE MOTOR FLUCTUATIONS?

It has been well known since the early days of LD that motor fluctuations and dyskinesias relate to therapy (2). Barbeau referred to it as the long-term levodopa syndrome (42). At that time, with no alternative treatments available, he indicated that its existence did not counterbalance the great usefulness of the drug. But what causes its onset and progression? The debate addresses whether it is disease progression or primarily LD itself or both. The answer is not totally clear but this question has been examined extensively in two ways: (1) evaluating patient populations and examining which of the two factors correlates with the onset of fluctuations and dyskinesias; (2) examining the actual response fluctuations in a controlled setting to determine possible etiological explanations. The conclusion to this debate is now more important than ever since alternative therapies are becoming available and the choice of which drug to use first is in question.

In a retrospective study, Lesser et al. (43) collected data from 131 PD patients relating to severity of disease and late complications and assessed whether these problems were attributed to duration of disease or LD therapy. A relationship was seen between the presence of fluctuations and duration of therapy whereby those with fluctuations tended to be treated for 4 years or more. This was not true for dyskinesias. They, therefore, associated fluctuations with LD therapy but did not rule out the possibility that those receiving LD longer had a more progressive disease. It was recommended that initiation of therapy be delayed until the patient “begins to function unsatisfactorily in occupational or social situations.” This is perhaps the most frequently quoted paper on the subject, but the authors themselves pointed out the flaws in a retrospective study and indicated the need for a prospective evaluation of the problem. In another retrospective study, de Jong et al. (44) examined 129 patients to determine the role of age of onset, predominant symptom (tremor vs. akinetic rigid PD vs. all three together), duration of therapy, and disease severity in the occurrence of motor fluctuations. There was no significant effect of age of onset, predominant symptom, and duration of disease prior to LD therapy (but there was a trend). However, those patients with later therapy showed a lower frequency of fluctuations. Those patients treated in the earlier stages of disease (Hoehn and Yahr stages 1 and 2) did significantly worse with regard to the onset of fluctuations than patients initiating therapy in later stages (Hoehn and Yahr stages 3 and 4), suggesting that LD should not be started until stage 3 disease.

Several studies have since been published which contradict these findings. Cedarbaum et al. (45) questioned the papers described above, indicating that the patients treated earlier had to be because of more severe disease prior to initiating therapy, continued to progress faster, and thus were more prone to the onset of motor fluctuations. They suggested that LD was not the cause of the late complications, nor did the drug itself lead to loss of efficacy. In their own retrospective study, 307 patients were surveyed or interviewed with regard to motor fluctuations and various demographic features and records were reviewed. Patients were evaluated as a whole and were divided into several subgroups based on duration of disease and duration of therapy. Analyses failed to show an association between initiation of LD therapy and fluctuations or dyskinesias. Both the duration of disease and duration of therapy were longer in the patients with motor fluctuations and dyskinesias than in the group as a whole. Despite these findings, detailed statistical analyses of subgroups failed to demonstrate that age of onset and duration of therapy influenced the occurrence of fluctuations and dyskinesias. Mean delay in LD therapy was the same for fluctuators and nonfluctuators. However, patients with dyskinesias were more than three times as likely to have had initiation of LD delayed more than 2 years from diagnosis. These authors did not advocate delaying LD therapy because it, in fact, increased the chances of dyskinesias. Blin et al. (46) agreed that the apparent acceleration of progression of disease after initiation of LD therapy related to the rapidity of progression prior to LD therapy and not the therapy itself. They also found that delayed initiation of LD led to quicker onset of dyskinesia. Caraceni et al. (47) performed a prospective study on 125 patients. The study followed patients for a mean of 6 years from initiation of LD therapy to evaluate any risk factors for motor fluctuations and dyskinesias. All patients were started on LD at first diagnosis. Using a multivariable analysis, they found the risk of late complications was greater in those with akinetic-rigid PD, younger-onset age, greater disability and duration of disease, and longer interval between initiation of disease onset and LD therapy. Duration and dose of LD therapy were not associated with onset of late complications. They concluded that LD did not accelerate the appearance of motor fluctuations and that these complications relate to the severity and progression of PD. Thus, they also concluded that there is no need to delay LD treatment. Hoehn (48) indicated, based on her comparison of patients in pre- and post-levodopa eras, that a delay in the introduction of LD but not duration of treatment was associated with a poorer outcome. Horstink et al. (49) examined the relationship of duration of disease and duration of LD therapy and onset of peak dose dyskinesias in 54 PD patients and found that both duration of disease and LD therapy were greater in the dyskinetic

