REVIEW ARTICLE

Levodopa in Parkinson's Disease: Current Status and Future Developments

Nicola Tambasco[1,*], Michele Romoli[1] and Paolo Calabresi[1,2]

[1]*Clinica Neurologica, Azienda Ospedaliera-Università di Perugia, Perugia, Italy;* [2]*IRCCS "Santa Lucia", Rome, Italy*

Abstract: ***Background***: Ever since the pioneering reports in the 60s, L-3,4-Dioxyphenylalanine (levodopa) has represented the gold standard for the treatment of Parkinson's Disease (PD). However, long-term levodopa (LD) treatment is frequently associated with fluctuations in motor response with serious impact on patient quality of life. The pharmacokinetic and pharmacodynamic properties of LD are pivotal to such motor fluctuations: discontinuous drug delivery, short half-life, poor bioavailability, and narrow therapeutic window are all crucial for such fluctuations. During the last 60 years, several attempts have been made to improve LD treatment and avoid long-term complications.

Methods: Research and trials to improve the LD pharmacokinetic since 1960s are reviewed, summarizing the progressive improvements of LD treatment.

Results: Inhibitors of peripheral amino acid decarboxylase (AADC) have been introduced to achieve proper LD concentration in the central nervous system reducing systemic adverse events. Inhibitors of catechol-O-methyltransferase (COMT) increased LD half-life and bioavailability. Efforts are still being made to achieve a continuous dopaminergic stimulation, with the combination of oral LD with an AADC inhibitor and a COMT inhibitor, or the intra-duodenal water-based LD/carbidopa gel. Further approaches to enhance LD efficacy are focused on new non-oral administration routes, including nasal, intra-duodenal, intrapulmonary (CVT-301) and subcutaneous (ND0612), as well as on novel ER formulations, including IPX066, which recently concluded phase III trial.

Conclusion: New LD formulations, oral compounds as well as routes have been tested in the last years, with two main targets: achieve continuous dopaminergic stimulation and find an instant deliver route for LD.

Keywords: Parkinson's disease, levodopa, pharmacology, IPX066, DM-1992, XP21279, ODM-101, LCIG.

1. A GLIMPSE ON LEVODOPA HISTORY

Parkinson's disease (PD) is a neurodegenerative disorder characterized by cardinal motor features, such as bradykinesia, rigidity, postural instability and resting tremor, and non-motor features, ranging from depression to hyposmia [1]. PD is the most common chronic neurodegenerative disease affecting motor behaviour, and its prevalence increases with age, from 2% in over 65 years old to 5% in over 85 years old [2]. PD is characterized by the progressive loss of dopaminergic (DA-ergic) pigmented neurons of the substantia nigra (SN) pars compacta, a small structure lying deep in the core of the human midbrain [3]. Among these neuronal cells, DA is synthesized from the L-3,4-dihydroxyphenylalanine (L-dopa) deriving from the hydroxylation of tyrosine [Nagatsu *et al*., 19644]. This pathway, the only one available in the SN for DA production [5, 6], leads to DA synthesis in its first stage, eventually producing other catecholamines, such as noradrenaline and adrenaline, by the action of aromatic L-amino acid decarboxylase (AADC; dopa decarboxylase, DDC) [7]. From the first depiction, dating back to Parkinson's original "*paralisis agitans*" [8], it took more than a century to unveil the distribution of cathecolamines among the central nervous system (CNS) [Sano *et al*., 1959], and almost 40 years to shed light on the dopamine (DA) deficiency peculiar of PD [10]. From such pioneering reports the levodopa (LD) era starts.

LD first synthesis dates back in 1911, when the efforts of Kazimierz Funk, a Polish passionate biochemist, paid with the identification of D,L-DOPA [10, 11]. The pure left enantiomer, LD, was isolated for the first time from the exotic bean plant *Vicia faba* by Marcus Guggeneheim, a Swiss biochemist, who described it as an inactive compound despite the violent vomiting he experienced after having tried a 2.5 g oral dose on himself, highlighting one of the most frequent systemic adverse events of LD in the pre-AADC inhibitors [12]. Even though being soon recognized as a cathecolamine precursor, the lack of a dopamine-centric vision of PD rele-

*Address correspondence to this author at the Clinica Neurologica, Azienda Ospedaliera e Universitaria di Perugia, Loc. S.Andrea delle Fratte 06156, Perugia, Italy; Tel: +39-075-5783830; Fax: +39-075-5784229; E-mail: n.tambasco@libero.it

gated LD to be investigated for its effects on blood pressure for almost half a century [13, 14]. A window of opportunity was opened by Arvid Carlsson, a pioneer of LD pharmacology, who tried to define monoaminergic systems in the brain, and their modulation with reserpine and LD [15-20]. Carlsson first reported a reserpine-induced depletion of norepinephrine and epinephrine in rabbit adrenal glands [18], and then proceeded investigating whether a serotonin/cathecolamine depletion could have accounted for the tranquilizer effect of reserpine on rabbit. In particular, he ruled out the participation of serotonin in such effect, since 5-hydroxytryptophan did not revert the reserpine-induced immobility. On the contrary, the effect of reserpine was consistently counteracted by the administration of D,L-3,4-dihydroxyphenylalanine. Moreover, such counteraction was enhanced with pre-administration of a monoamine oxidase inhibitor (MAO-I) such as iproniazid, suggesting that an amine, formed from the first compound, could have taken part in the reversal of reserpine-induced catatonia [15, 16]. Soon after, in 1960, at the Ciba Foundation Symposium on Adrenergic Mechanisms held in London, Carlsson challenged all the preconceptions about PD pathogenesis, proposing dopamine as the main involved neurotransmitter in this disorder [11, 16, 20]. Despite arousing a thorough rejection by the traditional scientific community at that time, both the hypothesis and the revolutionary technique, counting on spectrophotofluorimeters, were so attractive and foreseeing that little time passed before they were pursued. In the 60s, different and independent high profile researchers, such as Sano, Hornykiewicz and Ehringer, accurately described the localization of cathecolamines in the central nervous system, and pointed to dopamine as the depleted cathecolamine in PD, especially within the striatum [21-23]. Hence, LD was tested to replete the lacking DA in PD patients. Between 1960 and 1967 conflicting results emerged from LD treatment reports, mostly because of inhomogeneity in drug administration and dosages amid trials [6, 24-30]. In 1969, Cotzias and colleagues eventually reported clinical efficacy of high doses of LD (3-16 g/day), also limiting adverse events such as nausea, anorexia and vomiting through slow titration [31], paving the way for the use of the "platinum drug" in PD [30]. In the same year, Melvin Yahr and colleagues reported the results of the first double-blind, placebo controlled trial, reporting the efficacy of LD on akinesia, tremor and rigidity in patients with PD [32]. Both Cotzias and Yahr noticed adverse events, including involuntary choreiform and athetotic movements, with high dosages and long-term administration (6 months) of LD [31, 32]. Following the positive results, in 1970 the US Food and Drug Administration approved LD as a treatment for PD. The effects of LD treatment were also confirmed with post-mortem studies, showing dopamine concentrations up to 15 times higher in the putamen and caudate nuclei of treated patients compared to untreated ones [33]. The formulation evolved to be for oral route, since LD infusion would have been poorly feasible, highly impractical, and with local and systemic adverse events (LD is poorly soluble, and irritates vessels and soft tissues). However, in 1970, a revolutionary time for PD treatment, Hornykiewickz, saying that LD "is far from being perfect as a drug", foresaw what, in the following years, would have become the most important clue to PD diagno-

sis: the complications of long-term LD treatment [30, 34]. Among them, motor fluctuations have been, from the very beginning, one of the most compelling issues to target. Decades of research have been spent to understand the pathophysiology behind such fluctuations, and pioneering reports already demonstrated, back in the 70s, that continuous dopaminergic stimulation could provide a significant benefit over oral dosages [35, 36]. Such theory, further supported by the first report of the consistent improvement of motor fluctuations with continuous duodenal LD infusion *via* a nasogastric tube [37], has been pursued in the years, greatly improving patients quality of life. Ever since LD kicked in, millions of patients have been prescribed with it, and so they will, since it still represents the most effective drug in treating PD [35]. On the other hand, LD has pharmacokinetic and pharmacodynamics issues that, despite being faced since 1960, are yet to be tamed [11].

2. PHARMACOKINETIC AND PHARMACODYAMIC CHALLENGES: LEVODOPA AND PERIPHERAL METABOLISM INHIBITORS

LD absorption and bioavailability are one of the main pharmacological challenges being faced to date.

LD gastrointestinal uptake only occurs through a facilitated sodium dependent L-neutral amino acid transport in a small tract of the duodenum and proximal jejunum [30]. Moreover, in both these sites, as well as across the blood brain barrier, LD competes for uptake with other neutral amino acids derived from proteolysis of food [38-40]. The absorption of LD in the small intestine depends on 3 different transporters: b0,1AT-rBAT (apical), LAT2-4F2hc, and TAT1 (basolateral) [40]. Interestingly, none of the above-mentioned transporters activity is influenced by amino acid decarboxylase (AADC, Dopa decarboxylase) or cathecol-O-methyl transferase (COMT) [40].

LD absorption only represents the first issue of the more complex problem regarding its bioavailability. In fact, once reached the bloodstream, LD undergoes an extensive first-pass metabolism, a rapid distribution to the skeletal muscles, and a rapid degradation and clearance through different peripheral enzymes [30]. Both these mechanisms highly impact the half-life and bioavailability of LD: of the orally-administered dose, only 1% enters the brain to effectively act on dopaminergic transmission, and LD half-life only reaches an hour [11, 28]. The metabolism of orally administered LD has been defined by the identification, through gas-liquid chromatography, of urine metabolites, such as 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy- 3-methoxyphenylacetic acid (homovanillic acid, HVA) [6, 41]. In particular, peripherally, LD is primarily metabolized by AADC, monoamine oxidase (MAO), and catechol O-methyltransferase (COMT). The delineation of the pivotal effect of AADC on LD metabolism [42] led to the formulation of AADC inhibitors, to be co-administered with LD to increase its bioavailability [6, 28]. The co-administration of AADC, such as benserazide and carbidopa (CD), prolongs the efficacy and promotes the tolerability of LD [43-45]. Specifically, both these molecules reduce the plasma clearance of LD, thus prolonging its half-life to about 90 minutes, enhancing its clinical effect, and reducing the required over-

all dosage of 60-80% [28, 46-48]. Moreover, the combination of LD with AADC improves the tolerability profile of LD, minimizing the effects of peripheral decarboxylation into circulating dopamine, such as nausea, vomiting, hypotension [28, 49]. Clinical studies reported CD/LD to be associated with a significant improvement in nausea and vomiting, and a better control of PD clinical features [50, 51]. Despite improving pharmacokinetic, however, CD/LD does not affect pharmacodynamics issues: indeed long-term sustained benefit was found after 2 years of treatment only in 20% of patients, and more than 75% of them experienced dyskinesias [28, 51, 52]. Moreover, multiple doses of CD, as well as for benserazide, are needed to achieve a consistent AADC inhibition [43, 53]. Indeed, 75 to 100 mg per day only partially reduce AADC activity, whose complete inhibition needs very high drug dosage, being dose-dependent [30, 54, 55].

Of main importance is that AADC conversion of LD to dopamine should be avoided in the blood (to limit dopamine induced adverse events and pharmacokinetic issues) while it must be promoted as soon as it trespass the blood-brain barrier (BBB), to achieve dopamine repletion. AADCs do not pass the BBB, thus allowing free conversion of LD into dopamine within the central nervous system [35]. Moreover, to increase LD striatal conversion, research have prompted the use of viral vectors to deliver AADC, whose results need to be further confirmed [56-58].

Despite being pivotal in LD metabolism, the inhibition of AADC only allowed 5-10% of an oral dose of LD to reach the central nervous system [28, 59]. In the absence of the AADC activity, LD metabolism is consistently shifted to COMT. COMT, which can be found in soluble (S-COMT) and membrane-bound (MB-COMT) forms [60], is one of the main catabolic pathways of catecholamines, also producing 3-O-methyl-dopa (3-OMD), a competitive inhibitor of LD intestinal absorption, BBB transport, and a competitive substrate for CNS dopamine uptake [38, 60-62]. As soon as COMT inhibition was shown to increase LD availability in the CNS and limit the formation of 3-OMD, COMT inhibitors came into practice in PD [6, 63]. In particular, tolcapone, entacapone and opicapone are those to-date available. Among them, tolcapone is the only one able to block COMT also within the CNS, since it crosses the BBB, but is also much less tolerable compared to entacapone and opicapone. Moreover, tolcapone presents very rare but severe adverse events, such as liver failure, that have significantly limited its use in clinical practice and relegated it to the level B evidence in PD (entacapone has Level A evidence) [28, 64]. Multiple clinical trials have reported a decrease in the average LD daily dose, an increase in LD response duration, and an overall increase in LD bioavailability with a COMT inhibitor [6, 65, 66].

