UPPSALA UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1437

New Approaches for Levodopa Treatment in Parkinson's Disease

MARINA SENEK

ACTA UNIVERSITATIS UPSALIENSIS UPPSALA 2018

ISSN 1651-6206
ISBN 978-91-513-0256-0
urn:nbn:se:uu:diva-343036

Dissertation presented at Uppsala University to be publicly examined in Rudbeckssalen, Dag Hammarskjölds väg 20, Uppsala, Friday, 20 April 2018 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Professor Maral Mouradian (Rutgers - Robert Wood Johnson Medical School, NJ, USA).

Abstract

Senek, M. 2018. New Approaches for Levodopa Treatment in Parkinson's Disease. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1437. 84 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0256-0.

Parkinson's disease (PD) is characterized by degeneration of dopaminergic cells, which results in dopamine depletion. Levodopa is the most effective symptomatic treatment, however, disease progression along with the unfavorable pharmacokinetics of levodopa makes the disease increasingly difficult to treat with time.

This thesis focuses on two new approaches of levodopa treatments, the levodopa/carbidopa microtablets and the levodopa/entacapone/carbidopa intestinal gel, developed for patients with advanced PD.

To evaluate the microtablet pharmacokinetics and pharmacodynamics in advanced PD patients, a clinical study was conducted. Higher levodopa maximum plasma concentration and systemic exposure was observed in patients compared to healthy volunteers. A high variability, with respect to response and duration of effect, was found, highlighting the importance of individual assessment of motor function to optimize treatment effect. A population pharmacokinetic model for levodopa and carbidopa was developed and the impact of covariates were investigated on the pharmacokinetics. Disease stage and increasing carbidopa dose were found to decrease levodopa apparent clearance. Carbidopa apparent clearance was found to decrease with age. An observational study was conducted, including patients treated with microtablets, in order to evaluate the treatment in clinical practice. A majority reported that the dose dispenser simplified their treatment and improved adherence, while the motor function, with respect to bradykinesia and non-troublesome dyskinesia, was mainly improved or unchanged.

To investigate the pharmacokinetics and pharmacodynamics of the newly developed levodopa/entacapone/carbidopa intestinal gel treatment, a clinical trial was conducted, where it was compared to the conventional levodopa/carbidopa infusion. The new treatment was found to allow a lower amount of levodopa administration without worsening the treatment effect. An increasing plasma concentration was observed, and a population model was developed for investigation of appropriate dose adjustments. The conclusion was that the continuous maintenance dose could be decreased by approximately 35%, on a population level, compared to the patients' usual dose on the conventional treatment. An effect from entacapone was identified in all individuals, regardless of catechol-O-methyl transferase genotype (rs4680).

To conclude, both new treatments are promising alternatives to current strategies and the developed models may in the future be used for model-based treatment optimization.

Keywords: Parkinson's disease, Pharmacokinetics, Pharmacodynamics, Population modeling, Levodopa, Carbidopa, Entacapone, Microtablets, Intestinal infusion

Marina Senek, Department of Neuroscience, Box 593, Uppsala University, SE-75124 Uppsala, Sweden.

© Marina Senek 2018

ISSN 1651-6206
ISBN 978-91-513-0256-0
urn:nbn:se:uu:diva-343036 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-343036)

To curiosity

Cover made by Aleksandar Senek and
Robin Elo

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Senek M,** Aquilonius S-M, Askmark H, Bergquist F, Constantinescu R, Ericsson A, Lycke S, Medvedev A, Memedi M, Ohlsson F, Spira J, Westin J, Nyholm D. Levodopa/carbidopa microtablets in Parkinson's disease: a study of pharmacokinetics and blinded motor assessment. Eur. J. Clin. Pharmacol. 2017;73(5):563–571.

II **Senek M,** Hellström M, Albo J, Svenningsson P, Nyholm D. First clinical experience with levodopa/carbidopa microtablets in Parkinson's disease. Acta Neurol. Scand. 2017;136(6):727–731.

III **Senek M,** Nyholm D, Nielsen EI. Population pharmacokinetics of levodopa/carbidopa microtablets in healthy subjects and Parkinson's disease patients. *Submitted*

IV **Senek M,** Nielsen EI, Nyholm D. Levodopa-entacapone-carbidopa intestinal gel in Parkinson's disease: A randomized crossover study. Mov. Disord. 2017;32(2):283–286.

V **Senek M,** Nyholm D, Nielsen EI. Population pharmacokinetics of levodopa intestinal gel in advanced Parkinson's disease patients – effects of simultaneous entacapone infusion and consequence of genetic polymorphism. *In manuscript*

Reprints were made with permission from the respective publishers.

Related work

I **Senek M,** Nyholm D. Continuous drug delivery in Parkinson's disease. CNS Drugs. 2014;28:19–27.

II Aghanavesi S, Nyholm D, **Senek M,** Bergquist F, Memedi M. A smartphone-based system to quantify dexterity in Parkinson's disease patients. Inform Med Unlocked. 2017;9:11–17.

III Johansson, D., Ericsson, A., Johansson, A., Medvedev, A., Nyholm, D., Ohlsson, F., **Senek, M.,** Spira, J., Thomas, I., Bergquist, F. Individualization of levodopa treatment using a microtablet dispenser and ambulatory accelerometry. CNS Neurosci Ther. 2018;1-9.

IV Thomas I, Alam M, Nyholm D, **Senek M,** Westin J. Individual dose-response models for levodopa infusion dose optimization. Int J Med Inf. 2018;112:137–142.

Contents

Abbreviations

AUC	Area under the curve
AALASSO	Adjusted adaptive least absolute shrinkage and selection operator
CD	Carbidopa
CDD	Continuous drug delivery
CDS	Continuous dopaminergic stimulation
CI	Confidence interval
CL/F	Apparent clearance
Cmax	Maximum plasma concentration
COMT	Catechol-O-methyl transferase
DaT-SPECT	Dopamine transporter single-photon emission computed tomography
DDC	Dopa decarboxylase
fa	Fraction absorbed
FDOPA-PET	Fluoro-DOPA positron emission tomography
FOCEI	First order conditional estimation with interaction
Frel	Relative bioavailability
Hcy	Homocysteine
HY	Hoehn and Yahr
ka	Absorption rate constant
LCIG	Levodopa-carbidopa intestinal gel
LD	Levodopa
LECIG	Levodopa-entacapone-carbidopa intestinal gel
LEDD	Levodopa equivalent daily dose
LNAA	Large neutral amino acid transporter
LOD	Limit of detection
LOQ	Limit of quantification
MAO-B	Monoamine oxidase-B
MTT	Mean transit time
NLME	Non-linear mixed effects
NONMEM	Non-linear mixed effect modeling (software)
OFV	Objective function value
3-OMD	3-O-methyldopa
pcVPC	Prediction-corrected visual predictive check
PET	Positron-emission tomography

PD	Parkinson's disease
PsN	Pearl-speaks-NONMEM
Q/F	Apparent inter-compartmental clearance
RSE	Relative standard error
SD	Standard deviation
SE	Standard error
SIR	Sampling importance resampling
SNpc	Substantia nigra pars compacta
Tmax	Time to maximum concentration
TRS	Treatment response scale
t½	Half-life
UPDRS	Unified Parkinson's disease rating scale
V_C/F	Apparent central volume of distribution
Vp/F	Apparent peripheral volume of distribution
WT	Weight

Introduction

Parkinson's disease (PD) is named after James Parkinson who described the disorder in 1817 in "*An essay on the shaking palsy*".[1] It is a chronic, slowly progressing neurodegenerative condition affecting approximately 1% of the population over the age of 65 years.[2]

The disease results in increasing motor impairment and non-motor symptoms that affect the individual with the disease as well as family and caregivers indirectly. The disease is further complicated with time by the development of motor fluctuations, manifesting as end of dose deterioration and re-emergence of parkinsonism (wearing-off symptoms) between doses and/or dyskinesia (involuntary movements). This makes the disease increasingly difficult to treat, and leads to the need for individualized, fine-tuned treatments.

Pathophysiology

PD is characterized by degeneration of dopamine secreting neurons, which results in a dopamine deficiency. It is an idiopathic disease, meaning that the cause of cell loss is largely unknown.[3] Dopamine is a rapidly acting neurotransmitter and causes an acute response of the nervous system. It is secreted by neurons that originate in the substantia nigra and terminate mainly in the striatal region of the basal ganglia. The basal ganglia are connected via a number of circuits to the cortex and are involved in the control of cognitive, motor and emotional processes.[4] Consequently, the symptoms in form of movement disorders as well as behavioral and cognitive changes, as seen in PD, may be caused by a dysfunction in the basal ganglia.

The neurodegenerative process mainly targets substantia nigra pars compacta (SNpc), where the dopamine deficit begins and is most severe.[5] The nerve cell bodies of SNpc are colored black by the pigment neuromelanin.[6] In PD however, the coloring fades, due to the degeneration of the pigmented dopaminergic neurons. This is the main pathological finding that characterizes the disease.[7] It was first described in 1919 in a doctoral thesis by C. Trétiakoff.[8]

Lewy bodies, described in 1912 by F.H. Lewy, are inclusions primarily consisting of aggregates of a presynaptic protein, alpha-synuclein, and are a hallmark of neuron degeneration in patients with PD. They are found in the neurons of substantia nigra, but are also present in other parts of the brain.[9]

Lewy bodies can also be found in the peripheral nervous system and in the enteric nervous system in the gut. However, it is not yet fully understood why they form,[10] and the question whether PD starts in the gut or not remains to be answered.

Symptoms and disease progression

The clinical symptom debut is usually between the ages 50-70 years, and occurs when neuronal degeneration reaches approximately 50%, and the dopamine depletion falls below 80%.[5,11]

The main clinical signs of PD are slowness of movement (bradykinesia), tremor at rest, rigidity, postural instability and gait disturbances. The symptoms initially occur unilaterally, meaning one side of the body is more affected.[12] Bradykinesia is the most characteristic clinical feature of PD while resting tremor is the most easily recognized symptom, characteristically disappearing with action and during sleep. Tremors affect approximately 75% of the patients. Rigidity refers to the phenomenon of increased resistance when stretching a muscle passively.[13] Postural instability is generally a manifestation of late stages of PD, and together with the gait disturbances, it may lead to frequent falls, with an increased risk of fractures.[14] As the disease progresses, the increasing motor disability affects the activities of daily living and health related quality of life.

Some of the non-motor features of PD are thought to precede the motor symptoms, such as constipation, anosmia and rapid eye movement sleep behavior disorder. They have been discussed as a possible way of diagnosing PD earlier.[15] Depression has been related to other neurotransmitter dysfunctions such as serotonin and noradrenalin, and may also be a pre-motor symptom. Other non-motor symptoms that affect patients include slowed thinking, anxiety, fatigue and bladder disturbances. In early PD, non-motor symptoms have been reported to have higher impact on health related quality of life compared to motor symptoms.[16] They are often undeclared by the patients but are important determinants of quality of life.[17] Patients with PD also have an increased incidence of dementia.[18] In the later stages, depending on how aggressive the disease progression is, patients may need assistance for many activities of daily living, such as feeding and dressing.

Diagnosing Parkinson's disease

PD is difficult to diagnose during the first years of disease due to the slow symptom progression. The diagnosis can be set through patient history, through the use of stringent clinical criteria and neurological examination.[19] A meta-analysis evaluating the clinical diagnostic accuracy of PD reported it to

be from 80% at initial assessment and up to 84% after follow-up when performed by movement disorder experts.[20] The accuracy of clinical diagnosis made mainly by non-experts was 74%.

During examination, the patient should show signs of bradykinesia and have at least one of the other characteristic motor symptoms. There are no biological or imaging markers, therefore the diagnosis remains clinical. Some tools can be used to confirm the presence of dopaminergic denervation e.g. FDOPA-PET, DaT-SPECT or PE2I-PET (used in Uppsala, Sweden).[21,22] These tools are however not used for routine diagnosis of PD, because they can only be used to support diagnosis of dopaminergic parkinsonism, but cannot differentiate idiopathic PD from multiple system atrophy, progressive supranuclear palsy or Lewy body dementia.