group. The two variables are closely linked, so they then studied patients with significantly asymmetrical dyskinesias and found dyskinesia to be most prominent on the worst side, suggesting that disease severity is an important risk factor for dyskinesias, not duration of LD therapy. Roos et al. (50) retrospectively studied 89 PD patients and several clinical correlates with onset of response fluctuations (age of onset of PD, the presenting symptom, the duration of illness, stage of illness at initiation of LD, mean and last dose of LD). They used survival and covariate analyses. No correlation was found between the dose of LD and the onset of fluctuations. However, a rapid increase in LD dose rather than the total dose seemed to determine the onset of fluctuations. They suggested that this meant that fluctuations occurred in patients with a more rapidly progressive disease requiring a more rapid escalation in LD dose. They also concluded that there are no good reasons to delay LD therapy if disability dictates its need. Finally, Kostic et al. (51) recently examined the effect of stage of disease at initiation of LD on the development of motor complications. Of 40 consecutive PD patients, 17 were treated in stage 1, 13 at stage 2, and 10 at stage 3. They found that severity of disease was an important factor in the onset of fluctuations and dyskinesias. Those patients initially treated at stage 3 developed dyskinesias and fluctuations significantly earlier than patients did in stages 1 and 2. However, latencies from disease onset to development of fluctuations and dyskinesias were no different between groups. This suggested that onset of late complications relate to disease duration and severity and not LD therapy.

While questions remain, these data suggest that disease duration, progression, and severity are important risk factors in the development of motor fluctuations and dyskinesias. In accepting this conclusion one would agree that, based on the occurrence of motor complications, there is no reason to delay LD therapy. In fact, two of the studies indicate that a delay would increase the likelihood of dyskinesias. These findings are consistent with reports of patients with late-stage PD (1) or severe parkinsonism secondary to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) developing fluctuations soon after the initiation of therapy. This has also been seen in MPTP-treated nonhuman primates (52) and postencephalitic parkinsonism (53).

Several groups have studied the mechanism of motor fluctuations. The findings suggest that both duration of disease and LD therapy play a role. Work by Fabbrini and colleagues (54) has demonstrated that perhaps the initial feature that leads to onset of fluctuations is the degeneration of nigral dopaminergic neurons to a threshold level. Once this level is reached, motor fluctuations begin with wearing off. In their studies, they examined four groups of patients: levodopa-naive, levodopa-treated stable responders

(nonfluctuators), patients with wearing-off, and patients with unpredictable on/off. They treated each patient with a continuous intravenous infusion of LD for 16 hours and then abruptly stopped it. They found that there was no change in pharmacokinetics of LD in the more advanced patients. However, it was noted that there was a decay of antiparkinsonian effect, which worsened as the patients advanced from being LD-naive to having on/off phenomenon. The authors concluded from the study that the wearing-off effect is probably initiated as a consequence of the marked loss of presynaptic dopaminergic neurons. With loss to a threshold number of neurons, the dopamine system loses its ability to store and release dopamine and, thus, buffer fluctuations in serum and cerebral LD and dopamine levels. It is believed that LD is converted to dopamine in nondopaminergic cells that lack the ability to store and release it in the normally tonic fashion (21). Stimulation at postsynaptic dopamine receptors then becomes intermittent as a reflection of the peak and trough profile of oral LD therapy. It appears that as soon as this intermittent stimulation of dopamine receptors begins, postsynaptic changes are initiated. Studies have demonstrated a narrowing of the therapeutic window, alteration of threshold for onset of dyskinesias, and steepening of the anti-PD response slope, all which underlie progression toward a more unstable response to LD (55,56). These findings support the involvement of postsynaptic mechanisms, reflecting an increased sensitivity of clinical response to small fluctuations in dopamine levels and differing pharmacological mechanisms for antiparkinsonian response and dyskinesias.