Beyond the pharmacokinetic problems solved, there are even more to be faced. In particular, since a dopaminergic prolonged, or at best continuous stimulation, has been pointed at as the best available solution for motor fluctuations, researchers are struggling to provide it to the patients. However, continuous LD delivery has still been a pharmacological nightmare. Even though small as a molecule, in fact, LD does not pass easily through the skin, with the result

that only subcutaneous infusion is investigated to-date, with efficacy and tolerability yet to be extensively evaluated [11, 67]. Moreover, the issue of continuous dopaminergic stimulation is not just about pharmacokinetic, but deeply impacts pharmacodynamics, particularly on motor fluctuations.

One of the most demanding tasks of PD treatment is to deal with pharmacodynamics issues of long term LD treatment. In particular, motor and non-motor fluctuations develop in up to 75% of patients after 4 to 6 years of LD treatment, and negatively impact patients quality of life [28-30]. The mechanisms by which the effects of LD change over time, even though still impacting on patients motor symptoms also in late stages, are poorly understood to-date. At the same time, some motor fluctuations, such as wearing off and dyskinesias, have been inextricably linked to the inconsistency of LD provided to the dopaminergic circuits. Hence the demonstration that these relevant undesired complications could be significantly limited with a continuous infusion of LD, intravenous or subcutaneous [30, 68]. In addition, new oral formulations have been under investigation, with the aim of achieving a more practical route of administration, pursuing continuous dopaminergic stimulation [67]. However, especially in the late stages, fluctuations seem to reflect the loss of dopaminergic terminals and the consequent loss of dopamine conversion and storage [28, 69, 70]. In the early stages, surviving nigrostriatal terminals store and progressively release the dopamine derived from LD AADC conversion, ensuring steady synaptic dopamine levels irrespective of plasma levels [71]. On the contrary, in late stages, the loss of nigrostriatal terminals leads to compensatory storage in serotoninergic cells and other terminals, which can hardly deal with congruous dopamine release [71, 72]. Thus, dopamine concentration at the synaptic sites resembles the circulating one, with patients needing a constant stimulation to overcome the loss of dopamine storage and slow-release mechanisms failure [73]. Continuous stimulation has been shown to provide clinical benefit to patients, and to elude the lack of a short-term buffering of the LD plasmatic levels, despite dyskinesia can be experienced also with it [74, 75]. Moreover, the pulsatile stimulation to which dopaminergic neurons are exposed throughout disease progression induces postsynaptic downstream changes that alter the circuitry of the basal ganglia [28, 30, 73]. Several studies on animal models have unveiled that pulsatile stimulation contributes to the onset and perpetration of motor fluctuations, especially dyskinesias [70, 71]. On the contrary, sustained dopaminergic stimulation has been shown to be able to prevent such complications, also in clinical studies on humans [71, 76-78]. While dyskinesias have also been linked to postsynaptic mechanisms, the genesis of unpredictable "off" states, including freezing of gait, still has to be clarified [30]. For these specific issues, new LD formulations have been tested in the last 20 years, with two main targets: achieve continuous dopaminergic stimulation and find an instant deliver rout for LD (Table **1**).

3. LEVODOPA FORMULATIONS: ORAL ROUTE

After Yahr firstly reported the efficacy of LD in a placebo controlled trial [32], another milestone was achieved regarding LD treatment in PD. The Earlier versus Later LD

Table 1. Levodopa new oral and non-oral formulations.

Drug	Trial Status	Composition	Route	Doses	Action	Effect on "off" Time	Effect on Motor Symptoms	Effect on Dyskinesias	Adverse Events	Refs.
IPX066	Phase III FDA/EU approved	Capsule containing IR LD/CD + ER LD/CD (different dissolving rate)	Oral	LD 95, 145, 195, 245 mg	oral, immediate + long-acting	1.17 hrs ↓ in daily "off" time	vs Placebo: ↓ in UPDRS II and III (11.7, 12.9 and 14.9 pts with 145, 245 and 390 mg tid respectively) vs IR LD/CD: 3.57 pts ↓ in UPDRS II and III (5.71 pts ↓ with IPX066 vs 2.14 pts ↓ with IR LD/CD)	vs IR LD/CD: 0.05 pts ↑ (0.15 pts ↑ with IPX066 vs 0.1 with IR LD/CD)	Nausea, vomiting, headache, dizziness, insomnia, dyskinesias (in early PD), gait disturbances, psychosis, compulsive impulsive disorder (in advanced PD)	Hauser *et al.*, 2011 Hauser *et al.*, 2013 Pahwa *et al.*, 2014
AP LD/CD	Phase III ongoing	Accordion gastroretentive multi-layer pill	Oral	LD /CD 375/50 mg or 500/50 mg	oral, immediate + long-acting	1.9 hrs ↓ in daily "off" time	n/a	vs IR LD/CD: no increase in dyskinesias with AP LD-CD 275/50 mg; 40% ↑ with AP LD-CD 500/50 mg	No severe adverse events	LeWitt *et al.*, 2014 ClinicalTrials.gov NCT02605434
DM-1992	Phase II	Gastro-retentive core capsule containing IR LD/CD + ER LD/CD	Oral	IR LD/CD (30/7.5 mg) + ER LD/CD (200/65 mg)	oral, immediate + long-acting	vs IR LD/CD: 0.89 hrs ↓ in daily "off" time	vs IR LD/CD: 2.6 pts ↓ in UPDRS III (not statistically significant)	vs IR LD/CD: 0.03 hrs ↓ (0.6 hrs with IPX066 vs 0.63 with IR LD/CD)	Dizziness, abdominal pain, gait impairment, headache	Chen *et al.*, 2012 Verhagen Metman *et al.*, 2015
XP21279-CD	Phase II	Bilayer casule containing ER LD/CD	Oral	ER LD/CD 241/27 mg	oral, promotes absorption also in colon increasing bioavailability and plasma level steadiness	vs IR LD/CD: 0.3 to 2.8 hrs ↓ in daily "off" time	vs IR LD/CD: 6.5 pts ↓ in UPDRS III	vs IR LD/CD: no differences	Headache, dizziness, gastro-oesophageal reflux, dyskinesias, anorexia	LeWitt *et al.*, 2012 LeWitt *et al.*, 2014
ODM-101	Phase II	Capsule containing LD-CD-entacapone (LCE) with high CD concentration	Oral	LCE 101/65 mg or 101/105 mg	improve pharmacokinetic profile of LD with high CD concentration	vs standard LCE (100/25/200 mg): 0.62 and 0.66 hrs ↓ in daily "off" time with ODM-101/65 and ODM-101/105 repectively	vs standard LCE: no differences	vs standard LCE: no differences but dyskinesias more frequently reported with ODM-101/105 mg	Dyskinesias	Muller *et al.*, 2013
LC-5 (MyFid device)	EMA approved	Microtablets containing LD/CD	Oral (automated dispenser device)	LD/CD 5/1.25 mg	Oral, similar pharmacokinetic profile to standard LD/CD	vs LCE ↓ plasma LD fluctuations	n/a	no data on comparison with other formulations	n/a	Aquilonius and Nyholm, 2017
LCIG	Phase III FDA/EU approved	Carboxymethyl-cellulose gel with LD/CD suspension, intestinal deliver via PEG-J	Enteral via PEG-J	LD/CD 20 mg/mL (1 cassette=100 mL)	Constant infusion regimen	vs IR LD/CD: 1.9 hrs ↓ in daily "off" time	vs IR LD/CD: no statistically significant differences (1.5 pts ↓ with LCIG vs 2.9 pts with IR LD/CD)	vs IR LD/CD: no statistically significant differences (0.08 hrs ↓ with LCIG compared to IR LD/CD)	Mostly linked to procedural and device complication; polyneuropathy	Wirdefeldt *et al.*, 2016
ND0612	Phase II-III	Liquid formulation, subcutaneous via, patch-pump device	Trans-cutaneous	LD/CD 60/14 mg/mL (1 or 2 devices)	Constant infusion regimen	vs placebo: 2.1 to 2.4 ↓ in daily "off" time	n/a	0.47 hrs ↓ in daily "on with troublesome dyskinesias" time	Infusion site dermal reaction	Caraco *et al.*, 2013 Giladi *et al.*, 2015 Clinicaltrials.gov NCT02782481 - Phase III NCT02577523 / NCT20726386 - Phase II
CVT-301	Phase III	Inhalation powder	Lung-delivery	LD 35 or 50 mg	Instant delivery	0.9 hrs ↓ in daily "off" time	vs IR levodpa/CD: 7.0 pts ↓ in UPDRS III	n/a	Cough (reduced with subsequent inhalations), nausea, dizziness	LeWitt *et al.*, 2016 ClinicalTrials.gov NCT02240030

CD: carbidopa; CR: controlled release; ER: extended release; hrs: hours; IR: immediate release; LD: levodopa; LCIG: levodopa-carbidopa intestinal gel; PEG-J: percutaneous endoscopic gastrotomy; UPDRS: Unified Parkinson's Disease Rating Scale; vs: versus.

Therapy in Parkinson Disease (ELLDOPA) trial, specifically designed to assess the impact of LD treatment on PD progression, compared placebo versus different LD regimens (150, 300, or 600 mg daily) during a 40 weeks follow-up. The results pointed at a dose-dependent improvement in the severity of PD symptoms and signs [79-81]. Meta-analysis of available treatments, including dopamine agonists, COMT and MAO-B inhibitors, highlighted the pivotal role of LD in controlling PD, despite the higher risk of developing motor fluctuations compared to dopamine agonists and MAO-B inhibitors [81].

Slow release LD preparation has been the object of research interest from the 90s. Concerning motor fluctuations, Sage and Mark reported an increase in "on" time free of dyskinesia with controlled release (CR) LD/CD compared to standard formulation, among PD patients suffering from long-term motor fluctuations (5 years on average) [82]. The results were confirmed by Ahlskog and colleagues in an analogous 8-week, double-blind, double-dummy randomized trial comparing CR versus standard formulation of LD. In particular, patients receiving CR formulation had lower number of doses (7.0 vs 9.8), higher overall dosage (2 g/d versus 1.8 g/d), and less "off" hours (3.2 versus 3.7), compared to those receiving standard formulation, though not reaching statistical significance [79, 83]. A trend towards a 10% reduction in daily off periods was reported in a double-blind multicentre study, in favour of CR LD/CD versus standard formulation [84]. No difference regarding "wearing-off" phenomena was found between CR LD/CD and LD/CD standard formulations in a double blind crossover study [85]. The same comparison, in a 24 week randomized double-blind crossover study, did not reveal differences in UPDRS scores, "on" or "off" periods [86]. On the contrary, Jankovic and colleagues reported, in a 24 week double-blind comparative crossover trial including 20 PD patients with wearing off fluctuations, a decrease in "on" time with CR LD/CD compared to standard formulation, with also a trend to an increase in daily "off" time and no difference in dyskinesias [87]. A benefit in "on" time was also shown by a Dutch-British multicentre trial comparing CR LD/CD with standard LD/CD in 170 PD patients. In particular, the proportion of "on" time, assessed by self-scoring diaries, was significantly higher with CR formulation, while "off" periods were significantly limited [88]. CR LD/benserazide has also been tested versus standard LD/benserazide on nocturnal and early morning disability by the UK Madopar CR Study Group in a double-blind randomized crossover study, with the conclusion of an equivalent efficacy of the two formulations [89]. In a randomized double-blind parallel group multicenter study Dupont and colleagues compared clinical efficacy and side effects of slow release LD/benserazide vs standard LD/benserazide among 134 de novo PD patients followed up for 5 years [90]. Slow release formulation had higher impact than standard one on clinical rating scales (Unified Parkinson's Disease Rating Scale – UPDRS; Webster scores, North Western Disability Scale Score), though not reaching statistical significance. No differences in number of doses and overall dosage were found between the two formulations. Thus, slow release LD/benserazide was suggested to have a symptomatic efficacy similar to standard formulation as

monotherapy of de novo PD [79, 90]. Similar results were reported comparing immediate and CR CD/LD in a 5-year trial [91, 92], and in a crossover study comparing CR LD versus standard LD formulation [79].

