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Review

Levodopa delivery systems for the treatment of Parkinson's disease: An overview

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ABSTRACT

This review describes the different drug delivery systems containing levodopa that are used in the treatment of Parkinson's disease. Their composition, process of preparation, advantages, disadvantages and limitations are discussed as well as the major objective in the management of Parkinson's disease according to the pathology of the disease.

Contents

Abbreviations: AUC, area under curve; BELD, butylester levodopa; BS, benserazide; CD, carbidopa; COMT, catechol-*O*-méthyl transferase; CR, controlled-release; DR, dual-release; FMT, floating minitablets; GABA, gamma amino butyric acid; GI, gastrointestinal; GPe, external pallidal segment; Gpi, internal pallidal segment; GRDF, gastroretentive dosage form; GRT, gastric-residence time; HBS, Hydrodynamically Balanced System; IEDD, inhibitors of extracerebral dopa decarboxylase; IR, immediate-release; IV, intravenous; LDEE, levodopa ethylester; MAO, mono-amine oxidase; OMD, ortho-methyldopa; PD, Parkinson's disease; PLA, poly(L-lactides); PLAGA, poly(D,L-lactide-co-glycolide); PVA, polyvinylic alcool; RW, resultant-weight; SD, standard deviation; SNc, substantia nigra with its pars compacta; SNr, substantia nigra with its pars reticulata; SNT, subthalamic nucleus; SR, sustained-release.

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1. Introduction

The first clinical features of Parkinson's disease (PD) were described and published by James Parkinson in 1817 (Parkinson, 1817). In his work, Parkinson provided a visual but detailed description of the symptoms and also discussed the progressive worsening of the disorder. Nowadays, PD, being the second most common progressive neurodegenerative disorder, is a leading cause of neurologic disability. Its prevalence reaches 1–2% in people over the age of 50. It has a world-wide distribution and has no gender preference (Shastry, 2001).

The symptoms and signs of PD are related to a progressive loss of dopamine in the basal ganglia. Therefore, exogenous substitution with dopamine agonists or the dopamine's prodrug, levodopa, is used to correct the mechanical disorders at the early stage of the disease. After administration, levodopa is converted into dopamine and stored in dopaminergic neurons. This storage capacity buffers the fluctuations in plasma levels of levodopa that result from its variable oral bioavailability. Nevertheless, levodopa is still considered as the gold standard in the treatment of PD (Fahn, 2006).

The clinical response to levodopa can be described as a combination of a short-term and a long-term response. The short-term response is preserved more or less during the entire course of the disease and provides efficacy from PD symptoms, even in the very advanced stages. In contrast, the decrease in the duration of responsiveness to levodopa is related to the progressive degeneration of nigral dopaminergic neurons and the subsequent loss of dopamine buffering, resulting in motor fluctuations named dyskinesia and "on–off" effects. Due to the evolution of the disease, improvement can be sustained for several years but a gradual dose escalation is usually needed to prevent the mild decline in function that occurs within the first years of the therapy. It has been demonstrated that the appearance of the "on–off" effect is largely dependant on the dosage and the frequency of administration of levodopa. Therefore, the modern area of PD drug development and experimental therapeutics focusses on the concept of slowing and targeting the release of levodopa to prolong the therapeutic effect and reduce the number of administrations.

The efforts and resources implemented by the pharmaceutical field to provide drugs directed towards augmenting dopaminergic function in PD have developed dosage forms ranging from oral delivery systems to nasal inhalation to subcutaneously injectable agents to intraduodenal and even intravenous infusions of compounds.

As numerous dosage forms containing dopamine agonists (e.g. apomorphine), inhibitors of cholinesterase (e.g. donepezil) or inhibitors of D3 dopamine receptors (e.g. ropinirole) have been developed without providing a notable therapeutic benefit in the treatment of PD compared to those containing levodopa (Silverdale et al., 2004; Caroff et al., 2006; Hellmann et al., 2008), this review focusses on dosage forms containing levodopa as the only active agent. Moreover, as this prodrug has been widely studied over the past decades and the number of pharmaceutics concepts has been expanded simultaneously, it seemed useful to review and compare their advantages as well as their limitations in term of feasibility, compliance, safety, tolerability and short-term/long-term efficacy.

1.1. Circuit anatomy of the basal ganglia

1.1.1. Molecular metabolism of dopamine

Dopamine produced by neurons in the basal ganglia of the brain has a key role in coordinating complex movements.

The amino acid tyrosine is first converted into L-DOPA by the enzyme tyrosine hydroxylase. Thereafter, the conversion of L-DOPA requires the enzyme, aromatic amino acid decarboxylase, to produce dopamine, which is finally sequestered into storage vesicles by vesicular monoamine transporter 2 (Fig. 1) (Lowlor and During, 2004).

Dopamine is metabolized and inactivated in the postsynaptic cleft by the enzymes, catechol-*O*-méthyl transferase (COMT) and mono-amine oxidase (MAO). COMT degrades dopamine by incorporating a methyl group into the catecholamine function. The MAO catalyses the oxidative deamination of the monoamine group.

1.1.2. Anatomy and physiology

The basal ganglia include the neostriatum (caudate and putamen), the external and internal pallidal segments (GPe, GPi), the subthalamic nucleus (STN), and the *substantia nigra* with its *pars reticulata* (SNr) and *pars compacta* (SNc) (Fig. 2). The basal ganglia are located in the subcortical section of the midbrain, which integrates activity from the cortex in order to coordinate movement (Brown and Williams, 2005).

Information from the cortex passes through the basal ganglia to the thalamus and then returns to the supplementary motor area of the cortex through the dopaminergic pathway.

Under normal conditions, the striatum and the STN receive glutamatergic afferents from specific areas of the cerebral cortex or thalamus and transfer the information to the basal ganglia output nuclei, GPi and SNr. The projections between the striatum and GPi/SNr are divided into two separate pathways – direct connection and indirect projection – via the intercalated GPe and STN. Output from GPi/SNr goes to the motor thalamus which, in turn, projects back to the cerebral cortex and then to the striatum via the direct pathway (Bergman et al., 1998). The striatum also receives a non-negligible dopaminergic input directly from the SNc. This anatomic arrangement places the dopaminergic input in a position to regulate or gate the corticostriatal transmission. The D1-receptors are involved in the direct pathway, while the indirect pathway is mediated by the D2-receptors (Gerfen et al., 1990).

These pathways are thought to provide modulating antagonistic effects: direct pathway activation may inhibit GPi/SNr activity, thereby disinhibiting thalamocortical interactions. The indirect pathway activation does the opposite. Therefore, physiological dopaminergic stimulation may reduce GPi/SNr activity, thereby facilitating activity in thalamocortical projection neurons. Consequently, movement may be facilitated by the greater activation of the cerebral cortex (Galvan and Wichmann, 2008).

1.1.3. Receptors

There are five subtypes of dopamine receptors situated in various structures of the basal ganglia. Only D1 and D2 receptors, which are located in the striatum, play a role in the development of PD and in mediating the antiparkinsonian effects of dopamine substitutes (Deogaonkar and Subramanian, 2005).

There is some evidence of interactions between D1 and D2 output systems, probably due to their coupling with the G-proteins. For instance, stimulation of the direct pathway decreases the dopamine affinity of the D2-receptors. On the other hand, increasing the activity of D1- and D2-receptors enhances the dopamine response of both pathways (Marcotte et al., 1994).

All three glutamate receptor subtypes are found on dopaminergic neurons. Glutamatergic neurotransmission in the GPi/STN pathway is thought to be overactive following dopaminergic denervation (Calon et al., 2003).

1.2. Pathology and biochemical pathology of Parkinson's disease

Although the non-motor symptoms, such as constipation, aching shoulder, hypo-osmia and depression, appear prior to the motor disorders, a diagnosis of PD only follows from difficulties in motility.

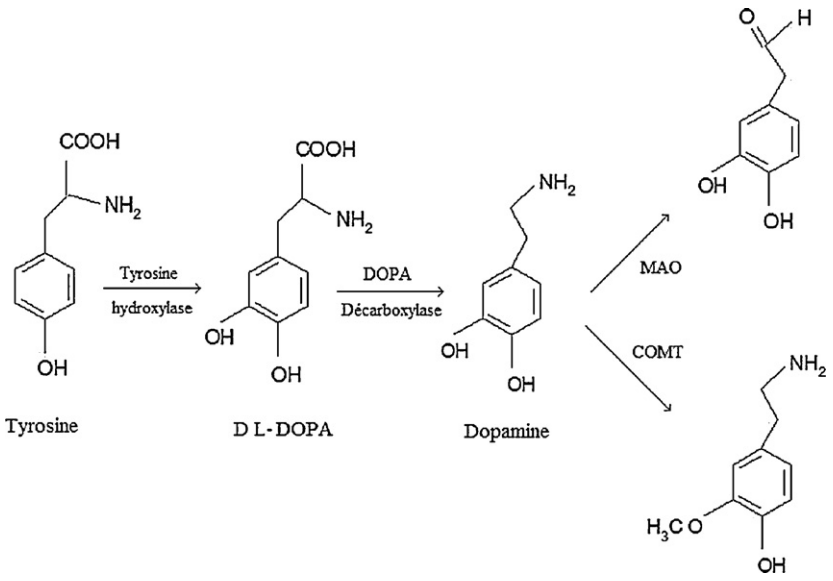

Fig. 1. Synthesis and degradation of dopamine.