In trials comparing LD to dopamine agonists, LD therapy leads to earlier onset and more frequent occurrence of dyskinesias and wearing-off. This would suggest that either the agonist prevents the onset of these problems or that LD therapy does have some role in causing them. Disease progression with loss of nigrostriatal dopaminergic neurons to a threshold level appears to be at the root of onset of motor fluctuations. LD plays a role, via intermittent stimulation of postsynaptic receptors, in the progression of fluctuations to a more unpredictable pattern. One needs to consider that onset and progression are probably caused by different scenarios. Delaying LD may delay this progression, but the symptoms would come on sooner after LD initiation, as previously demonstrated. The delay of therapy would deprive the patient of a period of known good response. Mouradian et al. (57) demonstrated that continuous infusion of LD can reverse motor fluctuations and dyskinesias. The same can be said about subthalamic nucleus (STN) stimulation. Some indicate that the reversibility of fluctuations implicates LD in the cause of the fluctuations (58,59) but that is not the only interpretation. It can also mean that the role LD plays in motor fluctuations is potentially reversible.

IS LEVODOPA TOXIC?

It has been suggested that LD may be toxic to dopaminergic neurons, leading to more rapid degeneration. This notion is based on the oxyradical hypothesis. There is evidence that oxyradicals play a role in the pathogenesis of cell death in PD (60). Dopamine, when metabolized by MAO or autooxidized, forms H_2O_2 , a precursor to the toxic hydroxyl radical. In PD, after loss of a substantial number of nigral cells, those surviving cells increase their dopamine metabolism, possibly increasing the risk of further degeneration, especially in an environment where protective mechanisms, such as glutathione, are diminished and iron has accumulated. The use of LD may lead to an increase in dopamine formation and, in turn, an increase in dopamine metabolism with greater free radical formation (61). While this theory has widespread appeal, and while laboratory evidence supports this possibility, the theory remains controversial (62). However, detailed reviews on the subject (63–65) have indicated that there is no convincing evidence to suggest that levodopa is toxic to our patients and that this concern should not govern how we treat our patients.

The evaluations for LD toxicity have included both *in vitro* (cell culture) and *in vivo* animal studies. In the cell culture studies, various cell types were used including fetal mesencephalic cells, neuroblastoma, fetal fibroblasts, pheochromocytoma PC12 cells, chick sympathetic neurons, and others (66). Results of these studies were variable because of the LD concentrations used and culture conditions. High doses of LD are toxic to dopaminergic neurons in pure neuronal cultures. Mechanisms of toxicity include oxyradicals, mitochondrial toxicity, or apoptosis (67–69). However, as the conditions are set to more accurately reflect *in vivo* systems, the toxicity disappears and the neurons are more able to resist injury. In fact, with exposure to medium doses (20–100 μM) and with glial cells present, LD actually has a trophic influence. The glial cells contain the protective enzymes catalase and glutathione peroxidase and provide a nutritive and protective environment. LD exposure to these cultures actually increases cellular concentrations of reduced glutathione peroxidase and may have other neurotrophic properties. At levels that are likely present in the extracellular fluid in the striatum of patients, as measured in animals by microdialysis (picomolar levels), it is unlikely that LD has any effect (65).

In vivo studies have included both unlesioned and lesioned animals. Several studies involved giving healthy animals LD for up to 18 months, and they demonstrated no loss of dopaminergic neurons (70,71). In one study, Cotzias et al. actually reported that mice given LD lived longer than controls not given LD (72). However, a controversy surrounds previously lesioned animals. Fahn (66) reviewed more than 15 studies of *in vivo* effects