Overall, studies on slow-release formulations suggest that they have no neuroprotective properties, are equally efficacious as standard LD regimen, and have no definitive evidences supporting a better control or prevention of motor fluctuations [79].

For the treatment of delayed-on and no-on motor fluctuations instant deliver novel formulation have been developed. Djaldetti and colleagues performed a randomized double-blind study comparing an oral solution of LD-ethyl ester and standard LD/CD, showing a reduced time-to-on and a significant limitation of no-on episodes with the former formulation [79, 93]. An effervescent tablet formulation of LD has been developed, under the name of melevodopa, which, compared with conventional tablets, is better absorbed in the stomach and duodenum, resulting in more rapid peak plasmatic levels [94, 95]. Melevodopa has been reported to be more effective than standard LD/CD in drug delivering, especially in cases of problematic LD absorption, and to provide a better overall control of motor symptoms [95, 96].

3.1. New Oral Compounds

Since 1980s, when first CR LD formulations appeared on the market, the target of best pharmacokinetic profile and better drug bioavailability has prompted several studies on different LD formulations.

3.1.1. IPX066 (Rytary ®)

IPX066 is a novel extended release (ER) LD/CD formulation approved in the USA in January 2015 for the treatment of PD independently from the stage [67, 97]. IPX066 combines LD and CD in a 4:1 ratio in a double dissolving tablet, containing both IR and ER formulations, allowing both early onset and longer duration of clinical benefit. Total LD doses per pill in clinical trials ranged from 95 to 390 mg [98, 99]. IPX066 has a Tmax (time to maximum concentration) similar to IR LD/CD, but provides longer timing with plasma concentration above 50% of Cmax (maximum concentration) (4.0 hours versus 1.4 hours) [67, 97, 100, 101].

The APEX-PD study was a randomized, double-blind, placebo-controlled fixed dose 30-week trial that reported the superiority of IPX066 over placebo in the symptomatic treatment of PD, with the evidence of significant benefit derived from the improvement of the UPDRS scores [99]. In the ADVANCE-PD trial IPX066 was compared with IR LD/CD among patients with PD experiencing motor fluctuations with IR LD/CD. In this randomized double-blind 22-week study 393 patients underwent a 3 week open label dose adjustment phase of IR LD/CD, followed by a 6 week period of conversion and dose adjustment for IPX066, after which they entered the double-blind phase. Compared to IR LD/CD, IPX066 was associated with a significant increase in "on" time without dyskinesias (1.1 hours) and a significant reduction in "off" time (1.17 hours overall, 2.18 vs 1.01 hours). Moreover, IPX066 had 3.6 daily doses versus 5.1 of

IR LD/CD, rendering it more suitable for patient compliance. No differences were reported in the incidence of dyskinesias between the two formulations in the ADVANCE-PD trial [97, 98].

The ASCEND-PD trial was a phase III double-blind randomized controlled crossover study designed to directly compare IR LD/CD plus entacapone versus IPX066 among PD patients with at least 2.5 hours/day in "off" time [102]. The protocol, involving 91 patients, contemplated a 6-week dose conversion from LD/CD plus entacapone to IPX066, followed by a two double-blind crossover periods separated by 1 week of open-label IPX066 treatment. Overall, IPX066 had a relevant impact on the reduction of "off" time (3.8 versus 5.2 hours/day), with an 8.5% gross reduction of "off" time/day, and was also associated with higher daily "on" time without dyskinesias (11.4 versus 10 hours/day). Despite a higher median dosage (1723 mg of LD with IPX066 versus 652 mg with standard formulation), daily dosing frequency was reduced with IPX066 treatment, and patient-reported treatment preference was highly in favour of IPX066 [102, 103].

Adverse events from IPX066 have been reported by each trials, and did not differ from those due to standard LD/CD formulations [97]. Nausea, headache, dizziness, insomnia, falls and dyskinesias were reported, with adverse events occurring in up to 43% of patients receiving IPX066. Moreover, in the ASCEND-PD trial, 6 serious adverse events were reported, though none had been linked to the drug [67, 97]. A recent 9-month open-label extension of a phase III trial of IPX066 in patients with early and advanced PD confirmed the efficacy and safety of this drug, the most common adverse events in the study period are dyskinesias (6.9%) and falls (6.6%) [104].

The results provided by the abovementioned trials highlighted the efficacy of IPX066 in reducing "off" times and improving both motor function and quality of life in all PD stages [105]. Hence, the US FDA and the EU approved IPX066 use in PD under the name of Rytary ® or Numient TM [67]. Various formulations are available with total LD dosage of 95,145,195, and 245 mg and 23.75, 36.25, 48.75, and 61.25 mg CD, respectively. Despite no ongoing trials are to date ongoing, it would be interesting to assess the ability to prevent the onset of motor fluctuations with IPX066 early use.

3.1.2. Accordion Pill CD-Levodopa (AP CD/LD)

AP CD/LD and DM-1992 are both LD formulations aimed at achieving better LD uptake through gastric retentive strategy.

AP CD/LD is a gastric-retentive CR formulation of LD/CD containing a multilayer planar structure of a biodegradable film folded in an accordion-like shape to build a standard sized capsule. This formulation dissolves over time as the accordion layers unfold to increase drug absorption, with gastric retention covering up to 12 hours [97, 106]. Phase II multicentre open-label randomized trials have evaluated the pharmacokinetic profile, safety and efficacy of AP CD/LD in patients with PD experiencing motor fluctuations [106, 107]. In particular, a significant reduction in

overall "off" time was reported with the AC CD/LD pill compared to the standard LD treatment (2.4 versus 4.3 hours, 44% reduction) [106]. Safety profile evaluation can only count on limited data, yet troublesome dyskinesia seemed to be reduced up to 40% with AC CD/LD [67, 106]. This promising formulation has been the object of a phase III multicenter double-blind randomized controlled trial, aimed at comparing AC CD/LD with IR LD/CD in PD patients experiencing motor fluctuations. This phase III trial, with stringent enrolling criteria, is currently ongoing and would be of highest importance in assessing clinical efficacy of AC CD/LD (ClinicalTrials.gov NCT02605434).

3.1.3. DM-1992 (DepoMed)

DM-1992 is a further attempt of improvement of the pharmacokinetic profile of LD through gastric retention strategy. DM-1992 is a bilayer formulation containing both an IR LD/CD and an ER LD/CD gastroretentive core. The gastroretentive ER part of LD/CD has been shown to swell upon contact with gastric juices, reaching a size that does not allow a progression through the pylorus. This allows gastric retention until complete dissolution, for up to 9 hours, with the result of a continuous release of LD to the upper small intestine, promoting a prolonged absorption and gradual delivery. DM-1992 twice daily has been reported to provide smoother plasma levels of LD and prolonged therapeutic levels compared to other gastroretentive formulations [108, 109]. A phase II pharmacokinetic/pharmacodynamics study compared DM-1992 to IR CD/LD in 34 PD patients on chronic LD treatment experiencing motor fluctuations [109]. Both formulations were assessed on day 10 in a 12-h assessment period after a single DM-1992 dose or an average four doses of IR CD/LD. Despite not differing significantly in terms of overall LD dosage (609 mg versus 704 mg respectively for DM-1992 and IR formulation), DM-1992 had a tenfold lower ratio of Cmax/Cmin compared to IR CD/LD (9.0 versus 91). Moreover, LD plasmatic levels pre-dosage were significantly higher with DM-1992 (823 ng/mL versus 94 ng/mL), suggesting a prolonged and steady release of LD. Despite no differences in UPDRS-III were reported, DM-1992 decreased daily OFF time compared with baseline by − 0.89 h, while IR LD/CD increased it by 0.19 h. Moreover, an increase in "on" time without troubling dyskinesias was also reported with DM-1992. Unfortunately, the tolerability profile of DM-1992 seemed inconvenient if compared to IR CD/LD, with higher rate of overall adverse events (35.3% vs 14.7%), the most frequent being worsening of parkinsonian gait (8.8 %), dizziness (8.8 %), worsening of hypertonia (5.9 %), headache (5.9 %), and abdominal pain (5.9 %). However, no serious adverse events or withdrawal due to adverse events have been reported, nor have been differences in dyskinesias between groups [67, 97, 109]. To-date no phase III clinical trials have been announced.

3.1.4. XP21279 (XenoPort Inc.)

XP21279 is an ER LD prodrug, composed of an ester-conjugate LD. This formulation is absorbed from the small and large intestine (including the colon and ileus) by high capacity nutrient transporters available in all the gastrointestinal tracts, and then metabolized to LD by carboxylesterases [67]. The aim of this formulation is to provide a better ab-

sorption profile, extending the delivery of LD. XP21279-CD (XP21279-CD) formulation contains 241 mesylate salt, equivalent to 104 mg LD, and CD 25 mg, and has a relative bioavailability of 88.2% of that of IR CD/LD [110, 111]. A phase 1b open-label two period trial was designed to evaluate pharmacokinetic and pharmacodynamics properties of XP21279-CD [110]. Patients received IR CD/LD for 2 weeks followed by XP21279-CD 3 times daily for 2 weeks. Pharmacokinetic results clearly highlighted a more steady plasmatic levels with XP21279-CD compared with IR CD/LD, with absolute deviation from average concentration 34.3 vs 41.6, reduced by 39.4% [111]. No significant differences in Cmax were found. XP21-279-CD treatment was associated with a reduction in daily "off" time of about 30% compared with IR CD/LD, with time to "on" after the first morning dose similar for the two formulations. However, such differences were not confirmed in a phase II double-blind crossover trial considering the efficacy of XP21279-CD on daily "off" period assessed through clinical diaries among 28 PD patients with motor fluctuation. A trend toward greater reduction in "off" time with XP21279 has been reported (3.3 hours/day versus 2.4 hours), but not reaching statistical significance. Adverse event profile was similar for XP21279-CD and IR CD/LD, though dyskinesias were slightly more prevalent with the former (11/12 patients versus 10/14), and dystonias with the latter (2 patients versus 4). Considering that patients already had motor fluctuations at recruitment, new or worsening dyskinesias were more commonly experienced with XP21279-CD (21%) compared to IR CD/LD (14%). All other adverse events, mild or moderate in intensity, such as headache, insomnia, somnolence, gastro-oesophageal reflux, had similar prevalence with both treatments [110]. To-date no plans for future developments of XP21279 have been declared.

3.1.5. ODM-101

ODM-101 is a LD-CD-entacapone (LCE) new oral formulation, containing LD 101 mg and more CD compared to standard LD/CD pills (65 or 105 mg versus 25 mg). ODM-101 has been reported to have a better pharmacokinetic profile than standard LCE preparations. To-date, the only available data on ODM-101 derive from the report, in abstract format, of the results of a phase II randomized double-blind crossover study considering clinical benefit among 117 PD patients with at least 3.0 hours/day in "off" state [112]. In this study, during 3 periods of 4 weeks, conventional LCE formulations have been compared with ODM-101/65 mg and ODM-101/105 mg, assessing "off" time, "on" time and UPDRS part II and III scores. Reduction in daily "off" time prevailed with ODM-101/65 mg compared to ODM-101/105 mg and standard LCE (-1.51 hours versus -1.29 and -0.92 hours respectively), while UPDRS scores and "on" time without significant dyskinesias did not differ significantly among different formulations. No safety concerns have been reported. Dyskinesias were more prevalent among patients receiving ODM-101/105 mg [112]. No studies are to-date ongoing to further investigate this drug.

3.1.6. LD/CD Microtablets (MyFid)

Pursuing the individualization of treatment in terms of dosing and timing, a rapidly soluble tiny tablet formulation

(3 mm in diameter) has been developed [113-115]. This tablet contains LD 5 mg and CD 1.25mg (LC-5, named Flexilev); 750 microtablets (3750 mg LD) are contained in an automatic dose-dispenser, a system named My Flexible Individual Dosing, MyFid [114]. The dose dispenser can be programmed by the physician, and reminds the patient with an alarm the optimal timing and dosage to take. The same dispenser can be used to record motor/non-motor symptoms, and data can be analysed through a dedicated software. LC-5 bioequivalence is similar to conventional LD/CD tablets [115]. In a healthy volunteers' cross-over study, MyFid halved plasma LD fluctuations compared to LD/CD/entacapone [115, 116]. Further investigations are needed to compare clinical efficacy of MyFid with other LD formulations among PD patients. The microtablet, LC-5, was approved by the Swedish MPA in 2014, and, from 2017, LC-5 has received EMA approval for 13 European countries.