Signs of parkinsonism comprise any combination of six specific, non-overlapping, motor impairments, which include tremor at rest, akinesia, bradykinesia, rigidity and loss of postural reflexes (Fahn and Przedborski, 2005). These features are hypothesized to be caused by a progressive massive degeneration of nigrostriatal dopaminergic neurons in the basal ganglia of the midbrain. However, approximately 80% of striatal nerve terminals and up to 60% of dopaminergic neurons in the SN are lost before clinical symptoms of PD become apparent (Shastry, 2001).

The underlying mechanisms of parkinsonism result from the combined action of multiple genes and environmental factors such as diet, toxins and exposure to drugs (Betarbet et al., 2000; Shastry, 2001). Oxidative stresses due to the accumulation of free radicals produced from dopamine metabolism are thought to be involved in cell death (Olanow, 1992). However, PD is considered as primary parkinsonism, which involves genetic ethiologies. Clinically, the last stage of PD is characterized by abnormal aggregation of a normally occurring synaptic fibrous protein, called α-synuclein, that plays a role in synaptic vesicle formation. These cytoplasmic inclusions are called Lewy bodies (McKeith, 2007). These deposits are known to deteriorate the nigrostriatal dopamine-producing neurons, causing dementia.

These physiological changes may take decades to develop. Dopamine depletion triggers changes in the density and sensitivity of dopamine receptors. Indeed, the number of D2-receptors and their corresponding binding sites has been shown to be increased

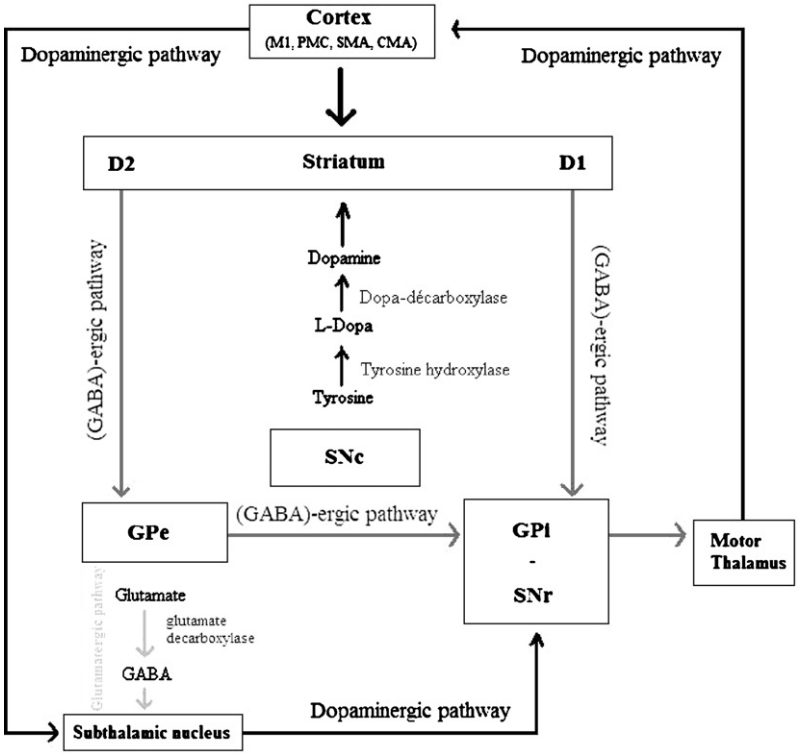

Fig. 2. Schematic activity in the basal ganglia-thalamocortical motor circuit (modified from Galvan and Wichmann, 2008).

in PD patients (Deogaonkar and Subramanian, 2005). Simultaneously, the proportion of D1- and D2-receptors changes due to modifications within the subcellular locations of D1-receptors, which move from cytoplasm to plasma membrane.

The physiopathological alterations in the level of the dopamine receptors affect neurological transmission in the basal ganglia. It has been demonstrated that parkinsonian animals have reduced activity in the D1-receptors. This observation can be correlated with the increased activation of the SNR and GPi reported in parkinsonian patients. On the other hand, responses to cortical stimulation in the indirect pathway are greater in dopamine-depleted models than in standard ones (Mallet et al., 2006). Given that normal dopamine physiology involves equilibrium of both direct and indirect pathways, the appearance of parkinsonism could be explained as the result of disturbance in the homeostasis of both dopamine pathways.

The loss of dopamine is known to increase D2-receptors' activity and decrease the activation of striatal D1-receptors. These phenomena lead to excessive activity of GPi/SNr, and increased inhibition in the thalamus and cerebral cortex, causing akinesia (Albin et al., 1989).

Besides the loss of dopamine, PD is also characterized by biochemical changes in the function of GABAergic and glutamatergic pathways in the basal ganglia.

2. Treatments

Current therapy for PD is essentially symptomatic. PD being characterized by dopamine depletion, the first curative treatments were based on exogenous dopamine supply to restore dopaminergic transmission at striatal synapses. However, trials with oral dopamine failed because dopamine cannot cross the blood–brain barrier, leading to severe peripheral adverse events. Following from this, George Cotzias demonstrated that high doses of D,L-DOPA promptly enhanced clinical function in patients with PD (Cotzias et al., 1967). Because D-dopa is not converted into dopamine and causes granulocytopenia, Cotzias et al. (1969) subsequently used L-DOPA to avoid these hematologic problems. Levodopa is a natural dopamine precursor that can cross the blood–brain barrier to reach the brain where it is converted into dopamine by peripheral decarboxylase and stored in vesicles in order to be progressively released onto postsynaptic receptors.

The duration and dosage of levodopa therapy being known to be the major risk factors in the appearance of motor complications (Poewe et al., 1986a), numerous strategies are now used to prevent dyskinesia and the "on-off" effect: (1) delay of the need for levodopa, (2) reduction of the cumulative dose of levodopa, (3) avoidance of the pulsatile stimulation of dopamine receptors, and (4) neuroprotection to slow down disease progression.

Concomitant administration of inhibitors of extracerebral dopa decarboxylase (IEDD) – e.g. carbidopa (CD) and benserazide (BS) – which do not cross the blood–brain barrier, have permitted the peripheral conversion into dopamine to be blocked and allowed a fourfold reduction in the levodopa dose requirement as its plasma elimination half-time has been shown to increase from 60 to 90 min (Pinder et al., 1976). Leppert et al. (1988) confirmed that the clearance of levodopa in rats significantly increased when it was administrated with CD.

As, like dopamine, levodopa contains a catecholamine group, it is also inactivated by COMT and MAO. Both MAO-A and MAO-B metabolize levodopa peripherally and centrally. The use of COMT inhibitors, such as tolcapone and entacapone, has been suggested in order to smooth out fluctuations in plasma concentrations of levodopa after oral administration, while selegiline is administrated as an MAO-B inhibitor.

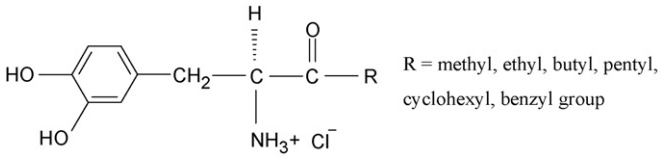

Fig. 3. Chemical structure of prodrug esters of levodopa.

There is also an increasing interest in developing more soluble but more lipophilic prodrugs to facilitate the absorption of levodopa. Carboxylic group alkyl esters of levodopa are very water soluble due to the molecular change in the chemical structure from that of a zwitterion to an amine salt of hydrochloride (Fig. 3). The higher lipophilicity can be reached after incorporation of a hydrophobic chain composed by alkyl groups. It promotes the passage of the ester through the plasmatic membrane by passive diffusion, thereby enhancing the resultant absorption of levodopa.

These esters have been shown to exert a pharmacological response similar to that of levodopa since the ester group is quickly hydrolysed by peripheral esterase. To our knowledge, ester prodrugs of levodopa have never been quantifiable in plasma samples, regardless of the administration route (Djaldetti et al., 2002). The major limitation in their evaluation is the species difference reported for esterase activity (Inoue et al., 1979).

As levodopa pharmacokinetic is not altered during the course of PD (Chan et al., 2005), the modern trend in prolonging the "on" response is to modulate or develop new dosage forms that are able to provide a constant and sustained supply of levodopa. Moreover, these dosage forms mostly contain the prodrug in association with IEDD in order to increase its plasma half-life. This becomes possible as the dosage form is able to control the release of both compounds.

2.1. Oral dosage forms

2.1.1. Immediate-release solid systems

The first marketed product containing a combination of levodopa and carbidopa was an immediate-release (IR) oral dosage form under the trade name of Simenet®. The other commercialized product, Madopar®, contained levodopa and the other peripheral decarboxylase inhibitor, benserazide.