Levodopa, a dopamine precursor, may also be included as a diagnostic test. Patients with suspected PD may be given the drug to see if the motor symptoms improve, which usually indicates PD, however the test is uncertain early in the disease and is not recommended as a diagnostic test.[23] Some patients with PD may not respond to treatment while others with atypical parkinsonism might do so and be misdiagnosed.[24]

Disease severity can be measured with rating scales used for monitoring PD-related disability and impairment. Clinical assessment can also be done using different non-motor symptoms questionnaires and questionnaires to evaluate health-related quality of life.

Rating scales

Several rating scales, beyond the ones mentioned here, are available for assessment of motor and non-motor disabilities, disease progression and treatment effect.[25]

The unified Parkinson's disease rating scale (UPDRS) is a widely used tool for assessment of different aspects of PD.[26] It was developed in 1987, and consists of four parts covering; behavior and mood (part I), activities of daily living (part II), motor examination (part III) and complications of therapy (part IV). Each part is rated on a scale from 0 to 4, or answered by a yes (score 0) or no (score 1). It can reveal changes in the course of the disease and be used in interventional studies.

The treatment response scale (TRS) is used to rate the motor function on a seven-step scale. It ranges from severe choreatic dyskinesia (score +3), to normal mobility (score 0), to severe parkinsonism (score −3).[27] In the case of mixed patterns, the instructions are to rate according to the dominating movement pattern, with the walking ability weighted as more important.

The Hoehn and Yahr (HY) scale was initially designed to give a simple descriptive general estimate of clinical function in PD, combining functional disability and objective signs of impairment.[28] It was developed in the pre-levodopa era, but has continued to be used widely. It was designed as a five-

point scale (1–5) based on the concept that parkinsonian dysfunction is related to bilateral motor involvement and compromised balance and gait. Stage 1 represents unilateral involvement only; Stage 2 is bilateral involvement, without impairment in balance; Stage 3 is first sign of impaired righting reflexes; Stage 4 is fully developed severely disabling disease and; Stage 5 is confinement to bed or wheelchair unless aided. A score of 0 indicates no signs of disease.

Levodopa

> *[...] if L-dopa works in parkinsonism by replenishing the missing dopamine in the striatum, then L-dopa is quite obviously the most natural substance we can have for treating what I should like to call "the striatal dopamine deficiency syndrome." However, it is quite clear to me as a pharmacologist that, whatever the mode and site of its action, L-dopa is far from being perfect as a drug.*
>
> *Oleh Hornykiewicz (1970)*

Pharmacokinetics

Levodopa (L-3,4-dihydroxyphenylalanin) is a naturally occurring large neutral amino acid, and a precursor to dopamine. Today, levodopa is the most effective symptomatic treatment throughout the course of PD. It ameliorates the symptoms and has served as a primary pharmacotherapy for almost 50 years. Levodopa can, unlike dopamine, cross the blood-brain barrier, and replenish the lost supply of dopamine.[29]

Levodopa is rapidly absorbed in the proximal small intestine after oral intake. The absorption from the stomach and colon is limited. It is transported across the intestinal endothelium, and across the blood-brain barrier by the saturable large neutral amino acid (LNAA) transporter system.[30] The LNAA transporters also transport other large neutral amino acids, therefore levodopa may compete for transport with dietary proteins.[31–33]

Orally administered levodopa undergoes considerable first-pass metabolism.[34] Administered alone, the bioavailability is approximately 30%. Levodopa is eliminated completely through metabolism and the metabolites formed are excreted mainly in the urine. The major metabolic pathways are the dopa decarboxylase (DDC) and catechol-O-methyl transferase (COMT) path-

ways.[35] DDC, responsible for the majority of the levodopa metabolism, is distributed in the gut, liver and kidneys. In a study in dogs, levodopa administration into the duodenum and injected into the hepatoportal vein showed that the main metabolism occurs in the intestine.[36] When administered alone, levodopa has a half-life of approximately 60 minutes[37], and less than 1% of a given dose reaches the brain, meaning that high doses are necessary for symptom relief.

Dopa decarboxylase inhibitors

Levodopa as a combination therapy with DDC inhibitors was first marketed in 1975.[38] Nowadays, levodopa is always administered with a DDC inhibitor, either carbidopa or benserazide, usually in a 1:4 ratio. The DDC inhibitors prevent metabolism of levodopa peripherally because neither carbidopa, nor benserazide crosses the blood-brain barrier at the doses administered. Addition of DDC inhibitors also reduces the severity of dopamine-mediated side-effects. The DDC inhibitors are clinically seen as interchangeable, although benserazide has been reported to be a more potent inhibitor of DDC,[39] resulting in higher levodopa peak plasma concentration.[40,41]

When levodopa is co-administered with carbidopa, the bioavailability increases to approximately 85%, the half-life increases to approximately 90 minutes and the clearance is reduced by roughly 50%.[37] The daily dose required to reach symptom relief can be reduced by about 70%.[42,43]

Carbidopa is a rapidly absorbed, competitive inhibitor of DDC.[43] It has a half-life of 2-3 hours. A daily dose of 75 to 100 mg of carbidopa is believed to be the requirement for DDC inhibition.[42–44]

In countries where levodopa-DDC products are not available, either due to affordability and accessibility, the use of *Mucuna pruriens*, which contains levodopa, has been investigated as an alternative.[45] The *Mucuna pruriens* seeds were roasted, powdered, and added to water for administration. It has shown non-inferiority in comparison to levodopa-DDC products.[46] While the treatment may be an option, and patients can learn to grow the vegetable and prepare the powder, the small study reported tolerability to be a limitation. Further studies on how to titrate the treatment to minimize side-effects are warranted.

The catechol-O-methyl transferase inhibitor entacapone

When the DDC pathway is inhibited, the metabolism of levodopa is shifted towards the COMT-pathway, which becomes the dominating metabolic pathway.[47] Entacapone is an orally administered, reversible, COMT inhibitor. The oral availability is reported to be 30-46%, and to increase with increased doses. A limitation is that entacapone has a short terminal half-life,[48] and requires frequent administration. The recommendations are to administer entacapone with every levodopa dose. The daily dose should not exceed 2000 mg.[49]

Maximum inhibition of COMT is reached within one hour, and full activity of the enzyme is regained within 8 hours. Entacapone administered with oral levodopa (combined with a DDC inhibitor), increases the area under the plasma concentration time curve (AUC) for levodopa,[50] which may cause dyskinesia if the levodopa dose is not adjusted.[51] The dose of oral levodopa can be decreased by 20-33% to reach equivalent levodopa concentrations.[52,53]

A COMT inhibition leads to less formation of the levodopa metabolite 3-O-methyldopa (3-OMD). The metabolite uses the same transporters as levodopa,[54] and therefore competes for transport, potentially limiting the penetration of levodopa into the central nervous system. 3-OMD has a half-life of approximately 15 hours and does not have higher affinity for the transporter system.[55] Challenges with 3-OMD have shown to reduce the clinical response to levodopa. However, 3-OMD concentrations were suggested to be low in comparison with other amino acids present in the body and during long-term treatment the concentration does not vary substantially during the day. It was therefore concluded that it does not explain the daily fluctuations in response.

Gastric emptying

The stomach has a limited capacity of absorbing levodopa, but controls the intestinal delivery of an ingested dose, and infrequent gastric emptying is one reason to why it is difficult to deliver the drug in a controlled manner.[56,57] Gastric emptying can become slowed, delayed and erratic as PD progresses. The erratic emptying is not only caused by reduced gastro-intestinal mobility due to the disease, but studies have also reported that levodopa, on its own, may affect the gastric emptying.[58,59] The erratic gastric emptying has been observed as double-peaks in paracetamol plasma concentration when it was co-administered with levodopa, but not when paracetamol was administered alone. The double-peak phenomenon has been observed in both healthy volunteers and in patients.[60] The resulting varying plasma concentration of levodopa can lead to an unpredictable response to treatment.

Dose administration immediately after food intake may also cause a complete lack of efficacy from the administered dose, called the “no-on” phenomenon, due to reduced drug absorption, delayed gastric emptying or competition for transport across the blood-brain barrier.[61]

Peripheral neuropathy in Parkinson’s disease

Since the introduction of levodopa/carbidopa intestinal gel, cases of symptomatic neuropathy, have been reported in patients receiving the infusion therapy.[62] Lately, cases in patients with oral treatment have also been described.[63] They are often associated with a B6/B12 vitamin deficiency and elevated levels of homocysteine (Hcy), as well as high levodopa dosage and high age of patients.[64,65] The clinical features of peripheral neuropathy may vary widely

and cause pain, weakness, altered sensation or autonomic symptoms.[66] The neuropathies in PD cases are mostly sensory or sensorimotor, meaning that the patients experience reduced sensation and movement.[67,68] The link between levodopa and the development of peripheral neuropathy still remains to be determined, and additional factors may be causing the side-effect, but some hypotheses have been presented.

The metabolism of levodopa to 3-OMD has been suggested to be associated with the development of peripheral neuropathy.[67,69] The metabolism is hypothesized to be a part of a cascade of events leading to vitamin B12, B6, and/or folate deficiency, and dysfunction of Hcy metabolism.[70] The conversion of levodopa to 3-OMD by COMT, requires methyl groups, and may lead to depletion of the methyl group reserves and thereby increased Hcy production. Subsequent Hcy re-methylation requires vitamin B12 as a co-factor and methyl groups. Hcy can also be trans-sulfurated which requires vitamin B6. A COMT-inhibition may thus, speculatively, reduce the risk of this side effect.[71]

Genetic polymorphism of enzymes

Genetic polymorphisms of the enzymes DDC and COMT may potentially be a cause for observed differences in levodopa pharmacokinetics and effect between individuals. Associations between different polymorphisms in the COMT and DDC genes have been investigated.

The polymorphism in the COMT gene (rs4680) results in a conversion of the amino acid valine to methionine.[72] Valine is associated with a higher COMT activity, while methionine is associated with a lower activity. Corvol et al., (2011)[73] investigated the effect of this polymorphism with respect to pharmacokinetics of levodopa administered with and without entacapone, and the efficacy assessed with UPDRS part III. Significant differences in both duration of time in on, levodopa AUC, half-life and clearance, were reported between patients with high activity and low activity. The authors de Lau et al., (2011) reported that patients with low activity have an increased risk of developing dyskinesia in a dose dependent manner. They suggested it to be because a lower activity results in higher dopamine concentrations centrally.[74] Contin et al., (2005)[75] conducted a study where the same genetic polymorphism was investigated, also with respect to levodopa pharmacokinetics and pharmacodynamics, but without the addition of entacapone. The effect was assessed with a finger tapping test as well as rating of dyskinesia. They reported no significant difference between the genotype subgroups.

Two variations (rs921451 and rs3937091) in the DDC gene have also been investigated with respect to levodopa pharmacokinetics and its effect.[76] A significantly lower improvement in motor function (assessed with UPDRS part III, as difference in AUC from baseline) was reported in the group with low/intermediate activity compared to the patients with high activity, based on the

polymorphism rs921451. The same was observed for patients with low/intermediate activity based on the polymorphism rs3937091 compared to the group with high activity. No differences were found in levodopa or dopamine pharmacokinetics.

Motor complications

Levodopa has an effect during the entire course of the disease and increases the life expectancy. Initially, most patients are in the so called "honeymoon period", meaning that they are in a stable phase. The motor function is normal ('on'-state) once the lower threshold for symptom relief is crossed, and the symptom control remains stable throughout the day.[77] Patients prescribed a low dose of levodopa three times daily may have a very good symptom control, despite the oscillating plasma concentration caused by the short half-life of levodopa, and plasma concentrations may decrease without the re-emergence of symptoms (Figure 1).