4. LEVODOPA FORMULATIONS: INTESTINAL DELIVERY

4.1. Levodopa-Carbidopa Intestinal Gel (LCIG)

LCIG is an LD-CD intestinal delivered carboxymethyl-cellulose acqueous gel, containing LD 20 mg/mL and CD 4.63 mg/mL. The suspension is provided in mono-use cassette containing LCIG 100 mL, equivalent to 2000 mg of LD and 463 mg of CD. The cassette has to be inserted in the portable infusion pump, which delivers LCIG directly in the jejunum *via* a percutaneous endoscopic gastrotomy (PEG-J) [67, 117]. Enteral jejunal infusion improves LD absorption bypassing gastric emptying issues, providing steady plasmatic levels, and reducing diet-related competition for intestinal uptake [97, 118, 119]. Bioavailability of LCIG is 97% of that of IR CD/LD, but LCIG has faster absorption than IR CD/LD. Moreover, pharmacokinetic investigations have shown that plasmatic levels of LD are highly restricted around Cavg over 2 to 16 hours of infusion with LCIG, with very low variations [120, 121]. A phase III double-blind randomized multicentre 12-week trial compared the efficacy of LCIG and IR CD/LD in 71 patients with advanced PD [122]. Patients were randomized to receive placebo intestinal gel infusion combined with oral IR LD/CD or placebo IR LD/CD combined with LCIG infusion. LCIG treatment was associated with a significant reduction in mean daily "off" time (-4.04 hours versus -2.14 hours, difference -1.91 hours), and a significant increase in mean daily "on" time (+4.11 hours versus +2.24 hours, difference +1.86), compared to IR CD/LD. In addition, "on" time with troublesome dyskinesias was reduced by 0.11 h/day in LCIG versus 0.03 h/day in IR CD/LD. Overall, LCIG was effective in the treatment of motor fluctuations in patients with advanced PD, significantly improving "on" time and limiting "off" time and dyskinesias [122].

An open-label prospective 54-week trial assessed the efficacy of LCIG in 192 PD patients experiencing motor fluctuations [123]. Compared with baseline status, LCIG treatment provided a decrease in "off" time of 3.9 hours/day (versus 3.2 at baseline), and an increase in "on" time without troublesome dyskinesias of 4.6 hours/day (*versus* 3.5). Moreover, at follow-up, which included 354 patients, the

benefit of treatment was confirmed, with 4.8 hours/day increase in "on" time and 4.4 hours/day decrease in "off" time [123].

A 2016 meta-analysis performed by Wirdefeldt and colleagues, including double-blind randomized controlled trials and observational studies with more than 10 patients, confirmed the net benefit of LCIG over other treatment strategies [121]. Overall, LCIG improved daily "on" time and motor symptoms, limited daily "off" time and dyskinesias, and improved patients quality of life [121].

In the GLORIA multicentre study, 375 patients have been prospectively enrolled to assess long-term LCIG benefit [124]. At 12 months 172 PD patients completed follow-up, with LCIG mean dosage of 1304 mg/daily. A significant reduction in daily "off" time (4.7 hours/day) and "on" time with dyskinesias (1.7 hours), together with an improvement in UPDRS part II and III scores and overall "on" time without troublesome dyskinesias was reported. Beyond motor control, also non-motor symptoms, assessed through Non-Motor Symptoms Scale (NMSS), were reduced with LCIG, with a mean reduction of NMSS score of -22.2 points. Quality of life was also improved with LCIG treatment, as shown by the reduction of -8.6 points in the Parkinson's Disease Questionnaire short version with eight items (PDQ-8), and by the improvement in the EuroQoL 5-Dimensions (EQ-5D) quality-of-life instrument descriptive scores and visual analog scale (VAS) scores [124].

Motor and cognitive impact of LCIG was also evaluated in long term follow-up studies with a 3-year and a 7-years follow-up [125, 126]. In a 3-year prospective study 25 patients receiving LCIG were evaluated, and a significant benefit in UPDRS part IV, with a score reduction from 8.6 to 5.6 points and a 42% reduction in dyskinesia duration, was reported. Moreover, "off" period duration was limited by 50%, while UPDRS part II and III worsened significantly compared with baseline, suggesting possible no impact on disease course. Interestingly, the PDQ-39 summary index significantly improved from 59.2 at baseline to 43.1 at the 3 year follow-up visit [125]. Quality of life was also reported as improved up to 90% in a 7-year follow-up study of 59 PD patients [126]. Also UPDRS part IV improved by 32%, and overall "off" time was limited by 49%. Only 11% of patients discontinued LCIG treatment before the term of the study [126].

Considering all the available literature, LCIG adverse events have been mostly related to technical and surgical issues (abdominal pain, dislocation of PEG-J, tube disconnections, PEG site infection or inflammation) [Kianirad and Simuni 2016]. Fernandez and colleagues reported adverse events prevalence of 87.5%, but only 7.6% of them leading to treatment dropout [123]. Similar rates were reported by Olanow and colleagues, with adverse events in 95% of patients, 89% being device-related complications, and 3% leading to treatment dropout [122]. In the recent meta-analysis abovementioned, adverse events were confirmed to derive mostly from device-related complications [121]. One of the most important, though not common, adverse events of LCIG is polyneuropathy, which seems to be different from that induced by oral LD treatment. In particular, with LCIG it can assume an acute clinical course, resembling that of Guillain-Barre syndrome. However, only 13.6% of patients experience such adverse event, with a mortality rate at 6 months in case of acute onset of 14% [127-130]. It is important to notice that the vast majority of cases had an axonal sensory-motor polyneuropathy and low vitamin levels [128, 130]. The origin of polyneuropathy, which can happen also with oral LD, has been linked to LD-induced high levels of homocysteine and methylmalonic acid, and reduced absorption of vitamins [128]. Indeed, significant improvement has been reported in these cases with vitamin supplementation, and, if needed, with LCIG cessation [127-129]. Since chronic LD treatment is associated with polyneuropathy and hyperomocysteinemia (LD is a co-enzyme in methionine breakdown), homocysteine and vitamin monitoring, especially B6 and B12, has been suggested [128]. LCIG has been used in Europe since 2004 and was approved by the FDA in 2015.

5. TRANSCUTANEOUS LEVODOPA FORMULATIONS

5.1. ND0612 (NeuroDerm Ltd.)

Trancutaneous LD delivery has been a nightmare for pharmacologists since LD, though small, has poor solubility. ND0612 is a liquid CD/LD formulation to be delivered subcutaneously *via* a small pump-patch device in order to achieve steady plasma levels. ND0612 is available in low dose (0.24 ml/8 hours *via* 1 infusion site, total 115 LD mg) and high dose (0.64 ml/8 hours *via* 2 infusion sites, overall 307 LD mg), but different CD dosages have been used in clinical trials [67, 97, 131]. Good tolerability and safety have been reported by a pilot phase I dose-escalating study on 54 healthy volunteers, with subcutaneous dose ranging from 0.08 to 0.24 ml/h (corresponding to LD 120 to 360 mg/day) and stable plasmatic concentrations from 400 to 500 ng/mL [132]. A phase II double-blind randomized controlled trial of adjunctive treatment with ND0612 low-dose reported a significant increase in plasma LD concentration steadiness compared with placebo [133]. Another phase IIa double-blind randomized placebo-controlled two-period trial of ND0612 (LD/CD 60/14 mg/ml) was performed in patients with PD with motor response fluctuations [134]. Patients, at first randomized to adjunct ND0612 or placebo, were further randomized to receive ND0612 monotherapy or ND0612 plus oral entacapone. Plasma LD levels were maintained at a mean of 550 ng/ml with ND0612 monotherapy and 800 ng/ml with ND0612 plus oral entacapone. With ND0612 the oral LD intake was reduced in average by 80%, and 3 patients also discontinued oral LD. Adjunct ND0612 treatment resulted in a 2 hours reduction in daily "off" time and a 0.47 hours reduction in troublesome dyskinesias compared to baseline [133, 134]. To date, ND0612 has been shown to be well tolerated, the most common adverse event being some small transient papules on injection site [67, 134].

Several ongoing clinical trials are assessing ND0612. Among them, 2 phase II trials are currently recruiting participants to assess efficacy and long term tolerability (Clinicaltrials.gov NCT02577523; Clinicaltrials.gov NCT02726386), while a phase III double-blind randomized controlled trial is ongoing though not recruiting (Clinicaltrials.gov NCT02782481).

6. INHALATION LEVODOPA FORMULATIONS

6.1. CVT-301 (Acorda)

Novel LD formulation also considers patients experiencing unexpected "off" motor fluctuations, such as no-on, delayed on or unpredictable off. In such cases, an instant delivery strategy, even faster than oral melevodopa, might be of great benefit.

CVT-301 is a LD inhalation powder consisting of microparticulate LD, dosed in inhaled puffs. The first development of this formulation has been described by Luinstra and colleagues, which reported a drug lung delivery *via* a high-dose dry powder breath activated inhaler (Cyclops) releasing co-micronized LD formulation with 2% L-leucine eccipient [135]. Lipp and colleagues tested CVT-301 in animal models, 18 healthy volunteers and 24 PD patients, showing a pharmacokinetic profile consistent with an immediate drug release: plasma LD peaked in all animals 2.5 min after administration, while in PD patients an increase in plasma LD concentration over 400 ng/mL was found in 77% of those receiving a 50 mg LD CVT-301 dose, versus only 27% receiving an oral dosing of CD/LD 25/100 mg. CVT-301 had a better pharmacokinetic profile in terms of peak time than oral LD, and also provided net benefit in UPDRS part III scores as early as 5 minutes after administration [136]. Regarding adverse events, the most common was cough, which occurred at inhalation, rapidly resolved, and became less frequent after initial dosing [136].

A single phase II 4-week double-blind randomized controlled trial evaluated CVT-301 versus placebo self-administration among PD patients to relieve "off" episodes [67, 137]. Overall, 86 PD patients with at least 2 hours daily "off" time despite oral LD four times daily were randomized to receive up to 3 daily doses of CVT-301 (titrated from 35 to 50 mg) or placebo for 4 weeks. Patients had their doses with an average of 2.1/day, and experienced a benefit after 10 minutes from drug inhalation. CVT-301 was superior to placebo in reducing UPDRS III score (7.0 points in average), and in limiting daily "off" time (0.9 hours/day compared to baseline) [137]. A phase III study assessing CVT-301 efficacy and tolerability among PD patients experiencing "off" fluctuations is ongoing (SPAN-PD trial), and has been extended from a 12 week to a 12-month follow-up study (Clinicaltrials.gov NCT02240030; Clinicaltrials.gov NCT02242487).

CONCLUSION

Despite more than 60 years old, LD still represents the gold standard of PD treatment. Adjunctive treatments have been developed in the last half century, and yet the most promising field remains the optimization of LD pharmacokinetic profile. Thus, novel formulations are on the verge of being investigated with large randomized double-blind trials to definitely allow their commercialization.

Two main aims animate the research in this field: first, to provide a more stable LD plasmatic concentration, and, second, to find a suitable route through which LD could act almost instantaneously.

The extension of LD half-life, bioavailability and delivery has been gradually implemented, and is to date significantly improved from the first reports of LD efficacy in PD. Providing a 16-hour long constant drug delivery, LCIG can ease motor symptoms, controlling fluctuations and, with extra-doses available, also having impact on sudden onset "off" episodes. A significant impact on "off" time and dyskinesias has been showed for different new oral formulations, such as IPX066, which also has safety and tolerability profiles similar to IR CD/LD. AP LD/CD, a gastroretentive slow-release LD new oral formulation, has been reported to have a highly reliable pharmacokinetic profile and improve daily "off" time, and is under investigation in phase III trials. The other gastroretentive formulation, DM-1992, though promising under a pharmacological profile, did not provide significant benefit in UPDRS scores, and despite having a significant impact on daily "off" time, needs further studies for its efficacy to be rigorously assessed. The same can be said for the prodrug XP21279, whose trials only showed a trend towards benefit in motor function and fluctuations. Results from the SPAN-PD trial, investigating a pulmonary delivered inhaled dry powder formulation of LD, CVT-301, are awaited with interest since preliminary reports reported significant impact on plasma LD concentration and almost instantaneous effect on UPDRS III score.