Under conventional oral medication, the response to levodopa in the early stages of the disease is very beneficial because the buffering and compensatory mechanisms are intact. With the progression of the disease, patients become very sensitive to rapid fluctuations in plasma levodopa concentrations. Dopaminergic terminals continue to degenerate and are no longer able to buffer the exogenous levodopa adequately. As a result, patients experience one or more periods during the day when the dose of levodopa wears off (Nyholm, 2007). After the administration of an IR tablet, the plasma levodopa level rises and falls rapidly because of the short plasma half-life of the drug (~1 h), its dependence to enzymatic conversion, and its narrow absorption window at the upper part of the small intestine. Dopamine receptors are thus stimulated in a frequent abnormal and intermittent fashion, thereby developing an oscillating clinical response during chronic treatment of PD.

Indeed, repeated dosing of levodopa leads to pulsatile stimulation of D1 and D2 receptors and subsequent desensitivation, which is known to induce dyskinesia. These abnormal involuntary movements occur in 75% of patients after about 6 years of levodopa therapy (Fahn, 1992). Moreover, pulsatile dopaminergic stimulation leads to upregulation of glutamate receptors via changes to kinase and phosphatase signalling pathway (Chase and Oh, 2000). These receptors become overactive and contribute to the appearance of motor dysfunction. In addition, nigrostriatal degeneration is also presumed to be an essential condition for the appearance of

Fig. 4. The dose dispensing device (from Bredenberg et al., 2003).

dyskinesia under levodopa medication (Chase et al., 1973). Therefore, motor fluctuations appear to be an all-or-nothing process, independent of the administrated dose (Nutt, 2000). Among these motor disturbances are choreatic movements, which are an expression of involuntary rhythmic contractions of the skeletal muscles (Yanagisawa, 2006), rigidity, which is an enhancement of the tonic stretch reflex (Pollock and Davis, 1930), and akinesia and bradykinesia, which are lack of movement and slowness of movement, respectively (Yanagisawa, 2006).

Besides dyskinesias, the sudden return of parkinsonian symptoms during asymptomatic episodes can be observed in the latest stages of the treatment. As PD evolves, the brain loses its ability to regulate dopamine function as both storage and release become impaired. Consequently, a long-duration response to a single levodopa dose is gradually replaced by a shortening interval of effect and a need for more drug (Stern, 2001). This "on–off" effect comprises an "on" state when the patient has a good response from levodopa and an "off" period characterized by a sudden loss of benefit when the plasma level of the drug falls. Usually, a wearing-off phenomenon can be defined to be present when an adequate dosage of levodopa does not last at least 4 h. However, in the late stages of the disease, the duration of the "on" response becomes shorter (Duvoisin, 1974).

Abnormal involuntary movements or tolerability phenomenon also appear with non-oral dosage forms that are unable to provide a prolonged constant supply of levodopa, thereby leading to fluctuations in plasma concentrations.

In addition to the apparent tolerance that appears with a chronic intake of levodopa, interpatient variability can result in adverse events or decreased efficacy (Sjöqvist, 1999). Conventional administration schemes having shown their limitations connected with motor fluctuations that appear over years of treatment, Bredenberg et al. (2003) proposed a new dispensing device that was able to deliver individual doses of levodopa and CD (Fig. 4). This automatic dose dispenser contained a cassette filled with up to 2000 microtablets, a dose adjustment electronic system, a battery-driven electronic motor and a photocell monitoring the number of microtablets dispensed from the cassette to a receiver compartment. The weight of the empty device was 232 g and the dimensions were 132 mm × 63 mm × 32 mm.

The microtablets were made by wet granulation followed by a compression step. The simple composition, comprising microcrystalline cellulose, PVP and magnesium stearate, provided an immediate release of the active drug. Each microtablet contained 5 mg of levodopa and 1.25 mg of carbidopa. Microtablets weighed 12 mg and had a height of 1.3 mm. They can be swallowed in either the dry or the dissolved forms.

A clinical study conducted on 20 PD patients showed that parkinsonism symptoms could be controlled with individual dosage as each PD patient controlled their own dosage of levodopa, according to need. Moreover, the device was well-accepted by all patients (Bredenberg et al., 2003).

Nevertheless, erratic tablet disintegration and gastric emptying variation still remained. Sublingual administration of levodopa methylester, evaluated to avoid intestinal absorption, failed to produce clinical effects because levodopa is not absorbed through the oral mucosa (Kleedorfer et al., 1991). Therefore, liquids and dispensable formulations were developed in order to avert unpredictable absorption connected with the variable drug supply observed after oral administration of conventional solid dosage forms. They enable an easier and faster gastrointestinal transit, independent of the interval for gastric emptying.

2.1.2. *Liquid and dispersible formulations*

Dispersible formulations are tablets characterized by a rapid disintegration when dispersed in liquid, which are then referred as "solutions". Ascorbic acid appears to be usually added to prevent the oxidation of levodopa and the associated IEDD (Pappert et al., 1996a).

It has been postulated that dispersible or liquid formulations of levodopa may decrease the t_{max} because their gastric emptying is less dependent on pylorus contraction compared to conventional solid dosage forms (Contin et al., 1999). However, a clinical study conducted by Marriott and colleagues on nine patients with moderate PD concluded that there was no statistical difference in terms of t_{max}, C_{max} and AUC between a levodopa/carbidopa solution and tablets containing the same amount of each drug (Marriott et al., 1998). Another trial showed that motor fluctuations were reduced in 23 parkinsonian patients when an oral solution of levodopa/carbidopa was administered instead of a solid form (Pappert et al., 1996b). The same trend was observed in eight patients with parkinsonism when an association of levodopa and BS was evaluated (Contin et al., 1999).

Despite the similar pharmacokinetic parameters, plasma levodopa levels were more stable under liquid treatment. This clinical benefit was probably due to the ability of patients to fractionate the liquid dose more easily, according to their requirements. Dispensable products could be more beneficial in treating

morning motor disturbance in PD patients undergoing long-term levodopa therapy. Indeed, long-term levodopa application causes increased levodopa bioavailability due to a deteriorated metabolism (Muhlack et al., 2004). With liquid formulations providing an immediate levodopa supply, the time taken to reach the "on" response in the morning could be shorter than that required with a standard solid dosage form.

In order to induce the beneficial clinical effect more rapidly, a liquid formulation of a highly soluble prodrug of levodopa was also investigated. Levodopa ethylester (LDEE) was thought to be absorbed faster, thereby providing an additional solution for PD patients with severe morning "off" states (Djaldetti and Melamed, 1996). A clinical study was conducted in 62 PD patients, who received either an LDEE-CD or LD-CD solution. The mean time to reach the onset response in the morning decreased after administration of the LDEE-CD solution (36 min) compared to that containing an equivalent dose of LD-CD (43 min). This observation could be correlated with the pharmacokinetic parameters as C_{max} increased by 33%, t_{max} was reduced by 53% and $AUC_{0-2\,h}$ increased by 18% in the LDEE-CD-treated group. However, dyskinesia appeared with both solutions. Moreover, LDEE-CD did not provide significant improvement in motor dysfunction compared to LD-CD (Djaldetti et al., 2002).

Solid dosage forms as well as liquid/dispersible preparations provide an immediate supply of levodopa and, associated with the short half-life of this drug, lead to a more intermittent delivery resulting in the appearance of peaks and troughs in plasma levodopa levels (Fig. 5). Ultimately, they failed to provide adequate relief because the therapeutic index narrows as PD progresses.

2.1.3. Sustained-release dosage forms

Sustained-release (SR) dosage forms provide immediate drug release after administration, with continued controlled drug release over an extended period of time. The main advantage of these dosage forms over IR dosage forms lies in the fact that the plasmatic concentrations can easily be levelled in the therapeutic range of the drug for an extended period of time with reduced dosing frequency (increased patient compliancy), thus limiting plasmatic peaks and reducing side effects.

In order to stabilize plasma levodopa concentrations, thereby improving clinical efficacy in the treatment of PD disease, sustained-release formulations have been developed, e.g. Sinemet® CR 50/200 and Madopar® CR 50/200. Slow-release preparations were also intended to increase clinical benefit in the treatment of night-time problems and early morning symptoms as IR formulations could not sustain sufficient plasma levodopa concentrations overnight (Jansen and Meerwaldt, 1990).

Numerous clinical trials have been conducted to compare the pharmacokinetic parameters and the efficacy on PD patients of the controlled-release (Sinemet® CR) versus the standard formulation (Sinemet®) (Poewe et al., 1986b; Cedarbaum et al., 1989a). Both types of formulation are composed of an association of levodopa and carbidopa. The CR system contains 200 mg of levodopa and 50 mg of CD, whereas a half-dose of both drugs is incorporated in the standard Sinemet® (Cedarbaum et al., 1989b). Nevertheless, Sinemet® CR presented a long latency to the patient turning "on", due to its pharmacokinetic characteristics. Indeed, no initial peak of levodopa was observed after oral administration of the controlled-release system (LeWitt et al., 1989). The t_{max} obtained for levodopa from Sinemet® CR (t_{max} = 4 h) was almost double that of the standard Sinemet® (t_{max} = 2.5 h) and was associated with a much slower increase in plasma levodopa levels (Stocchi et al., 1994).