As the disease progresses, the duration of benefit from a levodopa dose becomes shorter,[78] limited to a few hours. The drug wears off and parkinsonian motor and non-motor symptoms may reappear between doses. After five years approximately 50% of patients have reached this stage.[79]

With disease progression the therapeutic window continues to narrow. Choreatic dyskinesia (dancelike involuntary movements) may develop, typically as a peak concentration phenomenon, due to pulsatile drug delivery.[80,81] The symptom relief threshold rises, meaning that higher doses may be needed to reach symptom relief. An upper threshold is formed, where dyskinesias appear when it is crossed rendering the patient in a state of near normal motor function accompanied with involuntary movements.

The motor fluctuations, "off" (symptoms of parkinsonism), "on" (near normal motor function) and "on" with peak-dose dyskinesia (near normal motor function accompanied with involuntary movement),[82] have been correlated to levodopa plasma concentration in advanced PD patients. PET-studies in PD patients have shown that concentrations of dopamine after administration of levodopa correlate with symptoms and the extent of induced dyskinesias.[83–85]

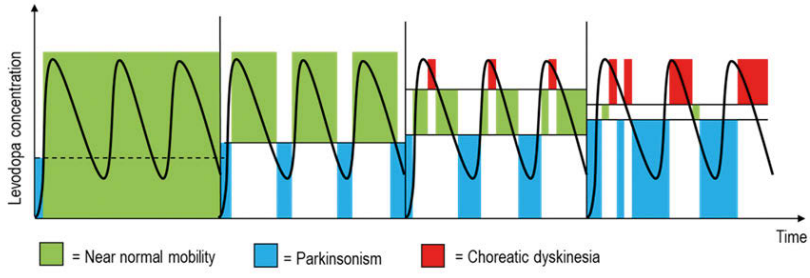

Figure 1. Schematic presentation of the pharmacokinetic-pharmacodynamic relationship of levodopa at different stages of PD.[80] Copyright: Adis

Some patients may continue to progress to severe on-off fluctuations, which seem to appear randomly, but are still often related to fluctuations in drug concentration. The therapeutic window is then very narrow, and normal mobility for a longer period of time is hard to achieve. The development of motor complications complicates the symptomatology and the disease becomes increasingly difficult to treat.

Treatment and the concept of continuous dopaminergic stimulation

Dopaminergic neurons usually fire in a tonic manner at a rate of 3-6 Hz and intermittently under stimuli.[86] Under normal conditions and initially during early PD, there is a sufficient amount of dopaminergic neurons and presynaptic nerve terminals that can store dopamine. When levodopa is administered, it is converted to dopamine within the dopaminergic neurons and stored and released in a usual manner. As PD progresses, there is a continued loss of neurons. Striatal uptake of radioactivity measured using PET has shown that the capacity of the striatum to retain tracer is impaired in PD patients compared to controls.[86] As the neurons degenerate, the administered levodopa is converted by the remaining dopaminergic neurons, but also by other cells that have little or no capacity to store dopamine.[87,88] The ability to buffer levodopa plasma concentrations is slowly lost, causing a pulsatile stimulation of the postsynaptic receptors. Intermittent doses induce discontinuous stimulation of the postsynaptic receptors, which is hypothesized to lead to molecular physiological changes and development of motor complications.[88,89]

A continuous dopaminergic stimulation (CDS) becomes desired to stabilize motor function. This may be achieved by a more continuous drug delivery (CDD), avoiding both the high peaks and the low troughs in drug concentration. A stable plasma concentration, and thereby a more natural continuous

stimulation of postsynaptic neurons, is thought to be essential for normal basal ganglia function.[80]

The choice to start treatment is based on consultation between the treating physician and the patient. If a patient is younger, the treatment can start with a dopamine agonist (could be in combination with levodopa/DDC inhibitor), in an attempt to delay the development of dyskinesia.[90] However, agonists are less potent at ameliorating the PD symptoms. Eventually most patients require levodopa, which remains the most effective treatment and is considered to be the "gold" standard.[91,92] As wearing-off symptoms start to develop, one strategy is to adjust the number of daily levodopa doses. Addition of enzyme inhibitors may also be done, such as monoamine oxidase-B (MAO-B) inhibitors, to decrease central dopamine breakdown, and COMT inhibitors, to increase peripheral levodopa concentrations.[93]

A fine-tuned, individualized oral levodopa dose providing stable plasma concentrations could reduce levodopa-related motor complications. Currently available oral strategies for extended levodopa action have so far failed to meet the need and they are not possible to fine tune with the available tablet strengths (lowest strength is 50 mg of levodopa). High levodopa doses have been associated with greater frequency of motor complications[7,51], which suggests that lower doses should be administered, in order to avoid dyskinesia. However, the doses need to be high enough so they do not become sub therapeutic.[94]

New strategies have been developed or are under development, aiming at providing a more stable levodopa plasma concentration and smoother postsynaptic stimulation. A new COMT-inhibitor that can be administered once daily, opicapone, was recently approved. It increases the levodopa exposure,[95] and was reported to be non-inferior compared with entacapone.[96] New extended release formulations are also under investigation, which combine an immediate release component and an extended release component.[97]

Infusion treatments that allow individual dosing, but also result in a steady levodopa plasma concentration, have shown the benefits of CDD on PD symptoms.[98,99] When the oral strategies can no longer provide enough time in "on", advanced device-aided strategies are available. These include deep brain stimulation (DBS), subcutaneous apomorphine infusion and levodopa/carbidopa intestinal gel (which is mentioned in a later section).[100]

Adherence to medication

Adherence to PD medication can be assessed as total adherence, which refers to total dose taken expressed as a percentage of total dose prescribed, and timing adherence, which is the percentage of doses taken at correct timing intervals. The adherence can be studied with different methods, e.g. pharmacy refill records, electronic monitoring bottles, pill-counts and questionnaires. In a

review article where results from nine publications were assessed, the prevalence of suboptimal medication adherence (defined as less than 80% of doses taken) was found to be between 10% and 67%.[101] One of the included studies was a single-center observational study including 112 patients.[102] Using an electronic monitor box, they found that the patients had a total adherence of 98%, while the timing adherence was only 24%. Some of the reported predictors of non-adherence are complex drug regimens, cognition, younger age, longer disease duration and poor knowledge of the disease.[101,103]

Individualized levodopa treatments for Parkinson's disease

The levodopa/carbidopa intestinal gel (LCIG, containing levodopa [20 mg/mL] and carbidopa monohydrate [5 mg/mL]; Duodopa®/Duopa®, AbbVie, Chicago, MI) is a treatment developed for patients with advanced PD.[104] It provides a stable levodopa plasma concentration, compared to oral administration, due to continuous infusion of levodopa and carbidopa into the duodenum/jejunum by a portable pump and intestinal tube.[99] The LCIG pump and cassette (100 mL) measure 100×197 mm, and the weight of the LCIG pump system with a full cassette is approximately 550 g (Figure 2). The tube placement allows the drugs to bypass the stomach making the drug delivery independent of gastric emptying, which leads to a significantly reduced within-subject variability in levodopa concentration.[99,105,106] By allowing individual doses[107] and a stable plasma levodopa concentration, it has proven to be successful in reducing motor fluctuations and improving health related quality of life.[108–110]

Levodopa/entacapone/carbidopa intestinal gel infusion

If LCIG is combined with orally administered entacapone, the daily levodopa dose may be lowered by approximately 20%.[52] The levodopa/entacapone/carbidopa intestinal gel (LECIG; LECIGON; LobSor Pharmaceuticals AB, Knivsta, Sweden) is a newly developed formulation for intestinal infusion. It contains levodopa [20 mg/mL], entacapone [20 mg/mL], and carbidopa monohydrate [5 mg/mL]. It is administered the same way as LCIG, via a gastrojejunostomy tube. LECIG is contained in 50-mL syringes attached to an infusion pump (CRONO S-PID 50, Cane, Italy), together measuring 55×150mm (Figure 2). The weight of the LECIG pump system with a full syringe is approximately 230 g.

Figure 2. **Left:** Pump used for administration of LECIG (levodopa/entacapone/carbidopa intestinal gel). Weight with full syringe: 230 g. **Right:** Pump used for administration of LCIG (levodopa/carbidopa intestinal gel). Weight with full cassette: 550 g.

Patients on LCIG infusion may have a higher than usual levodopa doses throughout the day compared with those on oral treatment. This because concomitant dopaminergic medication with conventional LCIG is less common. An addition of entacapone would reduce the daily dose needed, and could theoretically inhibit the depletion of vitamins and elevation of Hcy.[71] With LCIG, it has also been suggested that malabsorption of e.g. vitamins could be a possible reason for development of peripheral neuropathy.[111] The addition of entacapone, and a reduction in volume of gel could possibly reduce this risk.[65,112]

Levodopa microtablets

LCIG infusion has shown the importance of individualized doses and a stable levodopa plasma concentration. In theory, frequent oral levodopa administration could have similar effects, approaching a more continuous administration, when motor fluctuations start to occur. In previous studies, more frequent administration of liquid levodopa has shown to improve motor function in patients with fluctuations.[113–115] This is, however, limited by adherence.

To enable individualized dosing and overcome adherence problems, low dose dispersible levodopa (levodopa [5 mg] and carbidopa [1.25 mg]) microtablets have been developed. They are dispensed with a dose dispenser with an alarm and memory function (Flexilev® and MyFid®, Sensidose AB, Sollentuna, Sweden) (Figure 3).[116,117]

Figure 3. The automatic microtablet dose dispenser MyFID®

The microtablets could be useful when the wearing-off symptoms start. Instead of increasing the dose, which may lead to side-effects such as dyskinesia, one alternative is to adjust the dosing intervals so that they correspond to the duration of response. As a result, the dose may have to be adjusted slightly, i.e. fine-tuned, which is possible with the low-dose microtablets. This is usually already done in clinical practice, but small dose adjustments are not possible with available tablet strengths.

The dispenser saves records of dispensed doses and is pre-programmed with time points for administration. It also indicates on the screen if a dose is missed, helping the patients to keep track of their adherence. With its diary function it allows a better overview of symptoms for the patients, but also the healthcare personnel that titrate the doses and need to find the appropriate dosing frequency. The physician can also get an understanding of how the patient follows his or her treatment regimen and if extra doses are taken often, which could indicate a need for adjustments. Patients that take less medication than prescribed may more often report to be undertreated.[118] This scenario could lead to higher prescribed doses, and a more complex therapy, when it may not be necessary.

The pharmacokinetics of the microtablets have been investigated in healthy volunteers, where they were reported to be bioequivalent to levodopa/carbidopa immediate release tablets and levodopa/benserazide dispersible tablet.[40]

Pharmacometrics

Pharmacometrics is a quantitative science where mathematical and statistical methods are used to connect physiology, pharmacology, disease and treatment outcome to provide insight into the optimal use of drugs in clinical settings. The models are generally simplified descriptions of more complex systems,

useful for quantitatively describing and understanding e.g. the pharmacokinetic parameters and the time course of a drug effect.[119]

Non-linear mixed effects modeling

Non-linear mixed effects models (also called population models) use non-linear functions to describe processes related to pharmacokinetics, pharmacodynamics or disease. In a population model, all data are analyzed simultaneously, and one model is fit to the data from all subjects, however the information of the individuals is kept. It can be used as a tool for describing the mean tendencies in the population, identifying relationships between subjects' physiological characteristics and observed drug exposure or response, by accounting for different levels of variability such as inter-individual and inter-occasion variability at a population level.[120,121]

The population mean tendencies are described with fixed-effects parameter, i.e. a typical value, θ. It describes the structural elements such as clearance or volume of distribution. A random-effect parameter, η, describes the magnitude of the differences between the individual parameters from the typical value. It is assumed to be normally distributed with a mean of zero and an estimated variance of ω^2. An individual's parameter value, P_i, can be described according to:

$$P_i = \theta \times e^{\eta_i}$$

where η_i is the random effect describing the difference between the individual's parameter value from the typical parameter value.