The ultimate interrogative, beside patient short-term benefit, would be to assess whether a more CR of LD impacts on the future developments of motor fluctuations. If this is the case, and limiting pulsatile stimulation could allow a slower progression in disease course and disability, one may argue whether to start LCIG or other CR formulations as soon as diagnosis is made. This issue, of great interest and highly debated, still has to be addressed by ad-hoc designed studies.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

PC has received personal fees from UCB Pharma and Zambon for speaking and research grants from Merck Serono and Biogen. NT received personal fees from Abbvie, Chiesi, and Lundbeck for speaking. MR declare no conflicts of interest.

REFERENCES

[1]   Olanow, C.W.; Stern, M.B.; Sethi, K. The scientific and clinical basis for the treatment of Parkinson disease (2009). *Neurology,* **2009**, *72*(21)(Suppl. 4), S1-S136. [http://dx.doi.org/10.1212/WNL.0b013e3181a1d44c] [PMID: 19470958]

[2]   Rajput, A.H.; Birdi, S. Epidemiology of Parkinson's disease. *Parkinsonism Relat. Disord.,* **1997**, *3*(4), 175-186. [http://dx.doi.org/10.1016/S1353-8020(97)00029-1] [PMID: 18591073]

[3]   Jellinger, K.A. Morphological substrates of parkinsonism with and without dementia: a retrospective clinico-pathological study. *J. Neural Transm. Suppl.,* **2007**, *72*(72), 91-104. [PMID: 17982882]

[4]   Nagatsu, T.; Levitt, M.; Udenfriend, S. Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. *J. Biol. Chem.,* **1964**, *239*, 2910-2917. [PMID: 14216443]

[5]   Cooksey, C.J.; Garratt, P.J.; Land, E.J.; Pavel, S.; Ramsden, C.A.; Riley, P.A.; Smit, N.P. Evidence of the indirect formation of the catecholic intermediate substrate responsible for the auto-activation kinetics of tyrosinase. *J. Biol. Chem.,* **1997**, *272*(42), 26226-26235. [http://dx.doi.org/10.1074/jbc.272.42.26226] [PMID: 9334191]

[6]   Nagatsua, T.; Sawadab, M. L-dopa therapy for Parkinson's disease: past, present, and future. *Parkinsonism Relat. Disord.,* **2009**, *15*(Suppl. 1), S3-S8. [http://dx.doi.org/10.1016/S1353-8020(09)70004-5] [PMID: 19131039]

[7]   Lovenberg, W.; Weissbach, H.; Udenfriend, S. Aromatic L-amino acid decarboxylase. *J. Biol. Chem.,* **1962**, *237*, 89-93. [PMID: 14466899]

[8]   Parkinson, J. *An Essay on the Shaking Palsy*; Sherwood, Neely and Jones: London, **1871**.

[9]   Sano, I.; Gamo, T.; Kakimoto, Y.; Taniguchi, K.; Takesada, M.; Nishinuma, K. Distribution of catechol compounds in human brain. *Biochim. Biophys. Acta,* **1959**, *32*, 586-587. [http://dx.doi.org/10.1016/0006-3002(59)90652-3] [PMID: 14441532]

[10]   Hornykiewicz, O. L-DOPA: from a biologically inactive amino acid to a successful therapeutic agent. *Amino Acids,* **2002**, *23*(1-3), 65-70. [http://dx.doi.org/10.1007/s00726-001-0111-9] [PMID: 12373520]

[11]   Abbott, A. Levodopa: the story so far. *Nature,* **2010**, *466*(7310), S6-S7. [http://dx.doi.org/10.1038/466S6a] [PMID: 20739934]

[12]   Guggenheim, M. Dioxyphenylalanin, eine neue Amino- säure aus Vicia faba. *Hoppe Seylers Z. Physiol. Chem.,* **1913**, *88*, 276-284. [http://dx.doi.org/10.1515/bchm2.1913.88.4.276]

[13]   Oster, K.A.; Sorkin, S.Z. The effects of intravenous injection of L-dopa upon blood pressure. *Proc. Soc. Exp. Biol. Med.,* **1942**, *51*, 67-71. [http://dx.doi.org/10.3181/00379727-51-13831]

[14]   Holtz, P.; Credner, K.; Kroneberg, G. Über das sympathiko-mimetische pressorische Prinzip des Hirn ("Urosympathin"). *Arch. Exp. Path. Pharmik.,* **1947**, *204*, 228-243. [http://dx.doi.org/10.1007/BF00738347]

[15]   LeWitt, P.A.; Fahn, S. Levodopa therapy for Parkinson disease: A look backward and forward. *Neurology,* **2016**, *86*(14)(Suppl. 1), S3-S12. [http://dx.doi.org/10.1212/WNL.0000000000002509] [PMID: 27044648]

[16]   Carlsson, A.; Lindqvist, M.; Magnusson, T. 3,4-Dihydro-xyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature,* **1957**, *180*(4596), 1200. [http://dx.doi.org/10.1038/1801200a0] [PMID: 13483658]

[17]   Carlsson, A. Monoamine-depleting drugs. *Pharmacol. Ther. [B],* **1975**, *1*(3), 393-400. [http://dx.doi.org/10.1016/0306-039X(75)90045-8] [PMID: 772709]

[18]   Carlsson, A.; Hillarp, N.A. Release of adenosine triphosphate along with adrenaline and noradrenaline following stimulation of the ad-renal medulla. *Acta Physiol. Scand.,* **1956**, *37*(2-3), 235-239. [http://dx.doi.org/10.1111/j.1748-1716.1956.tb01359.x] [PMID: 13361903]

[19]   Carlsson, A. Treatment of Parkinson's with L-DOPA. The early discovery phase, and a comment on current problems. *J. Neural Transm. (Vienna),* **2002**, *109*(5-6), 777-787. [http://dx.doi.org/10.1007/s007020200064] [PMID: 12111467]

[20]   Carlsson, A. The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacol. Rev.,* **1959**, *11*(2, Part 2), 490-493. [PMID: 13667431]

[21]   Sano, I.; Gamo, T.; Kakimoto, Y.; Taniguchi, K.; Takesada, M.; Nishinuma, K. Distribution of catechol compounds in human brain. *Biochim. Biophys. Acta,* **1959**, *32*, 586-587. [http://dx.doi.org/10.1016/0006-3002(59)90652-3] [PMID: 14441532]

[22]   Sano, I. Biochemistry of the extrapyramidal system. *Shinkei Kenkyu No Shinnpo (Adv Neurol Sci)*, **1960**, 5, 42-48. (Translated into English by Sano, A. *Parkinsonism Relat. Disord.,* **2000**, *6*, 303-306.

[23]   Ehringer, H.; Hornykiewicz, O. Verteilung von noradrenalin und Dopamin (3-Hydroxytyramin) im nehirn des menschen und ihr verhalten bei erkrankungen des extrapyramidaren systems. *Klin. Wochenschr.,* **1960**, *38*, 1236-1239. [http://dx.doi.org/10.1007/BF01485901] [PMID: 13726012]

[24]   Birkmayer, W.; Hornykiewicz, O. The effect of l-3,4-dihydroxyphenylalanine (=DOPA) on akinesia in parkinsonism. *Parkinsonism Relat. Disord.,* **1998**, *4*(2), 59-60. [http://dx.doi.org/10.1016/S1353-8020(98)00013-3] [PMID: 18591089]

[25]   McGeer, P.L.; Zeldowicz, L.R. Administration of dihydroxyphenyl-lalanine to parkinsonian patients. *Can. Med. Assoc. J.,* **1964**, *90*, 463-466. [PMID: 14120951]

[26]   Bertler, A.; Rosengren, E. Possible role of brain dopamine. *Pharmacol. Rev.,* **1966**, *18*(1), 769-773. [PMID: 5904188]

[27]   Cotzias, G.C.; Van Woert, M.H.; Schiffer, L.M. Aromatic amino acids and modification of parkinsonism. *N. Engl. J. Med.,* **1967**, *276*(7), 374-379. [http://dx.doi.org/10.1056/NEJM196702162760703] [PMID: 5334614]

[28]   Hauser, R.A. Levodopa: past, present, and future. *Eur. Neurol.,* **2009**, *62*(1), 1-8. [http://dx.doi.org/10.1159/000215875] [PMID: 19407449]

[29]   Tambasco, N.; Belcastro, V.; Gallina, A.; Castrioto, A.; Calabresi, P.; Rossi, A. Levodopa-induced breathing, cognitive and behav-ioral changes in Parkinson's disease. *J. Neurol.,* **2011**, *258*(12), 2296-2299. [http://dx.doi.org/10.1007/s00415-011-6119-5] [PMID: 21647729]

[30]   LeWitt, P.A. Levodopa therapy for Parkinson's disease: Pharma-cokinetics and pharmacodynamics. *Mov. Disord.,* **2015**, *30*(1), 64-72. [http://dx.doi.org/10.1002/mds.26082] [PMID: 25449210]

[31]   Cotzias, G.C.; Papavasiliou, P.S.; Gellene, R. Modification of Parkinsonism--chronic treatment with L-dopa. *N. Engl. J. Med.,* **1969**, *280*(7), 337-345. [http://dx.doi.org/10.1056/NEJM196902132800701] [PMID: 4178641]

[32]   Yahr, M.D.; Duvoisin, R.C.; Schear, M.J.; Barrett, R.E.; Hoehn, M.M. Treatment of parkinsonism with levodopa. *Arch. Neurol.,* **1969**, *21*(4), 343-354. [http://dx.doi.org/10.1001/archneur.1969.00480160015001] [PMID: 5820999]

[33]   Lloyd, K.G.; Davidson, L.; Hornykiewicz, O. The neurochemistry of Parkinson's disease: effect of L-dopa therapy. *J. Pharmacol. Exp. Ther.,* **1975**, *195*(3), 453-464. [PMID: 489]

[34]   Hornykiewicz, O. *How does L-dopa work?*; Barbeau A, McDowell FH: Philadelphia, **1970**, pp. 393-399.

[35]   Fahn, S. Levodopa in the treatment of Parkinson's disease. *J. Neu-ral Transm. Suppl.,* **2006**, *71*(71), 1-15. [PMID: 17447410]

[36]   Shoulson, I.; Glaubiger, G.A.; Chase, T.N. On-off response. Clini-cal and biochemical correlations during oral and intravenous levodopa administration in parkinsonian patients. *Neurology,* **1975**, *25*(12), 1144-1148. [http://dx.doi.org/10.1212/WNL.25.12.1144] [PMID: 812004]

[37]   Kurlan, R.; Rubin, A.J.; Miller, C.; Rivera-Calimlim, L.; Clarke, A.; Shoulson, I. Duodenal delivery of levodopa for on-off fluctua-tions in parkinsonism: preliminary observations. *Ann. Neurol.,* **1986**, *20*(2), 262-265. [http://dx.doi.org/10.1002/ana.410200213] [PMID: 3752968]

[38]   Nutt, J.G.; Fellman, J.H. Pharmacokinetics of levodopa. *Clin. Neuropharmacol.,* **1984**, *7*(1), 35-49. [http://dx.doi.org/10.1097/00002826-198403000-00002] [PMID: 6367973]

[39]   Leenders, K.L.; Poewe, W.H.; Palmer, A.J.; Brenton, D.P.; Frack-owiak, R.S. Inhibition of L-[18F]fluorodopa uptake into human brain by amino acids demonstrated by positron emission tomogra-phy. *Ann. Neurol.,* **1986**, *20*(2), 258-262. [http://dx.doi.org/10.1002/ana.410200212] [PMID: 3092728]

[40]   Camargo, S.M.R.; Vuille-dit-Bille, R.N.; Mariotta, L.; Ramadan, T.; Huggel, K.; Singer, D.; Götze, O.; Verrey, F. The molecular mechanism of intestinal levodopa absorption and its possible impli-cations for the treatment of Parkinson's disease. *J. Pharmacol. Exp. Ther.,* **2014**, *351*(1), 114-123. [http://dx.doi.org/10.1124/jpet.114.216317] [PMID: 25073474]

[41]   Calne, D.B.; Karoum, F.; Ruthven, C.R.J.; Sandler, M. The metabolism of orally administered L-Dopa in Parkinsonism. *Br. J. Pharmacol.,* **1969**, *37*(1), 57-68. [http://dx.doi.org/10.1111/j.1476-5381.1969.tb09522.x] [PMID: 5343357]