On the other hand, the CR formulation provided less variability in plasma levodopa levels, probably due a lowered frequency of administration (Cedarbaum et al., 1987). In spite of the decrease in the number of administrations, the final total daily amounts of levodopa and carbidopa administered as CR were significantly greater than those observed with the standard dosage form (Goetz et al., 1988). Due to their narrow absorption window at the upper part of the small intestine and the sustained release of the incorporated drugs, the amount of levodopa and carbidopa that still remained in the dosage form became unavailable when the CR tablet reached the jejunum. The similar corrected bioavailability of levodopa and carbidopa for both evaluated systems was consistent with this hypothesis. Moreover, probably due to the narrow absorption window, it should be noticed that the bioavailability of Sinemet® CR was found to be lower in the fasting state than in fed condition, probably due to a faster gastric emptying (Yeh et al., 1989). Finally, long-term therapy showed that progressive dyskinesia appeared over years with Sinemet® CR, as well as with standard Sinemet® (Koller et al., 1999).

SR systems provide a smoother plasma levodopa profile but present a delayed response in reaching the antiparkinsonian effect compared to IR formulations. A single co-administration of both systems being unsuitable, the utilization of dual-release formulations should be an alternative solution.

2.1.4. Dual-release systems

Ideal dual-release (DR) characteristics would combine the advantages of early peak levels of levodopa in plasma with sustained plasma concentrations.

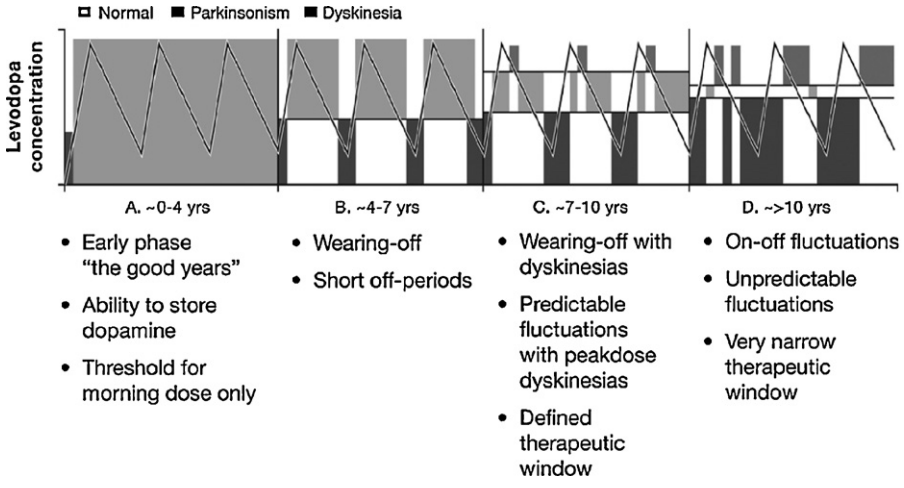

Fig. 5. Narrowing of the therapeutic window for oral levodopa over time (from Nyholm, 2007).

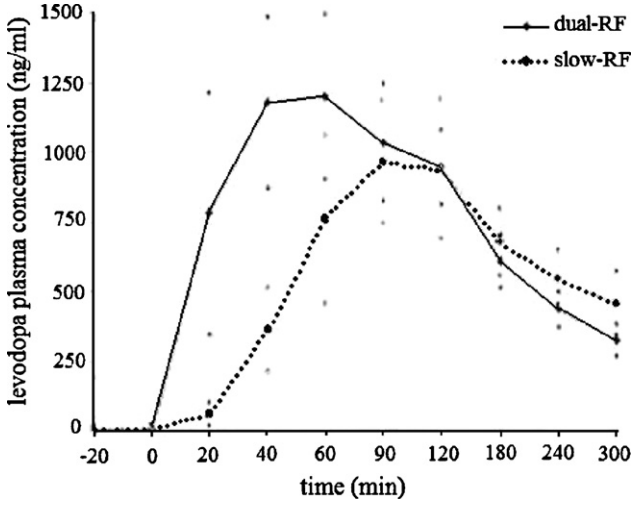

Fig. 6. Mean plasma concentration time profile of levodopa after a single dose of the DR formulation and SR dosage form; data expressed as mean ± SD (from Descombes et al., 2001).

In order to evaluate the effectiveness of such preparations, 18 healthy volunteers were enrolled to assess and compare the pharmacokinetic profile of levodopa following oral administration of a new DR (Madopar® DR) formulation to a standard SR dosage form. Both systems contained 100 mg of levodopa and 25 mg of BS and were administered in fasted condition. The total daily dose was 300 and 75 mg for levodopa and benserazide, respectively. Madopar® DR was a breakable three-layer tablet (IR, barrier, SR) resulting in DR properties (Gasser et al., 1998).

The plasma profile of levodopa following the administration of SR system was more evenly distributed than with the DR formulation. Indeed, single dose administration of Madopar® DR was characterized by a higher peak plasma concentration (C_{max}), which was reached faster (t_{max}) than that obtained with the conventional SR dosage form (Gasser et al., 1998). However, 3 h after administration, the plasma concentration–time profile of the DR system was similar to that representing the SR formulation, resulting in lower plasma fluctuations of levodopa levels compared to standard IR tablets (Granén et al., 1992). These pharmacokinetic characteristics resulted in similar bioavailability ($AUC_{0-\infty}$), regardless of the administered dosage form. Similar findings were obtained after multiple dosing (period of 7 days). It should be noticed that, in contrast to standard Madopar®, benserazide did not influence the systemic availability of levodopa released from Madopar® DR. On the other hand, the plasma levels of the other metabolized product, 3-*O*-methyldopa (3-OMD), were significantly higher after administration of DR and SR dosage forms than those resulting from IR systems (Gasser et al., 1998).

Thereafter, the same products were evaluated on 16 patients with idiopathic PD and wearing-off fluctuations. In this study, the dosage forms were administered in fed condition. The pharmacokinetic parameters (C_{max}, t_{max}, $AUC_{0-\infty}$) obtained with Madopar® DR and with the SR formulations revealed the same trend in PD patients as in healthy volunteers (Fig. 6).

As the DR product contained an IR layer, the mean onset of the "on" phase seemed to occur earlier and showed a trend for a longer response compared to the SR formulation. However, no statistical difference was found, due to the high intersubject variability. Probably due to the immediate supply of levodopa, which still provided a low peak and trough effect, the appearance of side effects was more frequent with Madopar® DR than with the standard IR formulation (Descombes et al., 2001).

Finally, Crevoisier and co-workers studied the influence of food intake on the pharmacokinetic parameters of levodopa on 19 healthy volunteers after administration of Madopar® DR. As fatty acids are known to decrease the motility of the stomach, it should be noted that the dosage form was administered after a high-fat breakfast when the post-prandial protocol was requested. The C_{max} value of levodopa was lower by one-third in the presence of food, while the t_{max} value was 3 h higher than that obtained in fasted condition (Fig. 7).

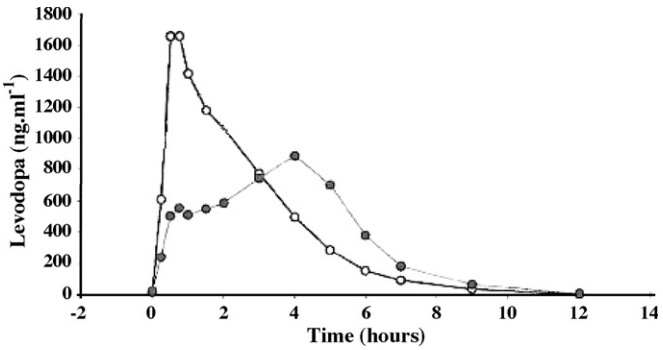

Fig. 7. Plasma concentration–time profiles of levodopa (○, fasting; ●, fed) after administration of Madopar® DR in fed and fasting state (arithmetic mean, $n = 19$) (modified from Crevoisier et al., 2003).

However, the AUC value was similar, regardless of the ingestion protocol. The fasting state provided the fastest "on" response. Food ingestion reduced the absorption rate of levodopa by delaying gastric emptying. This can be visualized as no initial peak plasma concentration of levodopa appeared in fed condition (Crevoisier et al., 2003). The effect of food on IEDD activity seemed to be much greater. Indeed, benserazide is less active than carbidopa in inhibiting the peripheral decarboxylase when given concomitantly with food; the inhibition property of carbidopa does not seem to be dependent on food intake (Dingemanse et al., 1997).

As previously observed, levodopa being characterized by a narrow absorption window at the upper part of the small intestine, this highly specific absorption mechanism implies that gastric emptying determines the rate and the extent of its absorption. Both IR and SR formulations failed to provide a constant supply of levodopa at its absorption site. Gastroretentive dosage forms were developed to obtain lesser-fluctuating plasma concentrations, thereby establishing a possibly more stable clinical effect.

2.1.5. Gastroretentive dosage forms

In addition to the motor complications consecutive to the chronic oral administration of IR dosage forms, a direct supply of levodopa may cause saturation of the specific transporters located throughout the duodenal wall and might limit the overall absorption of the drug as a consequence (Deleu et al., 2002). Gastroretentive dosage forms are able to stay in the stomach for a prolonged period of time, during which the drug is continuously released above the absorption site. Better spatial and temporal targeting decreases the frequency of administration and slows down the progressive tolerance to oral levodopa.

Different approaches have been proposed to prolong the residence time of delivery systems in the stomach, including the use of passage-delaying agents (Palin, 1985), swelling and expanding systems (Fix et al., 1993), bioadhesive systems (Park and Robinson, 1984), high-density products (Davis et al., 1986), and floating dosage forms (Seth and Tossounian, 1984).