of LD and dopamine. Four studies are of particular relevance to the issue of early LD therapy for PD. Blunt et al. (73) lesioned rats with 6-hydroxydopamine (6-OHDA) and gave LD to some. They then counted tyrosine hydroxylase (TH)-stained cells in the substantia nigra (SN) and ventral tegmental area (VTA). The unlesioned (healthy) side was unaffected by the LD, supporting the prior studies. The SN on the lesioned side lost 96% of its cells from the 6-OHDA. The VTA was less affected, with 23–65% of cells remaining. LD further reduced surviving cell numbers to 10–35%. They concluded that either LD suppressed TH activity or caused increased cell death, a conclusion that has been questioned (74). Fukuda et al. (75) used MPTP-lesioned mice and examined the effect of LD and bromocriptine on total and TH+ cell counts. LD further reduced cell counts in MPTP-treated mice but it was in TH- cells. TH+ cells were unaffected. Bromocriptine had no effect, but combined LD and bromocriptine resulted in a significant increase in surviving cells. Murer et al. (74) examined the effects of LD on nigrostriatal and VTA cells in rats with moderate and severe 6-OHDA lesions and sham-lesioned animals. They measured three dopaminergic markers—TH, dopamine transporter (DAT), and vesicular monoamine transporter (VMAT2)—via radio-immunohistochemistry in the SN, VTA, and striatum. They also examined rotational behavior to assess pharmacologically relevant doses and postsynaptic receptor binding. The study failed to demonstrate any significant difference on cell counts in SNpc and VTA in LD-treated animals compared to those treated with vehicle using all three markers. There was a trend toward increased TH staining in the SNpc of the moderately lesioned animals. At the level of striatum there was no effect of LD treatment in the sham-lesioned and severely lesioned animals, but in the moderate lesioned animals there was partial recovery of nerve terminals in the damaged area, suggesting a neuroprotective potential. The increased immunostaining in this region reached statistical significance compared to those rats treated with vehicle. It was suggested that this increased striatal activity with LD related to partial recovery via axonal sprouting by the remaining neurons. LD also tended to reverse increased binding (upregulation) of dopamine receptors and diminished the development of behavioral supersensitivity, indicating that the doses of LD were pharmacologically effective. These results indicate that LD did not damage the neurons or their terminals in normal and moderately or severely lesioned animals. It may promote compensatory mechanisms at the terminals and thus recovery of innervation of the striatum. Datla et al. (76) demonstrated similar findings. In the rats with 6-OHDA and ferric chloride (FeCl₃) lesions, LD had no short-term or long-term effects on the number of TH+ cells. In contrast, in the 6-OHDA model there may have been a protective effect since there was an increase in TH+ cells after 24 weeks. While results

of these animal studies appear to be conflicting, the latter studies seem to provide evidence that LD is not toxic. One could conclude that LD has no detrimental effect on dopaminergic neurons in healthy and compromised animals.

Human studies have also been nonsupportive for the possibility of LD toxicity. Quinn et al. (77) reported on the treatment of a non-PD patient who received high-dose LD for 4 years. Autopsy results demonstrated a normal SN. Rajput et al. (78) reported on five patients with similar results. Three patients had essential tremor, one had dopa-responsive dystonia, and the other was nonprogressive. Autopsies in two were normal. None of the essential tremor patients developed parkinsonism; the two other patients showed no progression of disease clinically. This would indicate that LD is not detrimental to patients with normal or dysfunctional SN. Yahr et al. (79) compared postmortem results in patients treated and never treated with LD and reported no difference in the pathology of the SNpc. Gwinn-Hardy et al. (80) examined the effect of LD on a family with autosomal dominant LD-responsive parkinsonism (mutation on chromosome 4p). There were 12 affected individuals, and survival duration and disease progression were compared in those treated and not treated. Survival was significantly different between the two groups, as was progression of disease, both in favor of LD therapy. Finally, a recent neuroimaging study compared progression of PD with a dopamine agonist versus LD (38,39). It utilized B-CIT SPECT imaging comparing LD and pramipexole. The decrease in binding was less over several years for the agonist than for LD. This may be an indicator that LD is toxic, that the agonist is neuroprotective, or it may reflect a differential pharmacological effect. At this point the answer is unknown.

When one looks at the data from cell culture, animals, and humans, there is so far no support for the notion that LD is toxic. There should be no concern about this when considering therapy in PD patients.

DOES TOLERANCE DEVELOP FROM LEVODOPA?

The lay literature is replete with information suggesting that LD loses its effect after about 5 years. This leads to some trepidation on the part of the patient and physician in initiating therapy. If that were the case, it would indicate that tolerance is a possible concern and such an occurrence would argue for delaying treatment. It is conceivable that, when all nigrostriatal cells are depleted, LD would lose all effectiveness since these are the cells that convert LD and release dopamine. Lesser et al. (43) found that longer duration of disease did not appear to adversely affect response to LD at the time of initiation of therapy, yet they demonstrated a deterioration in

response that did not correlate with duration of disease. Those receiving LD longer had more severe disease. The assumption made by the authors was that PD patients developed tolerance. Despite these findings, the authors did not rule out the possibility that those receiving LD longer had a more progressive disease. However, Blin et al. (46) noted that chronic treatment does not lead to decreased effectiveness. Evidence indicates that conversion of dopa to dopamine can occur at sites other than dopaminergic terminals in the striatum (43,54,58). Thus, LD continues to be effective throughout the course of disease. The potency of LD does not change with chronic use. Markham and Diamond (81,82) demonstrated this when they studied three groups of patients; those starting LD after 1–3 years of disease, 4–6 years, and 7–9 years. In this manner they could assess whether the apparent loss of efficacy could relate to the disease duration or the duration of drug therapy. After 6 years of follow-up they noted the following:

1. The disability scores were different for the three groups at initiation of LD and remained different thereafter.
2. Disability scores were the same for the three groups when they were matched for disease duration despite varied durations of therapy.
3. There was no significant difference with respect to the incidence of dyskinesias.

In projecting the course of disease it was found that all three groups ultimately followed the same predictable course of progression independent of the duration of LD therapy. This was confirmed after 12 years of follow-up of the first group (81,82). The authors concluded that LD works at all stages of PD, does not result in tolerance over time, but does not stop progression of disease. In other words, changes in disability of PD are related to duration of disease and not duration of therapy or tolerance to LD. Aside from progression of disease, another cause of the apparent loss of efficacy relates to narrowing of the therapeutic window—increased sensitivity to adverse effects such as dyskinesias and hallucinations (45,46). The worsening of disease also comes from the onset and progression of symptoms not attributable to dopamine systems, such as postural instability, freezing, and dementia (46).

MORTALITY OF PD WITH LEVODOPA

Several studies performed in the 1970s demonstrated that LD therapy improves mortality in PD. These studies compared the survival of LD-treated patients to the mortality rate demonstrated in the pre-levodopa Hoehn and Yahr study (2), which demonstrated that mortality was three

times greater than in the normal population. Nearly all studies indicated that LD improved survival with rates of 1.4–2.4 (83,84). Some investigators suggested that survival approached normal, while others indicated that the effect was only seen early in therapy and then disappeared. However, many of the studies have been criticized due to methodological flaws, problems with patient selection, and possible biases. One study of particular interest (83) utilized a population-based study design (retrospective) to avoid many of these flaws and examine the change in survival related to LD therapy. The study included patients treated from 1964 to 1978 to include patients treated early and late as well as untreated cases. Results indicated that survival for all patients was significantly poorer than that of the general population but was better in treated than in untreated PD. The improved survival over time was not linear. Throughout the entire 17 years of follow-up there was reduced risk of death with LD therapy.

One other area of interest relates to the timing of LD therapy. Does early or later intervention affect the survival rates? Diamond et al. (81,82) examined this question looking at 359 patients treated between 1968 and 1977. They divided patients into three groups: group one, 1–3 years of PD; group two, 4–6 years of PD; group three, 7–9 years of PD. They used observed-to-expected death rate (from a group of similar make-up in the general population) ratios as measures of survival. When duration of therapy is held constant at 15 years, the ratio was higher for patients with longer duration of disease. When duration of disease was held constant at 17 years, the patients in group one had a better mortality ratio than the other two groups. Thus, early initiation of LD therapy was beneficial to life expectancy. They suggested that the improved survival related to the symptomatic effect of the drug, keeping patients more active in the earlier years. In another study, Scigliano et al. (84) studied 145 patients seen from 1970 to 1983. Of those, 98 were treated for 2 or more years while 47 were treated for <2 years. Mortality was found to be 2.5 times greater among the patients treated later, but a multivariate analysis taking into account age and disease severity made the difference nonsignificant. However, there were biases that led to an underestimation of mortality in the delayed treatment group, including 47 patients who were lost to follow-up. They concluded that survival from early LD initiation is the same or better than late. Uitti et al. (85) examined the duration of the interval from onset of disease to treatment and found that it had no influence on subsequent mortality.

IS THERE AN ASSOCIATION BETWEEN LD THERAPY AND MELANOMA?

Previous reports as well as the *Physician's Desk Reference* caution against the use of LD in PD patients with a history of melanoma. As recently as 1998, Pfutzner and Przybilla reported that while no causal relationship has been proven, patients with a history of malignant melanoma receiving LD therapy should be carefully followed for the development of new pigmented lesions (86). Anecdotal reports exist in the literature of the potential carcinogenic effects of LD therapy and its potential to activate malignant melanoma (87). Because dopamine acts on and is produced by pigmented neurons, it has been proposed that levodopa may affect the activity of melanocytes, possibly promoting malignant transformation.