Additionally, a residual error model can be used to describe the unexplained variability that originates from different types of errors e.g. timing errors and bio-analytical errors. This variability is described by ε, which is assumed to be normally distributed with a mean of zero and an estimated variance of σ^2.

From the fixed effects and random effects, we can obtain each individual's parameter set, i.e. the empirical Bayes estimates, which can be used for the calculation of the individual predictions and in diagnostic plots.

In a model, covariates such as age, gender and weight, can be included to explain inter-individual variability, improve the model fit and/or reduce the unexplained random residual error.

NONMEM

There are several software packages available suitable for population pharmacokinetic-pharmacodynamic analysis. The software NONMEM[122] (Icon De-

velopment Solutions, Ellicott City, MD, USA, 2009), is a non-linear regression program, and the most widely used within the field of pharmacokinetic-pharmacodynamic modeling.[122]

In NONMEM the likelihood of the data, given the model and parameters, is estimated. The estimation is performed by minimizing -2log-likelihood of the data, which gives the objective function value (OFV). The computation of the likelihood function cannot be solved exactly, but numerical approximation methods can be used. In this thesis the First Order Conditional Estimation method with Interaction (FOCEI) was utilized.

Aims

The overall aim was to investigate new levodopa treatments for patients with Parkinson's disease

The specific aims were:

I To study the pharmacokinetics and the effect of single-dose administration of levodopa and carbidopa dispersible microtablets in advanced Parkinson's disease patients, and to evaluate the impact of covariates on the pharmacokinetics using a population modeling approach

II To evaluate the treatment concept of levodopa-carbidopa microtablets and an automatic dose dispenser with respect to effect, compliance and usability, in the first patients to ever try the treatment in clinical practice.

III To compare levodopa-entacapone-carbidopa (LECIG) intestinal gel and levodopa-carbidopa intestinal gel (LCIG) with respect to the systemic levodopa exposure and effect on motor function improvement in advanced Parkinson's disease patients, characterize the population pharmacokinetics to derive a suitable translation of dose from LCIG to LECIG treatment, and investigate the impact of common variations in the dopa decarboxylase and catechol-O-methyl transferase genes on levodopa pharmacokinetics.

Methods

Study Data

Two different treatments, the levodopa/carbidopa microtablets and the levodopa/entacapone/carbidopa intestinal gel, were studied in this thesis. All studies were approved by the Uppsala Ethical Review Board (Dnr 2015/100 for **Paper I** and **III**, Dnr 2015/397 for **Paper II**, Dnr 2015/073 for Paper **IV** and **V**, Dnr 2016/439 for **Paper V**). The studies were conducted in Sweden in accordance with the regulatory requirements, Good Clinical Practice and the ethical principles of the Declaration of Helsinki as adopted by the World Medical Association.

The Swedish Medical Products Agency approved the trial with levodopa-entacapone-carbidopa intestinal gel, NCT 02448914 (**Paper IV and V**). Written informed consent was obtained from all participants before entering the studies.

Study designs

Levodopa/carbidopa microtablets

In order to investigate the pharmacokinetics and pharmacodynamics of levodopa/carbidopa microtablets, a single center, open-label, single dose study was conducted. The study involved participation of 19 patients with advanced idiopathic Parkinson's disease, that, at the time of the study, were treated with levodopa. The patients had to experience wearing–off symptoms and/or dyskinesia with their current treatment.

Each patient was given 150% of their usual morning dose in the form of levodopa/carbidopa microtablets. The 50% increase in morning dose was given to enable the study of the motor response during the transition from off-state, to normal mobility and/or dyskinesia and the deterioration back to off-state. The patients came to the study site early in the morning and received the microtablet dose, on an empty stomach, after an 8-hour over-night washout. The doses were calculated based on the patients prescribed morning levodopa-DDC inhibitor dose and the doses of other anti-PD drugs. The conversion formula used to calculate the appropriate levodopa/carbidopa microtablet equivalents was based on the proposed conversion factors by Tomlinson et al., (2010)[53] and on results from a previously published study by Nyholm et al., (2012).[40]

For assessment of levodopa, carbidopa and 3-OMD concentration, blood samples were collected at pre-defined time points. One blood sample was drawn prior to dosing, one in conjunction with study dose administration at time 0, and thereafter at 15, 30, 45, 60, 80, 100, 120, 150, 180, 210, 240, 300 and 360 minutes after dose administration. Because the parkinsonian symptoms, when drug effect has worn-off, can be troublesome for the patients, they were allowed to discontinue prior to study stop if they could no longer withstand being without additional medication.

Clinical experience with the levodopa/carbidopa microtablets

To investigate the clinical experience of the levodopa/carbidopa microtablets, all patients previously or currently treated with the levodopa-carbidopa microtablets were included in an observational study (**Paper II**). A questionnaire was developed in Swedish. It comprised questions concerning patient experience of efficacy of the treatment, usability of the dose dispenser, the activity of daily living and whether the dose dispenser affected their treatment adherence. To investigate the patient perceived effect from the treatment, questions regarding motor function, i.e. troublesome dyskinesia, non-troublesome dyskinesia and bradykinesia with respect to frequency, duration and severity were included in the questionnaire. Information concerning demographics, number of dose adjustments, previous treatments, reasons to why patients switched to and from the microtablet treatment and other relevant information was collected retrospectively from the patient records.

For assessment of treatment adherence, dose dispenser reports were obtained from the patients whose dose dispensers had the software version 1.0.15. The timing adherence of the levodopa/carbidopa microtablet intake was defined as the mean value of the number of daytime doses taken on time (±15 minutes) divided by the number of daily doses. The total adherence was calculated from the mean daily amount (mg) of levodopa taken divided by the total daily levodopa dose (mg) prescribed.

Levodopa/entacapone/carbidopa intestinal infusion

To compare the levodopa/carbidopa intestinal gel (LCIG) treatment with levodopa/entacapone/carbidopa intestinal treatment (LECIG), a randomized, open label, crossover clinical trial was conducted (**Paper IV**). Patients that had not been exposed to entacapone within three months of screening, and that were on stable LCIG treatment, requiring less than 125 milliliter volume of gel per day, were eligible for inclusion. The study was conducted at the CTC (Clinical Trial Consultants AB) center at the Uppsala University Hospital between May and July 2015. The treatment is usually given as a morning dose, followed by a continuous maintenance infusion. If required, the patients can administer extra bolus doses during the day. The LCIG doses were individually optimized in routine care prior to study start.

The LECIG morning dose, continuous maintenance infusion dose and bolus doses were initially decreased by 20% compared to the patients usual LCIG doses. This was based on a previously conducted study where oral entacapone, 200 mg every 5 hours, was given to patients on LCIG treatment.[123] A 20% decrease of levodopa/carbidopa dose was found to be appropriate with the treatment combination. The included patients were randomly allocated in a 1:1 ratio to a treatment sequence, where they would either start with LCIG and then switch to LECIG or vice versa (Figure 4).

A pre-planned blinded, interim analysis was conducted after inclusion of the first 5 patients (cohort 1). The sample size was re-calculated based on the intra-individual coefficient of variation of $AUC_{0\text{-}14/dose}$ and it was then also decided that the morning doses of levodopa/carbidopa would be increased to 90% for the second patient cohort (cohort 2, n=6).

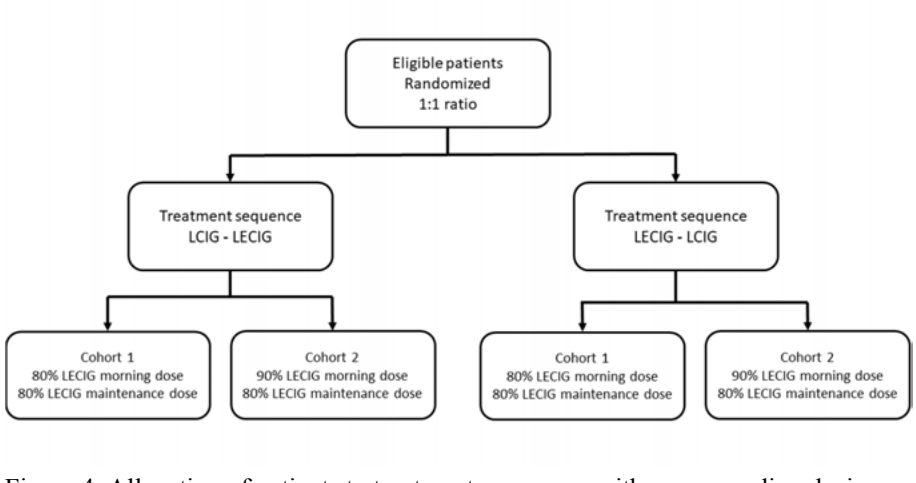

Figure 4. Allocation of patients to treatment sequences with corresponding dosing information.

The duration of treatment infusion was 14 hours, including the morning dose. After infusion stop it is necessary to flush the tube to prevent clogging overnight. The tube contains approximately 3 mL of gel and was flushed with water, which results in a fast bolus dose administration of 60/15 mg of levodopa/carbidopa and 60/60/15 mg of levodopa/entacapone/carbidopa. As night-time medication, patients were allowed to take levodopa/carbidopa immediate release oral tablets after infusion stop and up until three hours prior to infusion start.

During the study, low-protein meals were served at hours 1, 4, 7, 10 and 13 after infusion start. The mean (min, max) amount of protein in grams at each time point was 8.8 (5.7,12), 10.8 (9.3,12), 2.1 (2.0,2.3), 10.3 (8.9,11), 5.4 (3.0, 6.3) day 1 and 8.8 (5.7,12), 10.8 (9.3,12), 2.1 (2.0,2.3), 9.9 (5.8,11), 5.3 (3.0, 6.0) day 2.

Blood samples were drawn at pre-specified time points; immediately prior to dosing on day 1, half-hourly from 0-3 hours after infusion start and hourly from 3-14 hours. A blood sample was collected within 5 min after flushing the tube and thereafter half-hourly from 14.5 to 17 hours.

Levodopa/carbidopa microtablets in healthy subjects

Two studies had been previously performed with levodopa/carbidopa microtablets (Study 1 and Study 2, Table 1). Both studies included healthy volunteers. Study 1 was a single-center, open-label, single dose study where 18 healthy volunteers received 100/25 mg of levodopa-carbidopa microtablets.[40] They received the dose in fasting state, dispersed in a glass of water (100 mL). Blood samples were obtained once prior to dosing, every 10 minutes during the first hour after dose administration, every 20 minutes between hour 1 and 2, half-hourly between hour 2 and 3, hourly between hours 3 and 6, and then at 8, 10, 12 and 24 hours. Study 1 was included in the development of a population pharmacokinetic model for levodopa/carbidopa microtablets.

The second study was a single-center, open-label, multiple dose study including 10 healthy subjects (Study 2).[116] They received 75/18.75 mg levodopa/carbidopa microtablets as a morning dose and then five additional doses of 45/11.25 mg every 2.4 hours. The first dose was dispersed in 100 mL of water, and after intake an additional 100 mL of water was given. Thereafter the doses were given dispersed in 150 mL of water. Blood samples were collected 5 minutes prior to each dose administration and at 20, 40, 60 and 90 minutes after dose intake. The last blood sample was taken at 810 minutes from the first dose administration. Study 2 was used for external validation of the levodopa microtablet population pharmacokinetic model, i.e. it was not included during the model development process.

Table 1. Demographics, mean ± standard deviation [range].