[42]   Bartholini, G.; Burkard, W.P.; Pletscher, A.; Bates, H.M. Increase of cerebral catecholamines caused by 3,4-dihydroxyphenylalanine after inhibition of peripheral decarboxylase. *Nature,* **1967**, *215*(5103), 852-853. [http://dx.doi.org/10.1038/215852b0] [PMID: 6049735]

[43]　Nutt, J.G.; Woodward, W.R.; Anderson, J.L. Effect of carbidopa on pharmacokinetics of intravenously administered levodopa: implications for mechanism of action of carbidopa in the treatment of Parkinsonism. *Ann. Neurol.,* **1985**, *13*, 537-544. [http://dx.doi.org/10.1002/ana.410180505] [PMID: 4073849]

[44]　Jankovic, J. Levodopa strengths and weaknesses. *Neurology,* **2002**, *58*(4)(Suppl. 1), S19-S32. [http://dx.doi.org/10.1212/WNL.58.suppl_1.S19] [PMID: 11909982]

[45]　Pletscher, A.; DaPrada, M. Pharmacotherapy of Parkinson's disease: research from 1960 to 1991. *Acta Neurol. Scand. Suppl.,* **1993**, *146*, 26-31. [PMID: 8101413]

[46]　Papavasiliou, P.S.; Cotzias, G.C.; Düby, S.E.; Steck, A.J.; Fehling, C.; Bell, M.A. Levodopa in Parkinsonism: potentiation of central effects with a peripheral inhibitor. *N. Engl. J. Med.,* **1972**, *286*(1), 8-14. [http://dx.doi.org/10.1056/NEJM197201062860102] [PMID: 4550085]

[47]　Birkmayer, W.; Mentasti, M. [Further experimental studies on the catecholamine metabolism in extrapyramidal diseases (Parkinson and chorea syndromes)]. *Arch Psychiatr Nervenkr (1970),* **1967**, *210*(1), 29-35. [http://dx.doi.org/10.1007/BF01217333] [PMID: 4292871]

[48]　Huebert, N.D.; Palfreyman, M.G.; Haegele, K.D. A comparison of the effects of reversible and irreversible inhibitors of aromatic L-amino acid decarboxylase on the half-life and other pharmacokinetic parameters of oral L-3,4-dihydroxyphenylalanine. *Drug Metab. Dispos.,* **1983**, *11*(3), 195-200. [PMID: 6135575]

[49]　Salat, D.; Tolosa, E. Levodopa in the treatment of Parkinson's disease: current status and new developments. *J. Parkinsons Dis.,* **2013**, *3*(3), 255-269. [PMID: 23948989]

[50]　Markham, C.; Diamond, S.G.; Treciokas, L.J. Carbidopa in Parkinson disease and in nausea and vomiting of levodopa. *Arch. Neurol.,* **1974**, *31*(2), 128-133. [http://dx.doi.org/10.1001/archneur.1974.00490380076010] [PMID: 4834974]

[51]　Lieberman, A.; Goodgold, A.; Jonas, S.; Leibowitz, M. Comparison of dopa decarboxylase inhibitor (carbidopa) combined with levodopa and levodopa alone in Parkinson's disease. *Neurology,* **1975**, *25*(10), 911-916. [http://dx.doi.org/10.1212/WNL.25.10.911] [PMID: 1101099]

[52]　Calabresi, P.; Di Filippo, M.; Ghiglieri, V.; Tambasco, N.; Picconi, B. Levodopa-induced dyskinesias in patients with Parkinson's disease: filling the bench-to-bedside gap. *Lancet Neurol.,* **2010**, *9*(11), 1106-1117. [http://dx.doi.org/10.1016/S1474-4422(10)70218-0] [PMID: 20880751]

[53]　Cedarbaum, J.M.; Kutt, H.; Dhar, A.K.; Watkins, S.; McDowell, F.H. Effect of supplemental carbidopa on bioavailability of L-dopa. *Clin. Neuropharmacol.,* **1986**, *9*(2), 153-159. [http://dx.doi.org/10.1097/00002826-198604000-00006] [PMID: 3085927]

[54]　Ward, C.D.; Trombley, I.K.; Calne, D.B.; Kopin, I.J. L-dopa decarboxylation in chronically treated patients. *Neurology,* **1984**, *34*(2), 198-201. [http://dx.doi.org/10.1212/WNL.34.2.198] [PMID: 6538008]

[55]　Dingemanse, J.; Kleinbloesem, C.H.; Zürcher, G.; Wood, N.D.; Crevoisier, C. Pharmacodynamics of benserazide assessed by its effects on endogenous and exogenous levodopa pharmacokinetics. *Br. J. Clin. Pharmacol.,* **1997**, *44*(1), 41-48. [http://dx.doi.org/10.1046/j.1365-2125.1997.00610.x] [PMID: 9241095]

[56]　Eberling, J.L.; Jagust, W.J.; Christine, C.W.; Starr, P.; Larson, P.; Bankiewicz, K.S.; Aminoff, M.J. Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology,* **2008**, *70*(21), 1980-1983. [http://dx.doi.org/10.1212/01.wnl.0000312381.29287.ff] [PMID: 18401019]

[57]　Mittermeyer, G.; Christine, C.W.; Rosenbluth, K.H.; Baker, S.L.; Starr, P.; Larson, P.; Kaplan, P.L.; Forsayeth, J.; Aminoff, M.J.; Bankiewicz, K.S. Long-term evaluation of a phase 1 study of AADC gene therapy for Parkinson's disease. *Hum. Gene Ther.,* **2012**, *23*(4), 377-381. [http://dx.doi.org/10.1089/hum.2011.220] [PMID: 22424171]

[58]　Palfi, S.; Gurruchaga, J.M.; Ralph, G.S.; Lepetit, H.; Lavisse, S.; Buttery, P.C.; Watts, C.; Miskin, J.; Kelleher, M.; Deeley, S.; Iwamuro, H.; Lefaucheur, J.P.; Thiriez, C.; Fenelon, G.; Lucas, C.; Brugières, P.; Gabriel, I.; Abhay, K.; Drouot, X.; Tani, N.; Kas, A.; Ghaleh, B.; Le Corvoisier, P.; Dolphin, P.; Breen, D.P.; Mason, S.;

Guzman, N.V.; Mazarakis, N.D.; Radcliffe, P.A.; Harrop, R.; Kingsman, S.M.; Rascol, O.; Naylor, S.; Barker, R.A.; Hantraye, P.; Remy, P.; Cesaro, P.; Mitrophanous, K.A. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. *Lancet,* **2014**, *383*(9923), 1138-1146. [http://dx.doi.org/10.1016/S0140-6736(13)61939-X] [PMID: 24412048]

[59]　Contin, M.; Martinelli, P. Pharmacokinetics of levodopa. *J. Neurol.,* **2010**, *257*(Suppl. 2), S253-S261. [http://dx.doi.org/10.1007/s00415-010-5728-8] [PMID: 21080186]

[60]　Müller, T. Catechol-O-methyltransferase inhibitors in Parkinson's disease. *Drugs,* **2015**, *75*(2), 157-174. [http://dx.doi.org/10.1007/s40265-014-0343-0] [PMID: 25559423]

[61]　Männistö, P.T.; Kaakkola, S. New selective COMT inhibitors: useful adjuncts for Parkinson's disease? *Trends Pharmacol. Sci.,* **1989**, *10*(2), 54-56. [http://dx.doi.org/10.1016/0165-6147(89)90075-8] [PMID: 2655238]

[62]　Lee, E.S.; Chen, H.; King, J.; Charlton, C. The role of 3-O-methyldopa in the side effects of L-dopa. *Neurochem. Res.,* **2008**, *33*(3), 401-411. [http://dx.doi.org/10.1007/s11064-007-9442-6] [PMID: 17713853]

[63]　Tambasco, N.; Muti, M.; Chiarini, P.; Tarducci, R.; Caproni, S.; Castrioto, A.; Nigro, P.; Parnetti, L.; Floridi, P.; Rossi, A.; Calabresi, P. Entacapone reduces cortical activation in Parkinson's disease with wearing-off: a f-MRI study. **2014**, *9*(5), e96806 [http://dx.doi.org/10.1371/journal.pone.0096806]

[64]　Pahwa, R.; Factor, S.A.; Lyons, K.E.; Ondo, W.G.; Gronseth, G.; Bronte-Stewart, H.; Hallett, M.; Miyasaki, J.; Stevens, J.; Weiner, W.J. Practice Parameter: treatment of Parkinson disease with motor fluctuations and dyskinesia (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology,* **2006**, *66*(7), 983-995. [http://dx.doi.org/10.1212/01.wnl.0000215250.82576.87] [PMID: 16606909]

[65]　Kuruma, I.; Bartholini, G.; Tissot, R.; Pletscher, A. The metabolism of L-3-O-methyldopa, a precursor of dopa in man. *Clin. Pharmacol. Ther.,* **1971**, *12*(4), 678-682. [http://dx.doi.org/10.1002/cpt1971124678] [PMID: 5567811]

[66]　Bonifácio, M.J.; Palma, P.N.; Almeida, L.; Soares-da-Silva, P. Catechol-O-methyltransferase and its inhibitors in Parkinson's disease. *CNS Drug Rev.,* **2007**, *13*(3), 352-379. [http://dx.doi.org/10.1111/j.1527-3458.2007.00020.x] [PMID: 17894650]

[67]　Freitas, M.E.; Ruiz-Lopez, M.; Fox, S.H. Novel levodopa formulations for Parkinson's Disease. *CNS Drugs,* **2016**, *30*(11), 1079-1095. [http://dx.doi.org/10.1007/s40263-016-0386-8] [PMID: 27743318]

[68]　LeWitt, P.A.; Jennings, D.; Lyons, K.E.; Pahwa, R.; Rabinowicz, A.L.; Wang, J.; Guarnieri, M.; Hubble, J.P.; Murck, H. Pharmacokinetic-pharmacodynamic crossover comparison of two levodopa extension strategies. *Mov. Disord.,* **2009**, *24*(9), 1319-1324. [http://dx.doi.org/10.1002/mds.22587] [PMID: 19412946]

[69]　Obeso, J.A.; Rodriguez-Oroz, M.C.; Chana, P.; Lera, G.; Rodriguez, M.; Olanow, C.W. The evolution and origin of motor complications in Parkinson's disease. *Neurology,* **2000**, *55*(11)(Suppl. 4), S13-S20. [PMID: 11147505]

[70]　Olanow, C.W.; Obeso, J.A.; Stocchi, F. Drug insight: Continuous dopaminergic stimulation in the treatment of Parkinson's disease. *Nat. Clin. Pract. Neurol.,* **2006**, *2*(7), 382-392. [http://dx.doi.org/10.1038/ncpneuro0222] [PMID: 16932589]

[71]　Pilleri, M.; Antonini, A. Novel levodopa formulations in the treatment of Parkinson's disease. *Expert Rev. Neurother.,* **2014**, *14*(2), 143-149. [http://dx.doi.org/10.1586/14737175.2014.877840] [PMID: 24428803]

[72]　Contin, M.; Riva, R.; Albani, F.; Baruzzi, A. Pharmacokinetic optimisation in the treatment of Parkinson's disease. *Clin. Pharmacokinet.,* **1996**, *30*(6), 463-481. [http://dx.doi.org/10.2165/00003088-199630060-00004] [PMID: 8792058]

[73]　Olanow, C.W.; Obeso, J.A.; Stocchi, F. Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. *Lancet Neurol.,* **2006**, *5*(8), 677-687. [http://dx.doi.org/10.1016/S1474-4422(06)70521-X] [PMID: 16857573]

[74]　Nyholm, D. Pharmacokinetic optimisation in the treatment of Parkinson's disease : an update. *Clin. Pharmacokinet.,* **2006**, *45*(2),

109-136. [http://dx.doi.org/10.2165/00003088-200645020-00001] [PMID: 16485914]

[75] Meloni, M.; Solla, P.; Mascia, M.M.; Marrosu, F.; Cannas, A. Diphasic dyskinesias during levodopa-carbidopa intestinal gel (LCIG) infusion in Parkinson's disease. *Parkinsonism Relat. Disord.,* **2016**, *37,*92-96. [PMID: 28063683]

[76] Olanow, W.; Schapira, A.H.; Rascol, O. Continuous dopamine-receptor stimulation in early Parkinson's disease. *Trends Neurosci.,* **2000**, *23*(10)(Suppl.), S117-S126. [http://dx.doi.org/10.1016/S1471-1931(00)00030-6] [PMID: 11052229]

[77] Jenner, P.; McCreary, A.C.; Scheller, D.K. Continuous drug delivery in early- and late-stage Parkinson's disease as a strategy for avoiding dyskinesia induction and expression. *J. Neural Transm. (Vienna),* **2011**, *118*(12), 1691-1702. [http://dx.doi.org/10.1007/s00702-011-0703-9] [PMID: 21881838]

[78] Rascol, O.; Korczyn, A.; De Deyn, P.P.; Lang, A. Incidence of dyskinesias in a 10-year naturalistic follow-up of patients with early Parkinson's disease (PD) initially receiving ropinirole compared with L-dopa. *Mov. Disord.,* **2005**, *20*(Suppl. 10), S63.