Weiner et al. in 1993 (88) reviewed the literature and concluded that there is anecdotal evidence at best to support a link between LD and melanoma. They reported on nine patients with PD and a history of melanoma who were treated with LD, none of whom had a recurrence. They concluded that LD therapy could be used safely in PD patients with melanoma. Woofter and Manyam (87) reported on a 74-year-old man with PD who was treated with LD and whose malignant melanoma was later discovered. Prior to the diagnosis of melanoma, it was estimated that the patient received 5.7 kg of LD over a 6-year period. The patient continued with LD treatment for more than 10 years, with an additional 4.3 kg LD prescribed, and no recurrence of his melanoma was observed. They concluded that withholding LD therapy for fear of accelerating melanoma was unwarranted (87). Siple et al. reviewed 34 case reports found by literature review (January 1966–September 1999) and indicated that the association between LD and induction or exacerbation of malignant melanoma was unlikely (89).

Thus, despite the continued warning appearing in the prescribing literature for LD, there appears to be no causal relationship between LD therapy in PD and the occurrence of malignant melanoma. A history of melanoma in a PD patient should not prohibit the use of LD.

LEVODOPA CHALLENGE TEST

It can be difficult to accurately differentiate PD from other forms of parkinsonism, especially during early presentation. LD administration can be used for diagnostic purposes as PD patients respond more frequently and robustly to LD compared with other forms of parkinsonism.

Clarke and Davies recently published a review of 13 studies that examined whether an acute LD or apomorphine challenge test could aid in

the diagnosis of PD (90). Four studies examined de novo patients and nine examined patients with clinically established idiopathic PD. Although there was significant variability in the methodologies employed, abstracted sensitivity and specificity data were summarized from the studies and the two challenge tests compared as to their ability to accurately predict patients' diagnosis. The sensitivity for the diagnosis of established PD for apomorphine was 0.86 (95% CI), acute levodopa 0.75 (95% CI), and chronic levodopa therapy 0.91 (95% CI). The specificity for the diagnosis of established PD was apomorphine 0.85 (95% CI), acute levodopa 0.87 (95% CI), and chronic levodopa therapy 0.77 (95% CI). The number of patients positive for each test divided by the number with clinically diagnosed de novo disease was apomorphine 0.63 (95% CI), acute levodopa therapy 0.69 (95% CI), and chronic levodopa therapy 0.76 (95% CI). Twenty-one chronic LD patients described as having positive response were initially negative via acute LD.

The authors concluded that the accuracy of the acute levodopa and apomorphine tests was similar but not superior than that of chronic levodopa therapy and that these were not more accurate than the established accuracy of clinical diagnosis of PD (75–80% accuracy). In addition, given the additional costs and adverse effects associated with their use, they could not recommend using the challenge tests.

Rossi et al. (91) reported on the use of acute challenge with apomorphine and LD in patients with clinically defined forms of parkinsonism to assess the potential accuracy of the tests with regards to diagnosis. Motor responses to the acute administration of LD and apomorphine were analyzed in a series of 134 parkinsonian patients (83 with a clinical diagnosis of idiopathic PD, 28 patients with multiple system atrophy, 6 with progressive supranuclear palsy and 17 unclassified patients). The duration of disease or the clinical stage of the patients was not described. Patients received LD/AADI (250/25 mg) or subcutaneous apomorphine (1.5, 3, 4.5 mg). UPDRS motor scores were evaluated 1 hour following LD administration and 20 minutes after apomorphine injection. The motor evaluation was matched with the clinical diagnosis and the response to chronic LD therapy. Those patients who had improvement of at least 16% on their UPDRS were more likely to have PD when compared to non-PD patients. When comparing PD with MSA patients, those who improved at least 18% on their UPDRS were more likely to have PD rather than MSA. If a patient responded to the challenge test with at least 14.5% improvement in UPDRS, they were more likely to respond favorably to chronic LD therapy. The authors conclude that use of the challenge test was helpful in making treatment decisions regarding long-term LD therapy (91). It appears that an acute LD test is not very useful in

improving our ability to diagnose PD. Questions remain about its use in making treatment decisions.

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