Subjects	Sex (M/F)	Age (years)	Weight	Years since diagnosis	HY	Years on LD treatment	LD doses (mg)	CD doses (mg)	Last blood sample (min)
Healthy (Study 1)	9/9	26.0±6.2 [19-46]	71.7±11.3 [59-95]	NA	NA	NA	100±0	25±0	1440±0
Healthy (Study 2)	4/6	24.7±4.3 [20-32]	71.3±13.7 [52-100]	NA	NA	NA	300±0[a]	75±0	810±0

[a]Total drug dose administered, dosing interval 2.4 hours. M, Male; F, Female; HY, Hoehn and Yahr stage; LD, Levodopa; CD, Carbidopa; NA, Not applicable.

Bioanalysis

The methods used were validated in agreement with the ICH Validation of Analytical Procedures[124] and the Guideline on Bioanalytical Method Validation.[125]

Levodopa/carbidopa microtablets

All samples were analyzed at The Department of Pharmacology, University of Gothenburg, Sweden. Blood samples were collected in EDTA tubes, stored on ice, centrifuged (20 min, Sorvall SL50T, 3900 rpm) within 1 hour, and thereafter stored frozen at −80 °C until analysis. After thawing and protein precipitation, the plasma concentrations of levodopa and carbidopa were determined (Table 2). The limits of quantification (LOQ) were 10 and 20 ng/mL for levodopa and carbidopa, respectively.

Levodopa/entacapone/carbidopa intestinal gel

The blood sample analyses were conducted by OnTarget Chemistry, Uppsala, Sweden (at SVA laboratories). Concentrations of levodopa, carbidopa, 3-OMD and entacapone were determined in human plasma following protein precipitation (Table 2). The lower limits of quantification were 100 ng/mL for levodopa, 50 ng/mL for carbidopa, 600 ng/mL for 3-OMD and 20 ng/mL for entacapone.

Measurements of entacapone were missing for the first 5 patients due to degradation of entacapone by the stabilizer (sodium metabisulfite), which the blood collection tubes were primed with. Following this discovery, blood samples were collected in two different blood collection tubes, one with stabilizer, and one without.

Levodopa/carbidopa microtablets in healthy subjects

Blood samples were collected in EDTA tubes containing 143 IU of heparin, and immediately centrifuged (10 min, 3100 rpm). Fifty microliter of a 10% sodium metabisulfite solution was added and the samples were thereafter stored frozen at −75 °C until analysis. After thawing, and protein precipitation, the plasma concentrations of levodopa and carbidopa were determined (Table 2). The limits of quantification (LOQ) were 12 and 15 ng/mL for levodopa and carbidopa respectively.

Table 2. Bioanalysis equipment

	Levodopa/ carbidopa microtablets	**Levodopa/entacapone/carbidopa intestinal gel**	**Data form healthy subjects**
Chromatography	HPLC (Dionex Ultimate 3000 pump)	UPLC (Acquity)	HPLC (2250 Bischoff)
Detector	Waters 450 amperometric detector	Xevo-TQ-S tandem quadrupole mass spectrometer (Waters Corp., Milford, MA, USA)	Coulochem II multielectrode detector ESA (Chelmsford, Mass)
Column	C18 reverse phase column (Onyx) 2.0 mm x 200 mm	Waters BEH C18 (50 x 2.1 mm length x inner diameter, particle diameter 1.7 µm)	C18-AQ particle size 5 µm guard column (Reprosil-Pur)
Mobile phase	50 mmol/L phosphate buffer, pH 2.88 with EDTA 10 mg/L, methanol 4.0%, acetonitrile 1.5% and 1-octanesulphonic acid 100 mg/L	A: 0.5% Phosphoric acid in water containing 0.1% sodium bisulfite B: 5% formic acid in acetonitrile	100-mmol/L sodium dihydrogen orthophosphate, pH 3.0, containing 0.5-mmol/LOSA, 1-mmol/L EDTA, and 7% methanol
Tray cooling	+4 °C	-	+5 °C

HPLC, High performance liquid chromatography; UPLC, Ultra performance liquid chromatography.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic assessment

All analyses were performed in R 3.2.2. (**Paper I** and **IV**) and the non-compartmental pharmacokinetic analyses were performed using the ncappc package for R.[126,127] The blood samples that were not drawn on the exact time point were approximated to the pre-specified times for the statistical analysis.

The levodopa/carbidopa microtablet pharmacokinetic values (**Paper I**) calculated were time to maximum concentration (T_{max}), half-life ($t_{½}$), baseline and dose adjusted (to 100 mg for levodopa and 25 mg for carbidopa) maximum concentration of levodopa and carbidopa ($C_{max/dose}$) and baseline and dose adjusted area under the plasma concentration time curve ($AUC_{0\text{-}4/dose}$). The measured concentration at time 0 was subtracted from the rest of the measurements that were then divided with the individual administered dose of each compound. AUC was calculated using the trapezoid rule. Patients that remained without additional medication for at least 4 h were included in the analysis of $AUC_{0–4/dose}$. At least three descending measurements were required for the cal-

culation of $t_{½}$. The Welch two-sample t-test was used to compare the calculated parameters from patients with values from healthy subjects.[40] The Wilcoxon rank-sum test was used for statistical comparison of T_{max}.

For the comparison of the LECIG and LCIG treatments, $AUC_{0\text{-}14}$ and $AUC_{0\text{-}14/\,dose}$ for levodopa, carbidopa and 3-OMD (**Paper IV**) were calculated. The statistical comparison was done with paired Students t-test, two-tailed. It was done on the logarithmic values of AUC, with back-transformation to nominal values of point estimates and the 95% confidence interval (CI).

Population pharmacokinetic model development

One and two compartment disposition models were evaluated, parameterized in terms of relative bioavailability (Frel), absorption rate (ka), mean transit time (MTT), apparent volumes of distribution (V_C/F (central) and V_P/F (peripheral)), apparent inter-compartmental clearance (Q/F) and apparent clearance (CL/F). The inter-individual variability was included assuming a log-normal or normal (absorption related parameters) distribution of structural model parameters. Measurements below limit of detection (LOD) or LOQ were handled using the M6 method, where the first value below the limit is divided by 2, and subsequent measurements are deleted. In **Paper III**, 4.3% of the levodopa data and 5.6% of the carbidopa data were below the LOD. In **Paper V**, 1.9% of the levodopa data were below the LOQ.

The residual error models evaluated were additive, proportional, or combined additive and proportional error models.

Levodopa/carbidopa microtablets

The models for levodopa and carbidopa from microtablet administration were developed separately, and then combined for the covariate analysis. The patient population had a higher than expected plasma concentration at study start (mean 0.59 ± 0.93, range 0.01-3.41 μg/mL for levodopa and mean 0.03 ± 0.02, range 0.002-0.66 μg/mL for carbidopa). The plasma concentration prior to dose administration was estimated (with inter-individual variability), and assumed to be eliminated at the same rate as the individually estimated slopes (λ_2) of levodopa and carbidopa.

Double-peak plasma concentration profiles were present for both patients and healthy subjects. For a description of the pharmacokinetics, including the double-peaks, several models were investigated; parallel absorption compartments where fractions of the total dose administered are assumed to be fractionated into two separate dosing compartments (fraction was estimated on the logit transformed scale to constrain the parameter between zero and one), with transit compartments or lag-times separately estimated, and with and without the inclusion of a mixture model; an empirical model where two gastric emp-

tying rates are estimated and; a semi-mechanistic model where an effect compartment links the plasma concentration of levodopa which acts as a feedback mechanism on the rate of gastric emptying.

Because both levodopa and carbidopa were measured from the same blood sample and their residual error could be correlated, a part of the residual error was modeled as being shared between them.

Levodopa/entacapone/carbidopa intestinal gel

Both treatments (LCIG and LECIG) were modelled simultaneously, and differences in parameter estimates were investigated successively, to evaluate the impact of simultaneous entacapone infusion.

Only few patients took oral night-time medication. Because few blood samples were collected in relation to the oral treatment, the information was insufficient to allow for estimation of oral levodopa absorption related parameters. The absorption model for the oral treatment was described according to a previously published model[128]: one transit compartment between the depot and central compartment, a single transfer rate constant fixed to 2.4 h^{-1} and a difference in relative bioavailability of 3% for levodopa orally administered compared to intestinal infusion.

The difference in levodopa parameters for LECIG were investigated as a difference in relative bioavailability, absorption rate and apparent clearance. For illustration of a new dosing scheme on a population level, 1000 replicates of the dataset were simulated based on the study population, with altered doses.

Covariate model

Bodyweight (WT, subjects specific) was, in accordance to the allometric power model, included as a primary covariate, for both the microtablet model and the infusion treatment model, on all disposition parameters as shown in Equation 1 below:

$$P\theta_i = TVP\theta_1 \times \left(\frac{WT}{70}\right)^{P\theta_2} \qquad \text{Equation 1}$$

where $P\theta_i$ is the individual parameter value, $TVP\theta_1$ is the typical parameter value for an adult weighing 70 kg, and $P\theta_2$ is the allometric bodyweight exponent (fixed to 0.75 for CL/F and Q/F, and to 1 for V_C/F and V_P/F).[129]

For the microtablet model (**Paper III**), the secondary covariates assessed were age, sex, study association, Hoehn and Yahr score (HY, disease stage, set to 0 for healthy subjects), carbidopa dose, 3-O-methyldopa area under the curve (calculated with the trapezoid method), time since symptom onset, time since diagnosis and years with levodopa treatment. Initially, a graphically analysis was performed on all parameter-covariate relationships, by plotting empirical Bayes estimates versus covariates.

The adaptive least absolute shrinkage and selection operator (AALASSO)[130,131] as implemented in PsN[132] (version 4.7.0; Department of Pharmaceutical Biosciences, Uppsala University) was used for investigation of significant relationships (see section below, *Adjusted adaptive least absolute shrinkage and selection operator*). The data split was made on study association, to preserve the relative proportions in the cross-validation datasets.

Carbidopa is known to act as a peripheral DDC inhibitor, affecting the conversion of levodopa to dopamine. This effect of carbidopa dose on levodopa CL/F was evident in the initial covariate analysis. To further investigate the influence of carbidopa plasma concentrations on levodopa parameters, the levodopa and carbidopa models were combined, and the carbidopa dose and the individual model predicted concentration of carbidopa were investigated on levodopa CL/F, with a linear (Equation 2), or a non-linear covariate-parameter correlation (Equation 3):

$$P\theta_i = TVP\theta \times (1 + (P\theta_{Inter} \times (COV - \overline{COV})) \qquad \text{Equation 2}$$

$$P\theta_i = \frac{TVP\theta}{1+\left(\frac{COV}{P\theta_{Inter}}\right)} \qquad \text{Equation 3}$$

where $P\theta_i$ is the individual parameter value, $TVP\theta$ is the typical value of apparent levodopa clearance, $P\theta_{INTER}$ is the interaction factor representing the carbidopa potency as a competitive inhibitor, COV is the covariate representing carbidopa dose or model predicted concentration, and $\overline{COV}$ is the mean model predicted carbidopa dose. After investigating and including the effect of carbidopa, the AALASSO was repeated.

Adjusted adaptive least absolute shrinkage and selection operator

The adjusted adaptive least absolute shrinkage and selection operator (AALASSO) is a version of the least absolute shrinkage and selection operator (LASSO). It is a penalized estimation method where the covariates are standardized to a mean of zero and a variance of one.[130] The covariates are included according to a linear covariate-parameter correlation and the selection of covariates is carried out based on the tuning parameter (t-value). The estimated regression coefficients are restricted based on the t-value which restricts the model size. The coefficients within a cutoff value (in this case 0.005) are shrunk to zero, while the coefficients of the significant covariate-parameter relationships are estimated. The sum of the covariate coefficients has to be smaller than the t-value, which is estimated with a five-fold cross-validation. One advantage of the method is that all covariate-parameter relationships are tested simultaneously. This is in contrast to the stepwise covariate modeling

procedure (SCM) where one covariate is tested at a time and the model size depends on the P-value.