Only a small number of gastroretentive systems containing levodopa have been developed with success. Klausner et al. (2003) proposed a new controlled-release gastroretentive dosage form (CR-GRDF) based on unfolding membranes. The CR-GRDF were comprised of an inner layer composed of a polymer-drug matrix (ethylcellulose-levodopa 1:1) framed with rigid polymeric strips

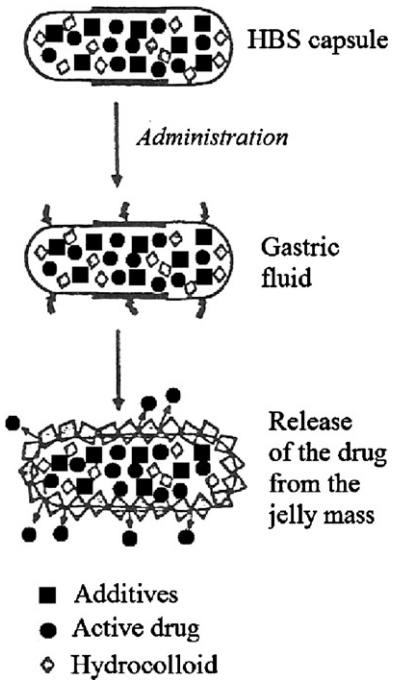

Fig. 8. Schematic representation of a HBS floating system (from Bogentoft, 1982).

(L-polylactic acid-ethylcellulose 9:1) covered on both sides by two outer layers (composed of enzymatically hydrolysed gelatin, methacrylic acid copolymer type B, glycerine and glutaraladehyde (48:30:20:2)). Dissolution tests showed that the release of levodopa could be sustained for between 2 and 14 h according to the membrane thickness. As it has been demonstrated that levodopa has very similar pharmacokinetic parameters in dogs and humans, the new CR-GRDF was then evaluated on beagle dogs in fasted condition. X-ray analysis showed that all of the CR-GRDFs were retained in the stomach for at least 24 h. As observed *in vitro*, the release of the drug was sufficiently sustained to ensure elevated levodopa concentrations for more than 9 h after drug administration (Klausner et al., 2003). Despite encouraging data, the therapeutic use of unfolding systems is usually avoided in humans due to the potential risk of an undesirable extended gastric retention time, which may cause GI problems.

Among the various attempts made to increase the gastric retention of an oral dosage form, it seems that the floating drug delivery systems have offered the most effective and rational protection against early and random gastric emptying. Supporting this, Seth and Tossounian (1984) developed their Hydrodynamically Balanced System (HBS^{TM}), based on a mixture of drugs and hydrocolloid. Upon contact with gastric fluids, the capsule shell dissolves and the hydrocolloid begins to swell (Fig. 8), maintaining a relative integrity of the initial shape, a bulk density lower than 1 g/ml, and regulating the drug release.

Goole and co-workers evaluated the floating properties of a marketed product, Prolopa® HBS 125 (Madopar® HBS), using the resultant-weight (RW) method (Timmermans and Moës, 1990). The HBS capsule presented no floating lag time due to its very low initial density. Its maximal RW value was obtained after 10 min and remained constant for about 1 h. Afterwards, its floating strength decreased as a result of the development of its hydrodynamic equilibrium (Fig. 9) (Goole et al., 2008). The dissolution tests showed that benserazide was released faster than levodopa (12 h vs. 24 h). The standard deviation noticed with Prolopa® HBS 125 increased, both for levodopa and BS, after 5 h due to the fragmentation of the jelly mass (Goole et al., 2008). In another study, the pharmacokinetic profile of the floating form, Madopar® HBS, and that corresponding to the "conventional" controlled-release system, Sinemet® CR, were compared in 18 healthy volunteers in fed condition. The HBS system was found to be bioequivalent to Sinemet® CR with respect to levodopa but exhibited a higher fluctuation index as the C_{max} was higher and the C_{min} lower than those obtained with Sinemet® CR (Granén et al., 1992). As both products provided a similar bioavailability, it seems that the conventional CR product was retained in the stomach for as long as the floating capsule. This was probably due to the presence of food, which slows down the pyloric passage. This observation demonstrated the significant role of the meal in proper achievement of the buoyancy retention principle.

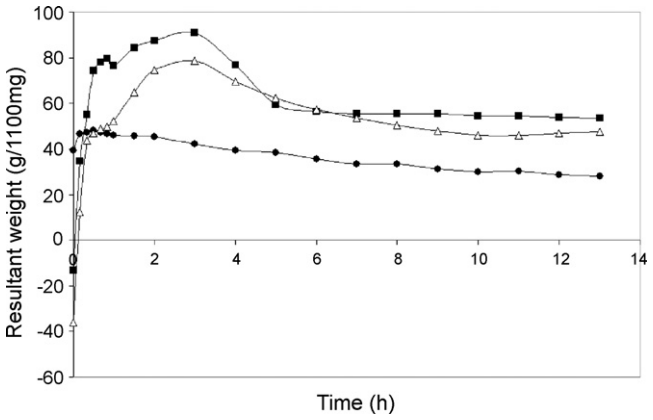

Fig. 9. Resultant-weight profiles obtained with the (■) swellable FMT, (○) coated FMT and (●) Prolopa® HBS ($n = 3$) (from Goole et al., 2008).

In vivo, most single-unit floating systems are generally unreliable and non-reproducible in prolonging the gastric residence time and providing stable plasma levels.

In contrast, multiple-unit dosage forms present a more reproducible gastric-residence time (GRT), a reduced inter-subject variability in absorption and offer a better dispersion throughout the gastrointestinal tract (Singh and Kim, 2000).

The development and evaluation of two concepts of floating minitablets (FMT) were reported. These minitablets were composed of granulates made by melt granulation (Hamdani et al., 2002) and containing an association of levodopa/CD, a meltable lipidic binder, and gas-generating agents. The first system developed contained Methocel® K15 M as a swellable polymer both to trap the generated carbon dioxide and to sustain the release of the active drug (Goole et al., 2008). For the second floating system developed, Methocel® K15 M was completely removed and a coating step was introduced in the manufacturing process in order to provide a coating layer capable of maintaining the generated carbon dioxide inside the dosage form for a prolonged period of time (Fig. 9) (Goole et al., 2008). *In vitro*, a sustained release of levodopa and CD occurred immediately after immersion with no burst effect, regardless of the FMT tested. The dissolution profiles of levodopa and the IEDD remained statistically similar until the complete release of both drugs. A pharmacoscintigraphic coupled with a pharmacokinetic study conducted on 10 healthy human volunteers in order to compare the novel FMT and Prolopa® HBS concluded that the three floating forms remained in the stomach for more than 4 h. They also provided a sustained pharmacokinetic profile of levodopa and CD for more than 8 h. BS offered a lower bioavailability than CD. Prolopa® HBS 125 and the coated FMT presented intragastric disintegration, resulting in the presence of a peak in the plasma levodopa profile and in higher variability in plasma levodopa levels than that observed after the administration of the swellable FMT (Goole et al., 2008). This observation could be correlated with the high number of dropouts observed after a 2-year follow-up study conducted

by Pacchetti et al. (1990) on 25 PD patients treated with the HBS form administrated alone. The FMT containing Methocel® K15 M provided the most evenly distribution of the plasma level values of levodopa, decreasing a possible pulsatile stimulation of dopamine receptors (Goole et al., 2008). No significant statistical difference was found between the GRT, AUC, C_{max} and t_{max} values obtained for the FMT and Prolopa® HBS 125.

Despite the numerous different systems developed to overcome the unpredictable gastric emptying process, erratic absorption of levodopa still appears with oral dosage forms. In addition to the inherent variability observed with the pharmaceutical form itself, it is well known that PD patients suffer from gastrointestinal dysfunctions connected with basal ganglia disturbance (Pfeiffer, 2003). Indeed, many clinical symptoms of PD have little or nothing to do with motor function. These abnormalities affect both men and women in the same proportion and are not correlated with the evolution of the disease (Fuh et al., 1997). GI dysfunctions include damage to the enteric nervous system caused by the formation of Lewy bodies, impaired gastric emptying and dysphagia.

The presence of Lewy bodies throughout the enteric nervous system slows down the intestinal motility, interfering with levodopa absorption (McKeith, 2007).

Gastric emptying is slower in PD patients. The excessive retention of levodopa in the stomach increases its exposure to dopa decarboxylase and causes its early conversion to dopamine (Pfeiffer, 2003).

Dysphagia is characterized by a delayed swallowing reflex due to abnormal control of the tongue, which provides the major driving force for pharyngeal pressure generation and clearance. Moreover, jaw tremor and rigidity make the ingestion of oral dosage forms difficult as they cause pain at the time of opening the mouth (Bushmann et al., 1989).

Dysphagia, unpredictable gastric emptying and subsequent erratic absorption of levodopa have lead drug development to focus on numerous alternative routes of administration. Non-oral formulations may be more suitable but also more reliable in avoiding pulsatile stimulation of dopamine receptors and the subsequent motor complications (Nyholm and Lennernäs, 2008).