[79] Goetz, C.G.; Koller, W.C.; Poewe, W. Management of Parkinson's disease: an evidence-based review. *Mov. Disord.,* **2002**, *17*(Suppl. 4), S1-S166. [PMID: 12211134]

[80] Fahn, S.; Oakes, D.; Shoulson, I.; Kieburtz, K.; Rudolph, A.; Lang, A.; Olanow, C.W.; Tanner, C.; Marek, K. Levodopa and the progression of Parkinson's disease. *N. Engl. J. Med.,* **2004**, *351*(24), 2498-2508. [http://dx.doi.org/10.1056/NEJMoa033447] [PMID: 15590952]

[81] Goetz, C.G.; Poewe, W.; Rascol, O.; Sampaio, C. Evidence-based medical review update: pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. *Mov. Disord.,* **2005**, *20*(5), 523-539. [http://dx.doi.org/10.1002/mds.20464] [PMID: 15818599]

[82] Sage, J.I.; Mark, M.H. Comparison of controlled-release Sinemet (CR4) and standard Sinemet (25 mg/100 mg) in advanced Parkinson's disease: a double-blind, crossover study. *Clin. Neuropharmacol.,* **1988**, *11*(2), 174-179. [http://dx.doi.org/10.1097/00002826-198804000-00009] [PMID: 3378226]

[83] Ahlskog, J.E.; Muenter, M.D.; McManis, P.G.; Bell, G.N.; Bailey, P.A. Controlled-release Sinemet (CR-4): a double-blind crossover study in patients with fluctuating Parkinson's disease. *Mayo Clin. Proc.,* **1988**, *63*(9), 876-886. [http://dx.doi.org/10.1016/S0025-6196(12)62690-1] [PMID: 3045435]

[84] Hutton, J.T.; Morris, J.L.; Bush, D.F.; Smith, M.E.; Liss, C.L.; Reines, S. Multicenter controlled study of Sinemet CR *vs* Sinemet (25/100) in advanced Parkinson's disease. *Neurology,* **1989**, *39*(11)(Suppl. 2), 67-72. [PMID: 2685652]

[85] Feldman, R.G.; Mosbach, P.A.; Kelly, M.R.; Thomas, C.A.; Saint Hilaire, M.H. Double-blind comparison of standard Sinemet and Sinemet CR in patients with mild-to-moderate Parkinson's disease. *Neurology,* **1989**, *39*(11)(Suppl. 2), 96-101. [PMID: 2685655]

[86] Lieberman, A.; Gopinathan, G. Miller, E.; Neophytides, A.; Baumann, G.; Chin, L. Ran- domized double-blind cross-over study of Sinemet-Controlled Release (CR4 50/ 200) versus Sinemet 25/100 in Parkinson's disease. *Eur. Neurol.,* **1990**, *30*, 75-78. [http://dx.doi.org/10.1159/000117314] [PMID: 2340838]

[87] Jankovic, J.; Schwartz, K.; Vander Linden, C. Comparison of Sinemet CR4 and standard Sinemet: double blind and long-term open trial in parkinsonian patients with fluctuations. *Mov. Disord.,* **1989**, *4*(4), 303-309. [http://dx.doi.org/10.1002/mds.870040403] [PMID: 2682215]

[88] Wolters, E.C.; Horstink, M.W.; Roos, R.A.; Jansen, E.N. Clinical efficacy of Sinemet CR 50/200 versus Sinemet 25/100 in patients with fluctuating Parkinson's disease. An open, and a double-blind, double-dummy, multicenter treatment evaluation. *Clin. Neurol. Neurosurg.,* **1992**, *94*(3), 205-211. [http://dx.doi.org/10.1016/0303-8467(92)90090-P] [PMID: 1327609]

[89] A comparison of Madopar CR and standard Madopar in the treatment of nocturnal and early-morning disability in Parkinson's disease. *Clin. Neuropharmacol.,* **1989**, *12*(6), 498-505. [PMID: 2691069]

[90] Dupont, E.; Andersen, A.; Boas, J.; Boisen, E.; Borgmann, R.; Helgetveit, A.C.; Kjaer, M.O.; Kristensen, T.N.; Mikkelsen, B.; Pakkenberg, H.; Presthus, J.; Stien, R.; Worm-Petersen, J.; Buch,

D. Sustained-release Madopar HBS compared with standard Madopar in the long-term treatment of de novo parkinsonian patients. *Acta Neurol. Scand.,* **1996**, *93*(1), 14-20. [http://dx.doi.org/10.1111/j.1600-0404.1996.tb00163.x] [PMID: 8825266]

[91] Block, G.; Liss, C.; Reines, S.; Irr, J.; Nibbelink, D. Comparison of immediate-release and controlled release carbidopa/levodopa in Parkinson's disease. A multicenter 5-year study. *Eur. Neurol.,* **1997**, *37*(1), 23-27. [http://dx.doi.org/10.1159/000117399] [PMID: 9018028]

[92] Koller, W.C.; Hutton, J.T.; Tolosa, E.; Capilldeo, R. Immediate-release and controlled-release carbidopa/levodopa in PD: a 5-year randomized multicenter study. *Neurology,* **1999**, *53*(5), 1012-1019. [http://dx.doi.org/10.1212/WNL.53.5.1012] [PMID: 10496260]

[93] Djaldetti, R.; Inzelberg, R.; Giladi, N.; Korczyn, A.D.; Peretz-Aharon, Y.; Rabey, M.J.; Herishano, Y.; Honigman, S.; Badarny, S.; Melamed, E. Oral solution of levodopa ethylester for treatment of response fluctuations in patients with advanced Parkinson's disease. *Mov. Disord.,* **2002**, *17*(2), 297-302. [http://dx.doi.org/10.1002/mds.10075] [PMID: 11921115]

[94] Stocchi, F.; Fabbri, L.; Vecsei, L.; Krygowska-Wajs, A.; Monici Preti, P.A.; Ruggieri, S.A. Clinical efficacy of a single afternoon dose of effervescent levodopa-carbidopa preparation (CHF 1512) in fluctuating Parkinson disease. *Clin. Neuropharmacol.,* **2007**, *30*(1), 18-24. [http://dx.doi.org/10.1097/01.WNF.0000236762.77913.C6] [PMID: 17272966]

[95] Stocchi, F.; Vacca, L.; Grassini, P.; Pawsey, S.; Whale, H.; Marconi, S.; Torti, M. L-Dopa pharmacokinetic profile with effervescent melevodopa/carbidopa *versus* standard-release levodopa/carbidopa tablets in Parkinson's Disease: A Randomised Study. *Parkinsons Dis.,* **2015**, *2015*, 369465. [http://dx.doi.org/10.1155/2015/369465] [PMID: 26171276]

[96] Silva, F. Effects of CHF 1512, a new combination of levodopa methylester and carbidopa, on motor fluctuations in Parkinson's disease: results from a European study. *Mov. Disord.,* **2006**, *21*(13), 119-120.

[97] Kianirad, Y.; Simuni, T. Novel approaches to optimization of levodopa therapy for Parkinson's Disease. *Curr. Neurol. Neurosci. Rep.,* **2016**, *16*(4), 34. [http://dx.doi.org/10.1007/s11910-016-0635-8] [PMID: 26898686]

[98] Hauser, R.A.; Hsu, A.; Kell, S.; Espay, A.J.; Sethi, K.; Stacy, M.; Ondo, W.; O'Connell, M.; Gupta, S. Extended-release carbidopa-levodopa (IPX066) compared with immediate-release carbidopa-levodopa in patients with Parkinson's disease and motor fluctuations: a phase 3 randomised, double-blind trial. *Lancet Neurol.,* **2013**, *12*(4), 346-356. [http://dx.doi.org/10.1016/S1474-4422(13)70025-5] [PMID: 23485610]

[99] Pahwa, R.; Lyons, K.E.; Hauser, R.A.; Fahn, S.; Jankovic, J.; Pourcher, E.; Hsu, A.; O'Connell, M.; Kell, S.; Gupta, S. Randomized trial of IPX066, carbidopa/levodopa extended release, in early Parkinson's disease. *Parkinsonism Relat. Disord.,* **2014**, *20*(2), 142-148. [http://dx.doi.org/10.1016/j.parkreldis.2013.08.017] [PMID: 24055014]

[100] Hauser, R.A.; Ellenbogen, A.L.; Metman, L.V.; Hsu, A.; O'Connell, M.J.; Modi, N.B.; Yao, H.M.; Kell, S.H.; Gupta, S.K. Crossover comparison of IPX066 and a standard levodopa formulation in advanced Parkinson's disease. *Mov. Disord.,* **2011**, *26*(12), 2246-2252. [http://dx.doi.org/10.1002/mds.23861] [PMID: 21755537]

[101] Mao, Z.; Hsu, A.; Gupta, S.; Modi, N.B. Population pharmacodynamics of IPX066: an oral extended-release capsule formulation of carbidopa-levodopa, and immediate-release carbidopa-levodopa in patients with advanced Parkinson's disease. *J. Clin. Pharmacol.,* **2013**, *53*(5), 523-531. [http://dx.doi.org/10.1002/jcph.63] [PMID: 23426902]

[102] Stocchi, F.; Hsu, A.; Khanna, S.; Ellenbogen, A.; Mahler, A.; Liang, G.; Dillmann, U.; Rubens, R.; Kell, S.; Gupta, S. Comparison of IPX066 with carbidopa-levodopa plus entacapone in advanced PD patients. *Parkinsonism Relat. Disord.,* **2014**, *20*(12), 1335-1340. [http://dx.doi.org/10.1016/j.parkreldis.2014.08.004] [PMID: 25306200]

[103] Nausieda, P.A.; Hsu, A.; Elmer, L.; Gil, R.A.; Spiegel, J.; Singer, C.; Khanna, S.; Rubens, R.; Kell, S.; Modi, N.B.; Gupta, S. Conversion to IPX066 from standard levodopa formulations in ad-

vanced Parkinson's disease: experience in clinical trials. *J. Parkinsons Dis.,* **2015**, *5*(4), 837-845. [http://dx.doi.org/10.3233/JPD-150622] [PMID: 26444090]

[104]   Waters, C.H.; Nausieda, P.; Dzyak, L.; Spiegel, J.; Rudzinska, M.; Silver, D.E.; Tsurkalenko, E.S.; Kell, S.; Hsu, A.; Khanna, S.; Gupta, S. Long-term treatment with extended-release carbidopa-levodopa (IPX066) in early and advanced Parkinson's disease: a 9-month open-label extension trial. *CNS Drugs,* **2015**, *29*(4), 341-350. [http://dx.doi.org/10.1007/s40263-015-0242-2] [PMID: 25895021]

[105]   Greig, S.L.; McKeage, K. Carbidopa/levodopa ER capsules (Rytary®, Numient™): a review in Parkinson's disease. *CNS Drugs,* **2016**, *30*(1), 79-90. [http://dx.doi.org/10.1007/s40263-015-0306-3] [PMID: 26692288]

[106]   LeWitt, P.A.; Giladi, N.; Gurevich, T. Accordion pill carbidopa/levodopa (AP CD/LD) for treatment of advanced Parkinson's disease (PD). *Mov. Disord.,* **2014**, *29*(1), S248. [PMID: 24339234]

[107]   LeWitt, P.A.; Friedman, H.; Giladi, N. Accordion pill carbidopa/levodopa for improved treatment of advanced Parkinson's disease symptoms. *Mov. Disord.,* **2012**, *27*(1), S408.