The AALASSO method includes the ratio of the standard error of the maximum likelihood estimator to the maximum likelihood estimator as the initial weight.[131] This method was suggested to overcome multicollinearity between covariates, and was compared to the adjusted LASSO (ALASSO) and the LASSO methods. AALASSO showed to have a better predictive performance with a low number of subjects and highly correlated covariates.

Software

The non-linear mixed effects modeling software NONMEM[122] (version 7.3; Icon Development Solutions, Ellicott City, MD, USA, 2009) was used for the development of the population pharmacokinetic models using the first order conditional estimation method with INTERACTION (FOCEI) and a user-defined model (ADVAN6 (**Paper III**) and ADVAN13 (**Paper V**) NONMEM Subroutine). The models were run using PsN[132] (version 4.7.0; Department of Pharmaceutical Biosciences, Uppsala University).

Model evaluation

The models were evaluated by scientific plausibility, goodness-of-fit plots, parameter precision and the objective function value (OFV). For the graphical display of the predictive performance of the model, the prediction corrected visual predictive check (pcVPC, 1000 replicates) plot was used to normalize for variability in independent variables, e.g. times, body weights and doses.[133] The OFV, was utilized in likelihood ratio testing to compare nested models (significance level 0.05, corresponding to ΔOFV of 3.84 for 1 degree of freedom). The Sampling Importance Resampling (SIR) procedure was used for calculation of parameter uncertainty on model parameters.[134]

Pharmacodynamics

The Unified Parkinson's Disease Rating Scale (UPDRS) and the treatment response scale (TRS) were used for motor function assessment. The TRS is a seven step scale ranging from -3 (severe parkinsonism) to 0 (normal mobility) to +3 (severe choreatic dyskinesia).[27] When mixed patterns of mobility were present (i.e. indications of both bradykinesia and dyskinesia), the instructions were to rate according to the dominating movement pattern, with the walking ability weighted as more important.

In the microtablet trial (**Paper I**) the motor function test was done in repeated test cycles, once before the study dose administration and then repeatedly every 20 minutes until 111 minutes, and thereafter every 30 minutes until 321 minutes (time of the last test) or until the patient could no longer remain

without medication. Each test cycle was video recorded for blinded (with respect to time) assessment by three movement disorder specialists. A computer program was used to randomize the video sequences to ensure that the rating was blinded. Six items were selected from the UPDRS motor symptom evaluation part (III), and each item was rated on a scale from 0 to 4, according to the UPDRS, per time point and item.[135] The items included were; finger tapping (item 23), rapid alternating movements of hands (item 25), tapping the heel (item 26, only rated by two of the raters), rising from chair with arms held across the chest (item 27), gait (item 29) and bradykinesia (item 31) (Figure 5). The gait included a walk of 4 meters, a turn and then a walk back. The three raters also rated the severity of dyskinesia on a scale from 0 to 4, where a score of 0 is given if there are no signs of dyskinesia, and the mobility was also rated according to the TRS. The raters' median scores for the six UPDRS item scores were summed up per time point into a total value.

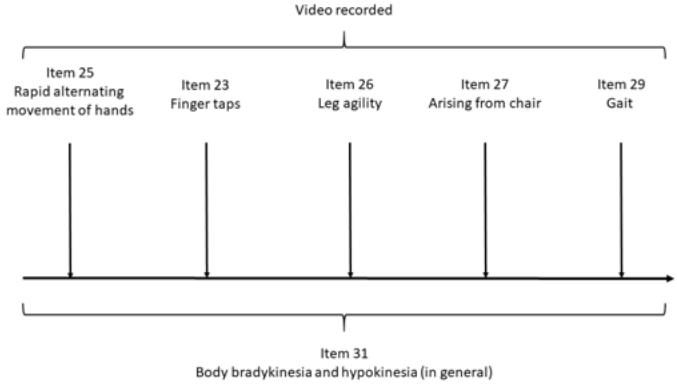

Figure 5. General overview of a test cycle, with the six included UPDRS items, rated by the movement disorder specialists (**Paper I**).

During the infusion treatment (**Paper IV**), study personnel were trained in motor function assessment according to the TRS[27] prior to study start. The assessment of patient motor function was done at the same time points as the pharmacokinetic sampling. The statistical analysis of the TRS scores was done by comparison of the ordinal mean TRS scores using Wilcoxon's signed rank test.

Genotyping of DDC and COMT

For genotyping of polymorphism of the COMT gene (rs4680), and polymorphisms of the DDC gene (rs921451 and rs3837091) (**Paper V**), genomic DNA

was extracted from an additionally given blood samples (Uppsala Clinical Research center and Uppsala Genome Center, Uppsala Sweden). The single nucleotide polymorphisms (SNP) with ID rs4680 ($COMT_{SNP}$) and with ID rs921451 (DDC_{SNP}) were analyzed by allelic discrimination TaqMan assay. The TaqMan SNP genotyping analysis was run on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). A substitution of A > G in the COMT gene (rs4680)[73,75] results in the conversion of the enzyme valine (158Val, associated with higher activity) to methionine (158Met, associated with lower activity). The nucleotide substitution of T > C in the DDC gene (rs321451)[76,136] is associated with lower expression and/or activity of DDC. The Sanger sequencing method was used for identification of the DDC gene (with ID rs3837091) polymorphism (DDC_{INDEL}). BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher) was used for the sequencing reactions. The fragments were sequenced with capillary electrophoresis with an automated sequencer (AB3730XL DNA Analyzer, Applied Biosystems, Foster City, CA, USA). The amplicons were compared to a GenBank-reference sequence, for identification of the polymorphism which is characterized by a 4-base pair deletion (AGAG). The deletion may cause lower expression and/or activity of DDC.[76] One control for each genotype and patient was analyzed. Graphical exploration of the difference in CL/F for the DDC_{SNP}, DDC_{INDEL} and $COMT_{SNP}$ were done based on empirical Bayes estimates.

Results

Study data

Levodopa/carbidopa microtablets

Nineteen patients experiencing wearing-off symptoms and/or dyskinesia (assessed at inclusion, based on the wearing-off questionnaire[137] and the UPDRS IV) were enrolled in the study (Table 3).

Table 3. Patient characteristics (n=19)

ID	Sex	Age	BMI	Symptom onset (years)	Diagnosis (years)	LD (years)	HY	UPDRS IV	Study dose LD/CD[b] (mg)	Last blood sample
A [a]	M	69	22.8	11	10	10	4	8	300/75	300
B	F	70	22.4	11	10	10	4	11	220/55	300
C	M	64	26.5	10	6	6	3	14	345/86.25	180
D [a]	M	66	25.5	17	15	14	3	4	410/102. 5	240
E [a]	M	61	22.3	13	11	11	3	5	360/90	240
F [a]	F	82	21.5	12	9	9	3	7	360/90	240
G	F	73	25.6	17	15	13	3	9	155/38.75	210
H [a]	M	79	27.7	6	4	4	3	4	370/92.5	240
I [a]	F	76	24.2	23	12	12	3	7	250/62.5	300
J [a]	M	61	24.5	7	4	4	2	3	270/67.5	300
K [a]	M	80	24.7	7	5	5	2	2	360/90	360
L [a]	M	74	23.4	8	8	8	4	4	110/27.5	360
M [a]	M	74	30.0	6	5	5	3	2	250/62.5	300
N	M	80	22.5	35	33	33	5	9	250/62.5	180
O [a]	M	73	22.2	7	6	6	2	5	180/45	360
P [a]	M	68	28.3	9	9	9	3	9	295/73.75	360
Q	M	69	20.0	17	13	13	5	7	365/91.25	300
R [a]	F	65	26.3	4	2	2	3	3	180/45	300
S [a]	M	72	28.7	12	7	7	2	5	195/48.75	360
Median	14/5	72	24.5	11	9	9	3	5	270/67.5	300
Range	-	61-82	20-30	4-35	2-33	2-33	2-5	2-14	110/27.5 – 410/102.5	180-360

[a]Included in the t½ and AUC_{0-4} calculation, [b]levodopa/carbidopa equivalents based on individual morning dose; LD, levodopa; CD, carbidopa; BMI, Body mass index; HY, Hoehn and Yahr.

Clinical experience with levodopa/carbidopa microtablets

A total of thirteen patients were (as prescribed by their treating physician) treated with, or had previously been treated with, levodopa/carbidopa microtablets. Eleven patients signed the informed consent form. Six patients had discontinued the treatment and five had ongoing treatment. Four dose dispenser reports and 11 patient records were obtained. All patients with ongoing treatment, and four of the patients who had discontinued treatment, answered the survey. The two patients that had discontinued the treatment and did not answer the questionnaire were judged by their physician to be too cognitively impaired to do so.

Levodopa/entacapone/carbidopa intestinal infusion

Eleven patients were included in and completed the infusion study (Table 4).

Table 4. Patient characteristics, n=11 (male n=7, female n=4)

	Age (years)	**Duration PD (years)**	**Body weight (kg)**	**LCIG Doses**		**LECIG Doses**	
				Morning dose (mg) n=10[a]	**Maintenance dose (mg)**	**Morning dose (mg) n=10[a]**	**Maintenance dose (mg)**
Mean (SD)	70 (4)	16 (4.8)	74 (15)	131 (56)	969 (277)	120 (49)	772 (226)
Median	70	14	73	130	1048	122	824
Range	63, 76	8, 23	51,99	41, 217	363,1367	41, 198	279,1107

[a]One patient did not have a morning dose prescribed. SD, standard deviation; PD, Parkinson's disease; LCIG, levodopa/carbidopa intestinal gel; LECIG, levodopa/entacapone/carbidopa intestinal gel.

Pharmacokinetics

Non-compartmental analysis of levodopa/carbidopa microtablets

The patients had a higher than expected plasma concentration prior to dose administration, and therefore the non-compartmental analysis was conducted on data adjusted for the measured concentration prior to dose administration. The baseline and dose adjusted levodopa maximum concentration ($C_{max/dose}$), was found to be higher for patients (p=0.026, n=19) compared to healthy subjects (sample mean difference 0.27, 95% CI: 0.035-0.51) (Table 5). Four patients were excluded from the comparison of systemic exposure and half-life estimation due to early drop-out. One patient was excluded due to an extremely high concentration prior to dose administration. The mean levodopa baseline and dose adjusted area under the curve ($AUC_{0-4/dose}$), calculated from

time zero to four hours also was found to be higher for patients (p=0.0008, n=14) compared to healthy subjects (sample mean difference 0.38, 95% CI: 0.18-0.58). The mean carbidopa dose and baseline adjusted $AUC_{0\text{-}4/dose}$ did not differ between patients and healthy volunteers, nor did the time to maximum concentration or $C_{max/dose}$. The carbidopa half-life was found to be longer for patients, compared to the healthy volunteers (p=0.029, sample mean difference 46, 95% CI: 5.2-87).