2.2. Intravenous injection

There is an apparent lack of data relating to the intravenous (IV) administration of levodopa. This is probably due to the evident difficulty in delivering the drug to the brain without producing the associated peripheral side effects. However, injection of levodopa directly to the systemic circulation may be needed for several reasons: (a) to overcome the unpredictable absorption after oral intake, (b) to provide a direct supply of the drug, (c) to maintain constant brain levodopa concentration and (d) to enhance bioavailability as well as reduce its variability with the age and sex (Bushmann et al., 1989).

The efficacy of a saline solution of levodopa (2 mg/ml) was evaluated on 27 PD patients in fasted condition. A 200 mg dose of CD was orally administered 1 h prior to starting the infusion. Firstly, a rapid IV loading (10 min) produced a transient peak in plasma levodopa concentration. Afterwards, a 90 min constant-rate infusion led to a target steady-state plasma concentration of 600 ng/ml (Black et al., 2003). This protocol mimicked a DR formulation, with an immediate supply of levodopa promptly after administration, followed by a progressive release of the drug. The final dose was biologically relevant both in terms of the bioequivalent daily 100 mg oral dose and the antiparkinsonian effect. The mean levodopa plasma concentrations after the infusion period were within 5% of the targeted tissue concentration. On the other hand, the common side effects were still observed: early conversion into dopamine produced nausea, sleepiness and dyskinesia (Black et al., 2003).

In addition to these biological adverse events, the compliancy of such preparations is relatively complex as IV solutions require invasive administration usually done in a medical institution by professional, high-cost personnel. Moreover, it has been reported that the stability of levodopa dramatically decreases in basic and neutral solutions (Coello et al., 2000). Indeed, after 10 min, the concentration of levodopa in solution was 2.1 mg/ml and fell to 1.9 mg/ml 1 h later.

Chronic IV administration of levodopa is not clinically practicable and cooperation is hard to obtain from patients with dementia, making injection quite difficult. Moreover, this technique does not bypass the passage through the blood–brain barrier, which limits the amount of the circulating dose available to the brain. Because of this, implantable systems that may provide a direct supply of levodopa to the brain were developed and evaluated.

2.3. Implantable systems

Implantable systems are designed for long-term therapy. They provide a continuous progressive supply of the incorporated drug for a prolonged period of time. Once they are implanted, no further invasive administration is needed until the complete release of the active drug. However, these systems are usually limited by their small size, and the constituting polymers must be biocompatible. Depending on the approach developed to achieve the controlled administration of the therapeutic agent (e.g. diffusion or activation), the type of polymer includes biocompatible compounds such as ethylene-vinyl acetate derivates, Polyethylene glycol, silicone elastomer, lipidic materials, PLA and PLGA (Chien, 1987a).

Arica et al. (2005) described microspheres containing levodopa prepared separately from microspheres loaded with carbidopa. Both were manufactured according to a solvent evaporation technique. The biodegradable polymers, poly(L-lactides) (L-PLA), poly(D,L-lactides) (D,L-PLA), and poly(D,L-lactide-co-glycolide) (PLAGA), were dissolved in dichloromethane prior to being added to a solution containing an aqueous emulsifying agent [PVA:SO or NaCMC:SO (4:1, w/v)]. After production, microspheres of size 20–40 μm were sterilized by gamma irradiation. Dissolution tests (USP I, HCl 0.1N, pH 1.2, 900 ml, 50 rpm) demonstrated that the polymers were able to provide a sustained release of levodopa and carbidopa for 10 h. The release profile depended on the diffusion of the drug through the pores or channels on and close to the surface of the microspheres, which were prepared in order to be porous. No erosion process was described. The loaded microspheres were surgically implanted into the striatum of lesioned rats presenting dopamine depletion in order to act directly on postsynaptic dopamine receptors involved in motor behaviour. The efficacy was assessed using the rotational behaviour technique compared to blank microspheres for 2 months. Levodopa was released slower in the brain tissue than *in vitro* due to the evident saturation appearing in the physiological condition.

Another invasive levodopa delivery method that avoids intestinal absorption concerned the intraperitoneal administration of prodrugs, [(*O*,*O*-diacetyl)-L-dopa-methylester]-succinyldiamide (Fig. 10), encapsulated in liposomal formulations. This system was composed of 3% (w/v) dimiristoylphosphatidylcholine and 0.3% (w/v) of cholesterol. The preparation of the liposomes yielded a low loading efficiency, with 95–97% of free drug unincorporated in the vesicular structure. The liposomes showed good chemical stability at acidic and physiological pH. After intraperitoneal administration in rats, levodopa levels in the striatum were higher with liposomial formulations than with free prodrug. The lipidic bilayer resulted from sustained delivery of prodrug-loaded liposomes to the brain. This property allowed the protection of the prodrug against degradation and increased the therapeutic effectiveness at a lower dose.

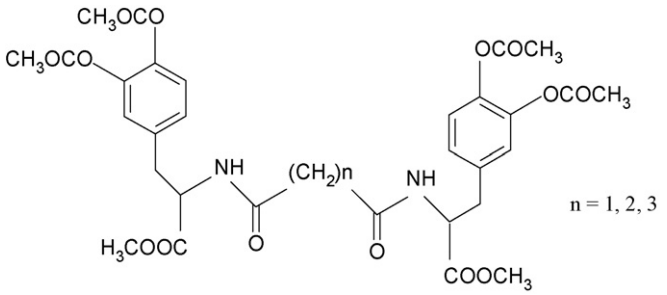

Fig. 10. Chemical formula for diamides of (*O*,*O*-diacetyl)-L-dopa-methylester (from Di Stefano et al., 2004).

Unfortunately, no clinical benefit in term of movement disorder was described (Di Stefano et al., 2004).

However, even if implanted microspheres or loaded liposomes may avoid peripheral side effects, provide an effective sustained-release of the drug, and constitute a specific site delivery method, a surgical intervention is an invasive procedure that should not be used in the first instance in the treatment of PD disease as the clinical benefit seems to be similar to that obtained with non-invasive delivery systems.

2.4. Pulmonary delivery

The pulmonary route is a non-invasive way of administration that may provide rapid and efficient delivery of levodopa to the brain due to the fast drug absorption through the alveoli (Jain, 2008). Interestingly, it could be used as rescue therapy when an immediate supply of levodopa is needed. As for IV administration, the pulmonary route avoids the first-pass liver metabolism.

The principal mechanisms contributing to lung deposition are inertial impaction, sedimentation and diffusion (Heyder et al., 1986). The major parameter influencing lung deposition is the aerodynamic diameter (d_a) of the inhaled particles. Although particles with a mean geometric diameter (d_g) ranged between 1–5 μm are known to provide an optimal deposition, large porous particles ($d_g > 5$ μm) have shown similar behaviour due to their low mass density (<0.4 g/cm^3). A new dry powder inhaler (AIR^{TM}) was specially designed to facilitate their dispersal deep into the lungs (Dunbar et al., 2002). To our knowledge, no clinical study has been conducted on humans using active ingredient incorporated in this system. However, inhalable particles loaded with levodopa were administered to a lesioned rat model of PD. These large porous particles were prepared by spray drying, and an equivalent of 2 mg of levodopa was administered to the rats via pulmonary insufflation. Another group orally received the same amount of levodopa suspended in a saline solution.

Pulmonary delivery yielded higher plasma levodopa C_{max}, which was reached faster (2 min vs. 30 min) than after oral gavages (Fig. 11).

Immediately after insufflation, plasma levodopa levels remained significantly elevated above oral levels for over 240 min. Consequently, the bioavailability of the drug was significantly higher following inhalation than after oral administration. Compared to oral therapy, pulmonary delivery provided levodopa and dopamine levels four- to twofold superior on both sides of the striatum, respectively. Therefore, restoration of movement was completed only 30 min after insufflation. In contrast, a similar improvement required at least 60 min after oral administration. However, the duration of motor improvement was comparable using either delivery route (Bartus et al., 2004). Given that pulmonary formulations cannot provide a prolonged supply of levodopa, they might have to be administered as frequently as an IR oral dosage form, leading to pulsatile stimulation of dopamine receptors as a consequence. The late adverse events observed in oral therapy might therefore not be avoided with pulmonary administration. This route should be reserved for "rescue therapy" due to the rapid onset response that it provides.

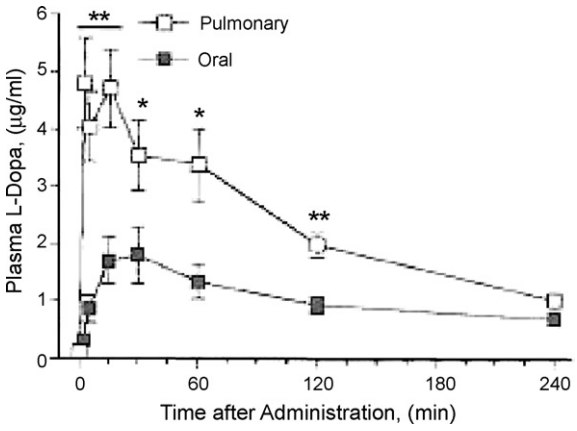

Fig. 11. Plasma pharmacokinetics of levodopa administered by oral and pulmonary routes. Each symbol represents the mean ± SD of data from 7 subjects in the oral group and 6 subjects in the pulmonary group (*, **, significantly different from oral levels, $p < 0.05$) (from Bartus et al., 2004).