[108]   Chen, C.; Cowles, V.E.; Sweeney, M.; Stolyarov, I.D.; Illarioshkin, S.N. Pharmacokinetics and pharmacodynamics of gastroretentive delivery of levodopa/carbidopa in patients with Parkinson disease. *Clin. Neuropharmacol.,* **2012**, *35*(2), 67-72. [http://dx.doi.org/10.1097/WNF.0b013e31824523de] [PMID: 22377973]

[109]   Verhagen Metman, L.; Stover, N.; Chen, C.; Cowles, V.E.; Sweeney, M. Gastroretentive carbidopa/levodopa, DM-1992, for the treatment of advanced Parkinson's disease. *Mov. Disord.,* **2015**, *30*(9), 1222-1228. [http://dx.doi.org/10.1002/mds.26219] [PMID: 25847690]

[110]   Lewitt, P.A.; Ellenbogen, A.; Chen, D.; Lal, R.; McGuire, K.; Zomorodi, K.; Luo, W.; Huff, F.J. Actively transported levodopa prodrug XP21279: a study in patients with Parkinson disease who experience motor fluctuations. *Clin. Neuropharmacol.,* **2012**, *35*(3), 103-110. [http://dx.doi.org/10.1097/WNF.0b013e31824e4d7d] [PMID: 22406623]

[111]   LeWitt, P.A.; Huff, F.J.; Hauser, R.A.; Chen, D.; Lissin, D.; Zomorodi, K.; Cundy, K.C. Double-blind study of the actively transported levodopa prodrug XP21279 in Parkinson's disease. *Mov. Disord.,* **2014**, *29*(1), 75-82. [http://dx.doi.org/10.1002/mds.25742] [PMID: 24339234]

[112]   Muller, T.; Kuoppamaki, M.; Vahteristio, M.; Aho, V. Novel levodopa product ODM-101 vs levodopa/carbidopa/entacapone in Parkinson's disease with response fluctuations. *Mov. Disord.,* **2013**, *28*, S146.

[113]   Bredenberg, S.; Nyholm, D.; Aquilonius, S.M.; Nyström, C. An automatic dose dispenser for microtablets--a new concept for individual dosage of drugs in tablet form. *Int. J. Pharm.,* **2003**, *261*(1-2), 137-146. [http://dx.doi.org/10.1016/S0378-5173(03)00294-1] [PMID: 12878402]

[114]   van Riet-Nales, D.A.; Hussain, N.; Sundberg, K.A.; Eggenschwyler, D.; Ferris, C.; Robert, J.L.; Cerreta, F. Regulatory incentives to ensure better medicines for older people: From ICH E7 to the EMA reflection paper on quality aspects. *Int. J. Pharm.,* **2016**, *512*(2), 343-351. [http://dx.doi.org/10.1016/j.ijpharm.2016.05.001] [PMID: 27150947]

[115]   Aquilonius, S.M.; Nyholm, D. Development of new levodopa treatment strategies in Parkinson's disease-from bedside to bench to bedside. *Ups. J. Med. Sci.,* **2017**, *122*(2), 71-77. [http://dx.doi.org/10.1080/03009734.2017.1285374] [PMID: 28276779]

[116]   Nyholm, D.; Ehrnebo, M.; Lewander, T.; Trolin, C.G.; Bäckström, T.; Panagiotidis, G.; Spira, J.; Nyström, C.; Aquilonius, S.M. Frequent administration of levodopa/carbidopa microtablets vs levodopa/carbidopa/entacapone in healthy volunteers. *Acta Neurol. Scand.,* **2013**, *127*(2), 124-132. [http://dx.doi.org/10.1111/j.1600-0404.2012.01700.x] [PMID: 22762460]

[117]   Seeberger, L.C.; Hauser, R.A. Carbidopa levodopa enteral suspension. *Expert Opin. Pharmacother.,* **2015**, *16*(18), 2807-2817. [http://dx.doi.org/10.1517/14656566.2015.1111336] [PMID: 26595228]

[118]   Nyholm, D.; Lennernäs, H. Irregular gastrointestinal drug absorption in Parkinson's disease. *Expert Opin. Drug Metab. Toxicol.,* **2008**, *4*(2), 193-203. [http://dx.doi.org/10.1517/17425255.4.2.193] [PMID: 18248312]

[119]   Nilsson, D.; Hansson, L.E.; Johansson, K.; Nyström, C.; Paalzow, L.; Aquilonius, S.M. Long-term intraduodenal infusion of a water based levodopa-carbidopa dispersion in very advanced Parkinson's disease. *Acta Neurol. Scand.,* **1998**, *97*(3), 175-183. [http://dx.doi.org/10.1111/j.1600-0404.1998.tb00633.x] [PMID: 9531434]

[120]   Nyholm, D.; Odin, P.; Johansson, A.; Chatamra, K.; Locke, C.; Dutta, S.; Othman, A.A. Pharmacokinetics of levodopa, carbidopa, and 3-O-methyldopa following 16-hour jejunal infusion of levodopa-carbidopa intestinal gel in advanced Parkinson's disease patients. *AAPS J.,* **2013**, *15*(2), 316-323. [http://dx.doi.org/10.1208/s12248-012-9439-1] [PMID: 23229334]

[121]   Wirdefeldt, K.; Odin, P.; Nyholm, D. Levodopa-carbidopa intestinal gel in patients with Parkinson's disease: a systematic review. *CNS Drugs,* **2016**, *30*, 1-24. [PMID: 26715389]

[122]   Olanow, C.W.; Kieburtz, K.; Odin, P.; Espay, A.J.; Standaert, D.G.; Fernandez, H.H.; Vanagunas, A.; Othman, A.A.; Widnell, K.L.; Robieson, W.Z.; Pritchett, Y.; Chatamra, K.; Benesh, J.; Lenz, R.A.; Antonini, A. Continuous intrajejunal infusion of levodopa-carbidopa intestinal gel for patients with advanced Parkinson's disease: a randomised, controlled, double-blind, double-dummy study. *Lancet Neurol.,* **2014**, *13*(2), 141-149. [http://dx.doi.org/10.1016/S1474-4422(13)70293-X] [PMID: 24361112]

[123]   Fernandez, H.H.; Vanagunas, A.; Odin, P.; Espay, A.J.; Hauser, R.A.; Standaert, D.G.; Chatamra, K.; Benesh, J.; Pritchett, Y.; Hass, S.L.; Lenz, R.A. Levodopa-carbidopa intestinal gel in advanced Parkinson's disease open-label study: interim results. *Parkinsonism Relat. Disord.,* **2013**, *19*(3), 339-345. [http://dx.doi.org/10.1016/j.parkreldis.2012.11.020] [PMID: 23287001]

[124]   Antonini, A.; Yegin, A.; Preda, C.; Bergmann, L.; Poewe, W. GLORIA study investigators and coordinators. Global long-term study on motor and non-motor symptoms and safety of levodopa-car- bidopa intestinal gel in routine care of advanced Parkinson's disease patients; 12-month interim outcomes. *Parkinsonism Relat. Disord.,* **2015**, *21*, 231-235. [http://dx.doi.org/10.1016/j.parkreldis.2014.12.012] [PMID: 25585993]

[125]   Zibetti, M.; Merola, A.; Ricchi, V.; Marchisio, A.; Artusi, C.A.; Rizzi, L.; Montanaro, E.; Reggio, D.; De Angelis, C.; Rizzone, M.; Lopiano, L. Long-term duodenal levodopa infusion in Parkinson's disease: a 3-year motor and cognitive follow-up study. *J. Neurol.,* **2013**, *260*(1), 105-114. [http://dx.doi.org/10.1007/s00415-012-6597-0] [PMID: 22772358]

[126]   Zibetti, M.; Merola, A.; Artusi, C.A.; Rizzi, L.; Angrisano, S.; Reggio, D.; De Angelis, C.; Rizzone, M.; Lopiano, L. Levodopa/carbidopa intestinal gel infusion in advanced Parkinson's disease: a 7-year experience. *Eur. J. Neurol.,* **2014**, *21*(2), 312-318. [http://dx.doi.org/10.1111/ene.12309] [PMID: 24313838]

[127]   Müller, T. Motor complications, levodopa metabolism and progression of Parkinson's disease. *Expert Opin. Drug Metab. Toxicol.,* **2011**, *7*(7), 847-855. [http://dx.doi.org/10.1517/17425255.2011.575779] [PMID: 21480824]

[128]   Müller, T. Detoxification and antioxidative therapy for levodopa-induced neurodegeneration in Parkinson's disease. *Expert Rev. Neurother.,* **2013**, *13*(6), 707-718. [http://dx.doi.org/10.1586/ern.13.50] [PMID: 23739007]

[129]   Müller, T.; van Laar, T.; Cornblath, D.R.; Odin, P.; Klostermann, F.; Grandas, F.J.; Ebersbach, G.; Urban, P.P.; Valldeoriola, F.; Antonini, A. Peripheral neuropathy in Parkinson's disease: levodopa exposure and implications for duodenal delivery. *Parkinsonism Relat. Disord.,* **2013**, *19*(5), 501-507. [http://dx.doi.org/10.1016/j.parkreldis.2013.02.006] [PMID: 23453891]

[130]   Uncini, A.; Eleopra, R.; Onofrj, M. Polyneuropathy associated with duodenal infusion of levodopa in Parkinson's disease: features, pathogenesis and management. *J. Neurol. Neurosurg. Psychiatry,* **2015**, *86*(5), 490-495. [http://dx.doi.org/10.1136/jnnp-2014-308586] [PMID: 25168395]

[131]   Caraco, Y.; Oren, S.; Yacoby-Zeevi, O.; Lewitt, P.; Giladi, N. ND0612, a novel formulation of levodopa/carbidopa for continuous, subcutaneous administration, achieves steady-state levodopa plasma concentration in Parkinson's disease patients. *Proceedings of the 17th International Congress of Parkinson's Disease and Movement Disorders, LBA26,* Sydney, Australia16-20 June 2013 **2015**.

[132] Caraco, Y.; Oren, S.; LeWitt, P. Constant therapeutic levodpa (LD) plasma concentrations maintained by continuous subcutaneous (SC) administration of ND-0612, a novel formulation of LD/ carbidopa (CD). *Mov. Disord.,* **2013**, *28*, S162.

[133] Giladi, N.; Caraco, Y.; Gurevich, T.; Djaldetti, R. Pharmacokinetics and safety of ND0612L (levodopa/carbidopa for subcutaneous infusion): results from a phase II study in moderate to severe Parkinson's disease [abstract]. *Proc. Am. Acad. Neurol.,* **2015**, *186*, Minneapolis.

[134] Giladi, N.; Caraco, Y.; Gurevich, T.R.; Djaldetti, Y.; Cohen, O. Yacobi-Zeevi.; Oren, S. Pharmacokinetic profile of ND0612L (levodopa/carbidopa for subcutaneous infusion) in patients with moderate to severe Parkinson's disease. Abstracts of the Nineteenth International Congress of Parkinson's Disease and Movement Disorders, S226. San Diego, CA, USA, 14-18 June 2015. International Parkinson and Movement Disorders Society, Milwaukee, **2015.**

[135] Luinstra, M.; Grasmeijer, F.; Hagedoorn, P.; Moes, J.R.; Frijlink, H.W.; de Boer, A.H. A levodopa dry powder inhaler for the treatment of Parkinson's disease patients in off periods. *Eur. J. Pharm. Biopharm.,* **2015**, *97*(Pt A), 22-29. [http://dx.doi.org/10.1016/ j.ejpb.2015.10.003] [PMID: 26453913]

[136] Lipp, M.M.; Batycky, R.; Moore, J.; Leinonen, M.; Freed, M.I. Preclinical and clinical assessment of inhaled levodopa for OFF episodes in Parkinson's disease. *Sci. Transl. Med.,* **2016**, *8*(360), 360ra136. [http://dx.doi.org/10.1126/scitranslmed.aad8858] [PMID: 27733560]

[137] LeWitt, P.A.; Hauser, R.A.; Grosset, D.G.; Stocchi, F.; Saint-Hilaire, M-H.; Ellenbogen, A.; Leinonen, M.; Hampson, N.B.; De-Feo-Fraulini, T.; Freed, M.I.; Kieburtz, K.D. A randomized trial of inhaled levodopa (CVT-301) for motor fluctuations in Parkinson's disease. *Mov. Disord.,* **2016**, *31*(9), 1356-1365. [http://dx.doi.org/ 10.1002/mds.26611] [PMID: 27090868]