Table 5. Pharmacokinetic parameters of levodopa and carbidopa

Patients	Levodopa					Carbidopa				
	N	Mean	SD	Median	Range	N	Mean	SD	Median	Range
$C_{max/dose}$ (µg/mL)[a]	19	1.17‡	0.43	1.16	0.31, 1.96	19	0.09†	0.03	0.09	0.03, 0.14
T_{max} (min)	19	32†	23	30†	15, 100	19	134†	47	120†	80, 240
$AUC_{0\text{-}4h/dose}$[b] (min×µg/mL/mg)	14	1.15‡	0.31	1.21	0,38, 1.74	14	0.67†	0.26	0.68	0.23, 1.14
$t_{½}$[d] (min)	14	106†	16	104	85, 144	13[e]	171‡	37	173	117, 248

Healthy volunteers[40]	Levodopa					Carbidopa				
	N	Mean	SD	Median	Range	N	Mean	SD	Median	Range
C_{max}[c] (µg/mL)	18	0.90	0.25	0.88	0.51, 1.38	18	0.09	0.05	0.09	0.03, 0.21
T_{max}[c] (min)	18	37	23	35	20, 120	18	109	48	100	40, 180
$AUC_{0\text{-}4h/dose}$[b] (min×µg/mL/mg)	18	0.77	0.17	0.75	0.53, 1.13	18	0.58	0.32	0.52	0.17, 1.44
$t_{½}$[c] (min)	18	91	34	85	45, 198	18	125	72	101	56, 315

[a]Baseline and dose adjusted (to 100 and 25 mg of levodopa/carbidopa). [b]Time points 0-4 hours (five patients were excluded); [c]Reused with permission from Wolters Kluwer Health, Inc. (Clinical Neuropharmacology); [d]At least three descending concentration time points were used for the calculation of t½; [e]Patient F did not have descending time points; †not found to be significant; ‡$p < 0.05$

Population pharmacokinetic model for levodopa/carbidopa microtablets

The final pharmacokinetic population model developed for levodopa and carbidopa (**Paper III**), was a two- and one-compartment model respectively. The final model parameter estimates, together with corresponding uncertainties are listed in Table 6. A two-compartment model for levodopa resulted in an OFV drop of 147.4 compared to a one-compartment model. The models were parameterized in terms of absorption compartment specific mean transit-time (MTT_1 and MTT_2), the fraction absorbed from the fast absorption compartment (fa_1), apparent volume of the central (V_C/F) and peripheral (V_P/F, for

levodopa only) compartment, apparent inter-compartmental clearance (Q/F, for levodopa only) and apparent clearance (CL/F).

A combined proportional and additive residual error model was separately estimated for carbidopa and levodopa and the different populations (PD patients and healthy subjects). The shared total residual error of levodopa and carbidopa was 28.5%, when the models were combined.

Double-peak profiles, observed in both healthy subjects and patients, were adequately described with parallel absorption compartments, and included five and six transit compartments for levodopa and three and 10 transit compartments for carbidopa (Individual plots, for illustration purpose, Figure 6).

Covariate model

In the initial covariate analysis, carbidopa dose was found to have a significant effect on levodopa apparent clearance. When described with a linear relationship, the decrease in OFV was 24. With a non-linear relationship the decrease in OFV was 30. The interaction between levodopa and carbidopa, described by the parameter $INTER_{CL/F,LD\text{-}CDAMT}$, was estimated to 86.7 mg (Table 6). This gives, for a typical subject of 70 kg, a levodopa apparent clearance of 49 L/h when administered with 50 mg carbidopa and 40 L/h with 75 mg carbidopa. The levodopa terminal (beta) half-life increases by approximately 9-12% with every 25 mg increment in carbidopa dose, with a lower increase as the carbidopa doses get higher. When the individual model predicted carbidopa concentration was used as a covariate on levodopa apparent clearance, there was a non-significant drop in OFV (less than 3.84).

The AALASSO method was chosen for investigation of influential covariates, and was performed on the separate models to reduce run-time. The final covariates included in the covariate analysis were: age, study association and HY on levodopa CL/F, carbidopa CL/F and levodopa F_{rel}; sex on carbidopa MTT_2 (carbidopa mean transit time between the second dose compartment and the central compartment) and years with levodopa treatment on levodopa CL/F.

The HY stage was in this analysis found to be the most influential covariate affecting the apparent clearance of levodopa (coefficient -0.062). The levodopa apparent clearance decreases by approximately 5 L/h with each stage.

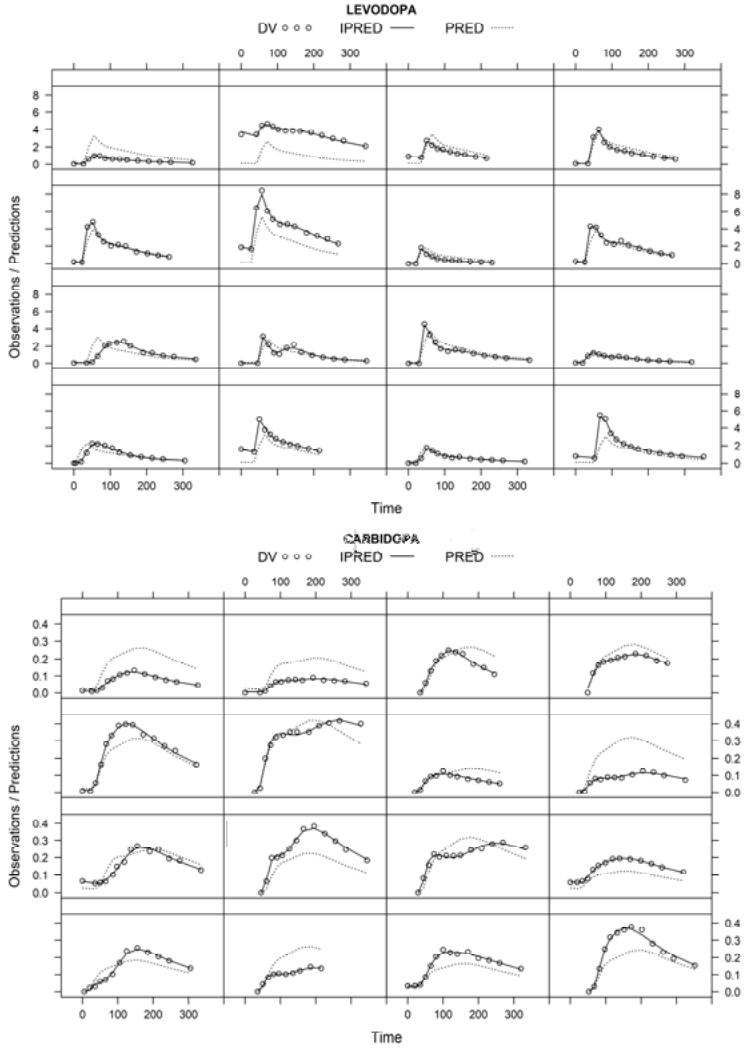

Figure 6. Individual plots, for illustration purpose, of levodopa and carbidopa plasma concentration over time. The points represent observations, the solid lines represent the individual model predicted plasma concentrations. The dotted lines represent the population predictions.

The levodopa relative bioavailability (F_{rel}) was found to modestly increase with age (3.4% between the age 60 and 80 years, coefficient 0.002).

Age and HY stage (coefficients -0.012 and 0.014 respectively) were found to significantly impact carbidopa apparent clearance, where the apparent clearance decreases by 15 L/h between the age 60 to 80 years old and increases modestly, by 0.9 L/h with increasing HY. Sex was found as a significant co-variate on carbidopa mean transit time for the second compartment (MTT_2,

coefficient 0.178), meaning that the estimated mean transit time was on a population level 22 minutes longer for women.

The pcVPCs, for the final models, stratified by study association, are shown in Figure 7.

Table 6. Parameter estimates for the final LD/CD population pharmacokinetic model and results from the SIR evaluation.

Parameter	**LD (%RSE)[c] [%Shrinkage]**	**CD (%RSE)[c] [%Shrinkage]**	**LD SIR (%RSE)[c] [95% CI]**	**CD SIR (%RSE)[c] [95% CI†]**
CL/F (L/min/70 kg)	1.31[a] (10.7)	1.05 (8.46)	1.31 (10.6) [1.08-1.63]	1.05 (8.57) [0.881-1.23]
V_C/F (L/70 kg)	45.4 (9.85)	168 (8.50)	46.0 (9.17) [38.6-55.2]	167 (8.13) [142-195]
Q/F (L/min/70 kg)	0.667 (15.7)	-	0.667 (11.2) [0.548-0.837]	-
V_P/F (L/70 kg)	44.9 (5.90)	-	44.8 (6.01) [40.0-50.3]	-
MTT_1 (min)	16.1 (6.15)	34.6 (6.20)	16.1 (6.05) [14.3-18.2]	34.7 (5.73) [30.9-38.7]
MTT_2 (min)	86.2 (6.39)	121 (6.01)	85.9 (4.69) [78.1-94.4]	121 (5.27) [109-134]
F_{REL}	1 FIX	1 FIX	1 FIX	1 FIX
fa_1[b]	0.749* (5.35)	0.570* (7.10)	0.749 (4.67) [0.678-0.816]	0.570 (6.61) [0.50-0.65]
Pre-dose concentration (µg/mL)	0.120 (42.3)	0.0256 (38.8)	0.129 (45.9) [0.0542-0.268]	0.0256 (32.5) [0.01-0.04]
$INTER_{CL/F\text{-}CDAMT}$ (mg)	86.7 (36.3)	-	94.6 (32.8) [52.0-161.5]	-
CL/F-AGE	-	-0.0119 FIX	-	-0.0119 FIX
CL/F-HY	-0.0616 FIX	0.0141 FIX	-0.0616 FIX	0.0141 FIX
F_{REL}-AGE	0.00172 FIX	-	0.00172 FIX	-
MTT_2-SEX	-	0.178 FIX	-	0.178 FIX
$IIV_{CL/F}$	15.0 (31.8) [25.8]	20.9 (16.0) [18.4]	16.4 (27.6) [8.45-23.7]	21.8 (19.8) [14.5-29.6]
$IIV_{VC/F}$	39.5 (22.8) [17.3]	-	41.2 (17.4) [28.2-53.9]	-
IIV_{MTT1}	34.5 (13.8) [6.49]	31.9 (10.6) [6.55]	35.3 (14.3) [26.6-45.9]	32.9 (12.7) [25.6-40.9]
IIV_{MTT2}	15.2 (34.7) [18.0]	27.2 (19.5) [3.98]	16.5 (28.5) [8.67-24.5]	27.6 (15.2) [20.6-36.2]
$IIV_{fraction\ absorbed}$[b]	99.6 (15.8) [10.0]	81.2* (19.2) [9.53]	104 (17.0) [77.6-139]	84.7 (16.4) [62.9-112]
IIV_{Frel}	28.3 (20.6) [4.89]	48.1 (9.69) [1.60]	28.7 (14.3) [21.2-36.4]	49.5 (12.7) [38.5-61.8]
$IIV_{Pre\text{-}dose\ concentration}$	180 (11.3) [28.3]	89.6 (24.9) [59.3]	187 (15.1) [138-239]	102 (36.3) [55.6-156]
Proportional error				
Healthy (%)	15.0 (11.3)	7.70 (14.1)	15.0 (6.14) [13.5-17.1]	7.70 (11.4) [6.08-9.49]
Patients (%)	7.02 (14.6)	3.86 (35.3)	7.07 (6.51) [6.22-8.04]	3.86 (20.2) [2.21-5.27]
Additive error				
Healthy (µg/mL)	0.00406 (16.6)	0.00415 (14.2)	0.00406 (9.15) [0.00343-0.00491]	0.00418 (5.85) [0.00372-0.00468]
Patients (µg/mL)	-	0.00684 (28.3)	-	0.00693 (13.5) [0.00526-0.00880]

[a]per milligram carbidopa; [b]logit transformed; [c]Point estimate and the associated % relative standard error (% RSE, reported on the approximate standard deviation scale (SE/variance estimate)/2). LD, levodopa; CD, carbidopa; CI, confidence interval; IIV, inter-individual variability (CV%). SIR, sampling importance resampling procedure.