2.5. Nasal administration

Conventionally, the nasal route has been used for delivery of drugs in the treatment of local diseases. However, the last decade has recognised the importance of the nasal cavity as a potential route for non-invasive drug delivery. The nasal cavity possesses many advantages, such as a large surface area for absorption with a highly vascularised subepithelial layer. Moreover, the direct transport of absorbed drug into the systemic circulation avoids the first-pass metabolism by the liver, bypasses the blood–brain barrier and results in preferential absorption to the cerebrospinal fluid (Sakana et al., 1991). The small volume of the aqueous secretions present in the nasal cavity limits the dissolution of an instilled compound. Utilization of water soluble prodrugs of levodopa provides a suitable alternative. As an example, butylester levodopa (BELD) was selected because it offered the best solubility and lipophilicity compared to the other alkyl ester prodrugs of levodopa. Using a rat model, the absolute bioavailability of levodopa following nasal administration of BELD was found to be around 90% (vs. 5% *per os* (Goodman Gilman et al., 1980)). Nasal administration of BELD resulted in higher levodopa levels in the cerebrospinal fluid than following IV perfusion of the non-esterified drug. Moreover, the early conversion of BELD into dopamine in the nasal cavity minimized the peripheral side effects, probably because dopamine delayed its own absorption due to its vasoconstrictive effect.

The major limitations connected with nasal administration are the low permeability of nasal mucosa for polar molecules, and the mucociliary clearance mechanism, which set a limited time available for drug absorption within the nasal cavity (Soane et al., 1999). In the case reported above, another limitation seems to be the amount of drug that must be instilled to obtain the desired clinical effect (Goodman Gilman et al., 1980). For instance, BELD was administrated at a 20 mg/kg levodopa-equivalent dose in rats, which corresponds to 1.4 g for a 70 kg man.

2.6. Rectal formulations

Rectal administration is another non-invasive technique that may be used to deliver levodopa to PD patients. Unfortunately,

when levodopa was given rectally alone, there was no rise in plasma level and no clinical benefit (Eisler et al., 1981). This lack of absorption was attributed to the relative alkanility of the rectal secretions. Clinical improvement following rectal administration of a strongly acidic suspension of LD-CD in a hospitalized PD patient supported this hypothesis (Cooper et al., 2001). As no pharmacokinetic evaluation was performed, the observed clinical improvements were not correlated with plasma levodopa levels.

Rectal perfusion of a microenema solution in rats demonstrated that the absorption of levodopa could be enhanced when associated with salicylate. This phenomenon was shown to be concentration- and pH-dependent. Salycilate facilitates the rectal absorption of numerous drugs, especially in their ionic form. Although the disappearance of levodopa in the perfusate was higher at pH <5 and >7, the enhancement of the drug absorption by salicylate was not due to the formation of a complex as the absorption rate of the adjuvant administered alone did not depend on the presence of levodopa in the perfusion. Nevertheless, both salicylate and drug had to be administered simultaneously in the same perfusion. Indeed, when the adjuvant was intravenously injected immediately before rectal administration of levodopa, the active drug was not found in the plasma (Nishihata et al., 1982). As observed for pulmonary administration, the rectal route cannot provide a prolonged release of the drug. Moreover, the use of irritating adjuvants, such as salicylate, to enhance the absorption of levodopa should be avoided for patients weakened by the disease.

In order to avoid the use of absorption enhancers, rectal administration of aqueous solutions (pH 5.5) containing several short-chain alkyl esters prodrugs of levodopa was evaluated in rats, mice and beagle dogs (Fix et al., 1989). In all species, the absorption of the prodrugs and the resulting bioavailability were greater than those obtained with levodopa itself. The esters were not converted into levodopa in the rectum but an enzymatic cleavage occurred immediately after absorption. A slower absorption rate for the 4-hydroxybutyl ester might prove to be a useful advantage in terms of designing a sustained-release formulation. However, the use of a sustained release dosage form may be restricted by the poor rectal absorption of carbidopa and the resultant potential risk of discharge (Fix et al., 1990).

2.7. Transdermal application

The transdermal route was then thought to be a better route for providing a progressive supply of levodopa to the systemic circulation without the adverse complications appearing with the oral route and IR systems. Moreover, this route of administration is very useful for drugs that undergo extensive first-pass metabolism such as levodopa.

However, the percutaneous permeation of the highly hydrophilic drug, levodopa, ($\log K_{o/w} = 4.7$) across the dense barrier structure of the stratum corneum is extremely restricted. The excellent barrier of the skin is notably maintained by ceramides, the main polar lipids that constitute a major portion of intercellular lipid matrix in the stratum corneum. Using a rat model, acetone was found to be the most effective solvent in extracting sphingosine, due to its very low dielectric constant (21.5). This perturbation allowed an enhanced permeation of levodopa across the skin (Gupta and Tiwary, 2002). Unfortunately, the barrier status of the skin was entirely restored after 24 h. In order to prolong the perturbation effect of acetone, β-chloroalanine may be used to selectively inhibit serine palmitoyl transferase, an enzyme that catalyzes the synthesis of ceramides (Holleran et al., 1991). Therefore, the most interesting pharmacokinetic data were obtained when the integrity of the skin was primarily perturbed by acetone, followed by the application of a patch containing an association of levodopa and carbidopa loaded with a high amount of β-chloroalanine. The inhibition of sphingosine synthesis was maintained for more than 36 h and the effective level of levodopa was sustained for 28 h (Gupta and Tiwary, 2002). Due to the apparent difficulty in applying acetone prior to the therapeutic patch, it seemed that another kind of transcutaneous system might be more attractive for delivering levodopa without pre-treating the skin with an organic solvent.

Kankkunen et al. (2002) proposed a controlled transdermal delivery system based on iontophoresis and ion-exchange fiber. Since only dissolved levodopa could be loaded in a cation-exchange resin, the optimal pH of the solution was fixed at 2.0 to avoid the alkaline oxidation of the colorless hydroquinone groups to the corresponding colored quinone functions. Probably because of its zwitterionic form, the entire dose of levodopa was already released after 2 h when the dosage form was immersed in an *in vitro* saline solution. In contrast, permeation studies performed on cadaver human skin showed that only 25% (w/w) of levodopa crossed the epithelium barrier after 2 h. Therefore, it seemed that the ionic-exchange system failed to control the release of levodopa since the release rate of the drug was determined by the skin. A constant iontophoretic current of 0.5 mA/cm^2 was then applied to promote the transport of the ionic compound through the skin. A three- to fourfold increase in transdermal levodopa permeation was clearly observed (Hirvonen and Guy, 1997). Despite the encouraging results obtained by iontophoresis, the incorporation of transdermal permeation enhancers in the dosage form seemed to be more practical and less expensive than the systematic use of a current generator.

In this way, the development and evaluation on a rat model of a hydrogel formulation containing levodopa, using ethanol and menthol as cutaneous permeation enhancers, was reported (Sudo et al., 1998). The influence of the enhancer concentration was studied, with an increasing percentage of ethanol ranging from 20% to 40% (v/w). The hydrogel was attached to the shaved abdominal skin of the rat with an adhesive and the plasma concentrations of levodopa and dopamine were determined. A marked elevation in the diffusion parameter value of levodopa was observed with the system containing 40% ethanol and 2% L-menthol. A higher amount of ethanol provided a more effective perturbation of the *stratum corneum*, allowing an easier cutaneous permeation of levodopa. Interestingly, the plasma level of levodopa rose until 180 min, while the dopamine level reached a plateau after 30 min. Nevertheless, peripheral side effects were observed as no IEDD was incorporated in the hydrogel. Moreover, the high percentage of ethanol incorporated in the formulation could produce a non-negligible dermal irritation. Finally, the levodopa hydrogel became blackish within several days after its preparation. This coloration was attributed to the instability of levodopa in the hydrogel.

Therefore, the same research group developed a similar transcutaneous dosage form containing two different layers (Iwase et al., 2000). The first sheet contained a lyophilized preparation of levodopa and was in direct contact with the skin. The second sheet encompassed a hydrogel including 40% (w/v) of ethanol and 2% (v/v) of L-menthol as cutaneous permeation enhancers. The preparation of the levodopa-containing layer offered a low yield of 50%, probably limiting any industrial production. As it was stored in a dark box, the hydrogel remained colorless for at least 12 weeks. Since light is known to accelerate the oxidation of the hydroquinone groups, the apparent stability of levodopa could be attributed to the storage conditions instead of the dosage form itself (Coello et al., 2000). Nevertheless, the lyophilisation process ensured dry storage conditions, thereby limiting the effect of water on the oxidation of the molecule. The plasma concentration of levodopa reached a peak at 30 min due to an initial burst effect. Its level then fell 1 h after application, following by a progressive increase until 180 min (Fig. 12).

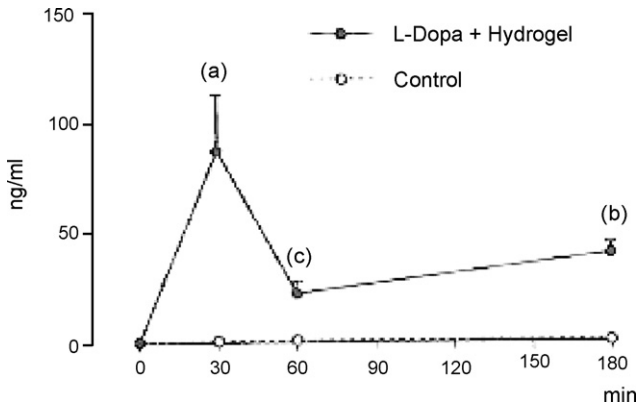

Fig. 12. Plasma level of levodopa during cutaneous attachment of levodopa and hydrogel compared with the control at identical time points (from Iwase et al., 2000).

The burst effect in the release of levodopa, the abolishment of which was attempted in the non-oral routes of administration such as transdermal delivery systems, is considered to be disadvantageous for PD patients exhibiting a wearing-off phenomenon.

In order to provide a progressive elevation in the plasma levodopa levels directly after the initial increase, thereby avoiding the peak and trough fluctuations, a pro-drug of levodopa was synthesized (Vaughan and Joyce, 1953). The lipophilic derivate, BELD, was incorporated in the same double-layered transdermal system as that evaluated on rats. The *in vivo* cutaneous study indicated that the application of BELD elevated the plasma level of levodopa more effectively than the application of the drug itself. However, the initial burst effect was still present and the plasma level of dopamine was much higher as it rose throughout the entire experimental period (Sudo et al., 2002). Consequently, the use of levodopa-butylester for transdermal administration seemed to be inappropriate.

With a thickness of only a few millimetres, the skin serves as a protective barrier against physical, chemical and microbial attacks. The stratum corneum, which constitutes the first layer of the epidermis, consists of many layers of compacted, flattened, dehydrated and keratinized cells. Due to this highly impermeable and tiny hydrophobic structure, the permeation of exogenous compounds, such as active drugs, is limited to small-sized neutral molecules, which are absorbed by passive diffusion. In addition to these physiological limitations, a membrane permeation-controlled transdermal drug delivery system usually failed to provide a constant plasma level of the active drug in the therapeutic window. Indeed, after an initial burst effect, the release of the drug is limited by both membrane and skin permeability (order 1). Moreover, during the release process, an inhomogeneous dispersion of the active drug is created in the transdermal system due to the theoretical adjacent layers of matrix constituting the thickness of the dosage form (order 1/2) (Chien, 1987b). Although these limitations may be partially avoided using iontophoresis, permeation enhancers or multi-layered patches, the complexity of such advanced delivery systems usually leads to a high-cost manufacturing process for a relatively small therapeutic benefit.

As has been noted, most drug delivery systems failed to provide a constant supply of levodopa. Consequently, the plasma level cannot be sustained for a prolonged period of time, thereby involving fluctuations in the therapeutic response. According to the drug delivery system considered, the inability to provide suitable plasma concentrations of levodopa is partially created by the complexity of the transport processes and the extensive metabolization of the drug. One interpretation is to deliver levodopa directly to its absorption zone in order to avoid the problems connected with the route of administration and with the pharmaceutics concept of the dosage form.

2.8. Intraduodenal infusion

Continuous enteral infusion of levodopa/carbidopa constitutes a useful method for the most severely fluctuating PD patients. A constant supply of dopamine can mimic the tonic dopaminergic stimulation seen in the normal state by avoiding the fluctuations in dopamine levels that accompany intermittent oral levodopa dosing, thus facilitating more normal control of movement (Nutt et al., 2000).

Consequently, a water-based gelling suspension of levodopa and CD (Duodopa®, Solvay Pharmaceuticals) that may be administered intraduodenally by a portal pump through a percutaneous endoscopic gastrostomy was developed. The dispersion contains micronized levodopa (20 mg/ml) and carbidopa (5 mg/ml) dispersed in a 1.8% aqueous methylcellulose solution (Bredberg et al., 1993).

Numerous clinical trials were conducted to evaluate the therapeutic benefits provided by Duodopa® in short-, medium- and long-term therapy. Prediction of the final therapeutic response obtained with infusion treatment may be firstly evaluated non-invasively with a nasoduodenal tube prior to surgical tube placement.

A 3-week follow-up study conducted on 30 PD patients showed that the more severe parkinsonian symptoms the patients had during their oral treatment, the more improved they became with infusion. This was especially marked for motor fluctuating patients as they usually developed a narrower therapeutic window for levodopa during their oral therapy. Only slight improvement was observed in less severely affected patients (Westin et al., 2006).

A 6-month trial followed by 25 advanced idiopathic PD patients demonstrated that subjects on levodopa infusion spent a larger proportion of the day in the "on" state. Motor fluctuations were significantly improved. After 1 week of dose adjustment, a duodenal tube was introduced through endoscopic gastrostomy, allowing a 24-h infusion. A continuous around-the-clock drug delivery especially improved quality of life for patients with severe night-time disability (Isacson et al., 2008).

Baumhack reported the improvement of motor functionalities in 4 advanced PD patients for 10–15 months. Nevertheless, a dislocation of the intestinal tube from the duodenum into the stomach happened in every case, although could be easily managed (Baumhack, 2007).

The effectiveness of the intestinal levodopa/carbidopa gel infusion was also evaluated on 22 PD patients that were followed for up to 2 years. Compared to oral administration, the duration of the "off" period and the appearance of motor fluctuations were statistically reduced in all patients ($P < 0.01$). The daily dose of levodopa was never increased during the study period. Only 2 patients withdrew because of adverse events (Mancini et al., 2007).

As the course of PD is a slow progression of symptoms, increasing dosage of levodopa had to be applied for longer duration therapy (e.g. 4–7 years). Although reduced variation of levodopa delivery to the absorption site reduced several long-term pathophysiological changes, the loss of dopamine neurons progressively diminished the synaptic storing capacity. Video scoring showed the negative evolution of the disease on patients under duodenal levodopa infusion. They spent more time in a normal state after 8 months than after 7 years. The mean time spent in the "off" state had also increased as a consequence. However, improvement was still higher as compared to oral therapy (Fig. 13) (Nilsson et al., 2001).

In addition to its heavy cost, the most frequent problems commonly reported with infusion treatment were technical difficulties

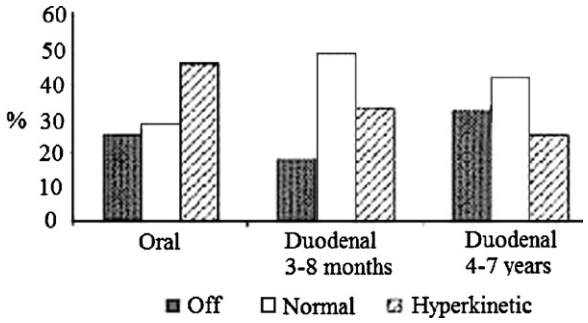

Fig. 13. Percentage of time spent in different motor states during oral therapy and after 3–8 months and 4–7 years of duodenal infusion, $n = 6$ (from Nilsson et al., 2001).

with the pumps, transient infection within the gastrostomy, change or adjustment of the catheter by gastroscopy or X-ray guidance, and sliding back of the tip from the duodenum into the stomach during infusion. After several years of treatment, the tube in use also had to be replaced due to increased resistance to infusion. Moreover, these mechanical limitations were more frequent in patients with severe parkinsonism as they developed dementia with major tremor.

On the other hand, duodenal infusion was shown to be feasible for a home therapy and to improve the quality of life of patients with severe PD. Moreover, as the evolution of the disease is connected with pulsatile stimulation of dopamine receptors, a constant supply of levodopa by duodenal infusion should also be useful in early treatment.

3. Conclusion

As it can be seen, drug delivery systems used in the treatment of PD provide both advantages and limitations connected to the route of administration. It seems that none of them is able to manage PD for a long-term therapy without the appearance of side effects, late adverse events or diminishing the evolution of the disorder. This is due to their inability to provide a constant and sustained supply of the active drug for a prolonged period of time. Simultaneously, the progressive degeneration of dopaminergic neurons leads to a decrease in the efficacy of such therapies. Therefore, gene therapy and neuronal replacement therapy using human embryonic cells were introduced as alternative strategies (Fraix, 2004).

However, due to the potential risk connected with such surgical interventions and the lack of data available in the literature, administration of active compounds such as dopamine agonists and inhibitors of cholinesterase remains the major commonly used treatment.

Long-term levodopa treatment coupled with disease progression leads to narrowing of the therapeutic window for levodopa. If the dose of levodopa administered is too low, the clinical effect will be insufficient and will wear off quickly. Therefore, motor complications have limited the usefulness of levodopa. However, levodopa currently remains the most powerful drug available to treat PD. No other drug that acts directly on the dopamine receptor is as powerful as levodopa. In order to increase its plasma half-life, levodopa may be associated to IEDD. The modern area of PD drug development and experimental therapeutics is focussed on the concept of slowing and targeting the release of levodopa to prolong the therapeutic effect and reduce the number of administrations.

Bredberg et al. (1994) reviewed the pharmacokinetics of levodopa and CD in the rat after different routes of administration. They showed that the bioavailability of levodopa is influenced by the presence of carbidopa and greatly depends on the mode of administration. They concluded that the oral route should not be considered as the best route for levodopa therapy. Nevertheless, it still remains the standard one due to the greater convenience that it provides for PD patients.

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