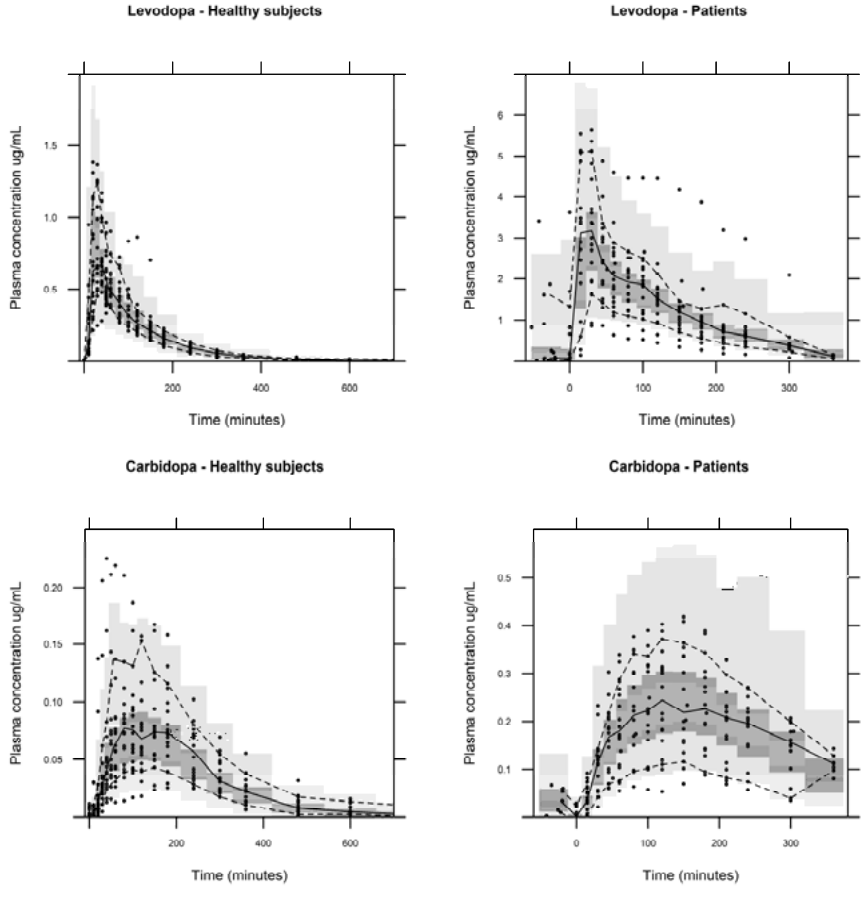

Figure 7. Prediction corrected visual predictive check (1000 replicates) for levodopa and carbidopa after covariate model selection, stratified on healthy volunteers and PD patients. The solid line is the median of the observed data. The dashed lines represent the observed 10th and 90th percentile of the observations. The top and bottom light grey areas are the 95% confidence intervals for 10th and 90th percentiles of the simulated data. The middle dark grey area is the 95% confidence interval for the median of the simulated data.

External validation

The final levodopa model was used for prediction of data from an external dataset, where healthy subjects received multiple lower doses of levodopa/carbidopa microtablets every 2.4 hours[116] (Figure 8). As done for the single-dose studies, the total dose of carbidopa that was administered during the study period was used to describe the interaction between levodopa and carbidopa. The plasma concentration after first dose is over predicted for the population, however the observations, especially at later time points, are relatively well-captured by the model predictions.

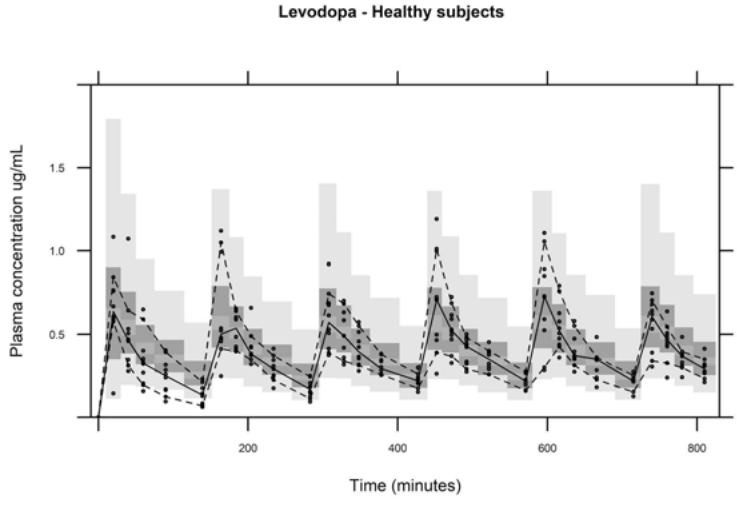

Figure 8. External evaluation of the predicitive performance of the final levodopa model with covariates (1000 replicates) based on data from 10 healthy subjects. The solid line is the median of the observed data. The dashed lines represent the observed 10th and 90th percentiles of the observations. The top and bottom light grey areas are the 95% confidence intervals for 10th and 90th percentiles of the simulated data. The middle dark grey area is the 95% confidence interval for the median of the simulated data.

Non-compartmental analysis of levodopa/entacapone/carbidopa intestinal gel

The mean plasma concentration of levodopa, carbidopa, 3-OMD and entacapone are shown in Figure 9. The non-compartmental analysis results showed no significant difference in $AUC_{0\text{-}14}$ for levodopa between LECIG and LCIG infusions (AUC ratio LECIG/LCIG of 1.10 (95% CI: 0.951; 1.17)) (Table 7). The levodopa $AUC_{0\text{-}14/dose}$ was found to be higher during LECIG treatment (AUC ratio 1.34 [95% CI: 1.19; 1.45]). The mean $AUC_{0\text{-}14}$ for carbidopa was significantly lower with LECIG compared with LCIG, AUC ratio 0. 938 [95% CI: 0.815; 0.990], while the $AUC_{0\text{-}14/dose}$ for carbidopa was significantly higher during LECIG (ratio 1.15 [95% CI: 1.02; 1.22]). When investigating individual systemic exposures, it was found that two patients did not reach the target 20% increase, three patients had the expected increase, and six patients had an increase higher than the expected 20%. The 3-OMD plasma concentration was decreasing during the LECIG administration.

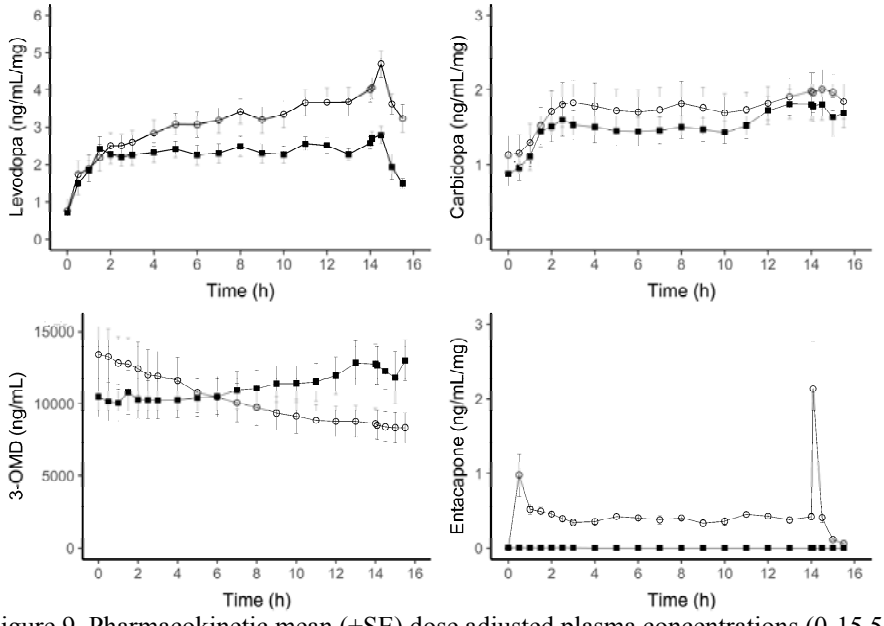

Figure 9. Pharmacokinetic mean (±SE) dose adjusted plasma concentrations (0-15.5 h) of levodopa (n=11), carbidopa (n=11), 3-O-methyldopa (n=11) and entacapone (n=6). Filled squares represent levodopa/carbidopa infusion (LCIG), open circles represent levodopa/entacapone/carbidopa infusion (LECIG).

When the morning bolus dose was increased from 80% to 90% of levodopa LCIG dose, the initial increase in plasma concentration of levodopa, during the first hours was faster (Figure 10).

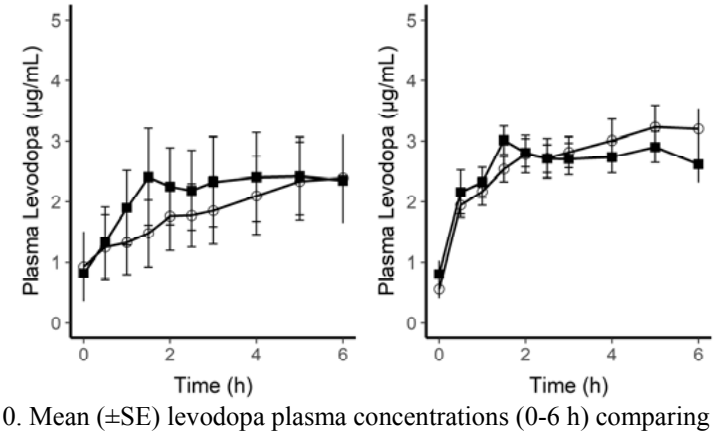

Figure 10. Mean (±SE) levodopa plasma concentrations (0-6 h) comparing 80% (left, n=5) and 90% (right, n=6) morning bolus dose administration of levodopa/entacapone/carbidopa infusion (LECIG, open circles), with levodopa/carbidopa infusion (LCIG, filled squares).

Table 7. Pharmacokinetic parameters of LCIG and LECIG during 0 to 14 hours; mean (SD) values (n=11).

	Treatment		P Value	Ratio LECIG/LCIG (95% CI)
	LCIG	LECIG		
Levodopa				
$AUC_{0\text{-}14}$[a] (h*ng/mL)	35479.1 (14693.0)	39016.1 (17327.6)	0.27	1.10 (0.95; 1.17)
$AUC_{0\text{-}14/dose}$ (h*ng/mL)/mg	31.9 (9.4)	42.7 (14.1)	0.00013	1.34 (1.19; 1.45)
C_{max}[a] (ng/mL)	3269.0 (1140.4)	3668.0 (1481.1)	0.089	1.12 (0.98; 1.19)
Carbidopa				
$AUC_{0\text{-}14}$[a] (h*ng/mL)	5950.1 (3236.3)	5582.4 (3605.3)	0.03	0. 938 (0.815; 0.990)
$AUC_{0\text{-}14/dose}$ (h*ng/mL)/mg	20.9 (7.7)	24.1 (11.3)	0.03	1.15 (1.02;1.22)
C_{max}[a] (ng/mL)	559.3 (292.4)	498.1 (297.8)	0.02	0.89 (0.79; 0.98)
3-OMD				
$AUC_{0\text{-}14}$[a] (h*ng/mL)	154714.1 (56931.0)	145745.7 (61182.8)	0.21	0.94 (0.79; 1.01)
$AUC_{0\text{-}14/dose}$ (h*ng/mL)/mg	-	-		
C_{max}[a] (ng/mL)	13281.8 (4861.0)	13518.2 (6116.2)	0.74	1.02 (0.82; 1.15)
Entacapone				
$AUC_{0\text{-}14}$[a] (h*ng/mL)	-	5205.9 (1073.7)		
$AUC_{0\text{-}14/dose}$ (h*ng/mL)/mg	-	5.6 (1.1)		
C_{max}[a] (ng/mL)	0.03[b]	935.3 (550.9)		

[a]Results are presented as mean values (SD) for area under the curve from 0 to 14 hours ($AUC_{0\text{-}14}$), dose adjusted $AUC_{0\text{-}14/dose}$, and maximum plasma concentration (C_{max}).
[b]Geometric mean.

Population pharmacokinetic model for levodopa/entacapone/carbidopa intestinal gel

The disposition of levodopa following continuous infusion was described with a one-compartment model (**Paper V**). The absorption rate constant (ka) was estimated to a high value, and therefore fixed to 50 for both treatments, which was the lowest value that did not result in a significant increase in OFV. Estimation of a two compartment model resulted in model instability and the distribution phase was estimated to be very fast, with high uncertainty on the

estimated parameters. When the effect of entacapone was estimated as a shift in typical value of levodopa apparent clearance, including an inter-individual variability in the shift parameter, the OFV decreased by -436. For LCIG, the population parameter for apparent clearance was estimated to 27.9 L/h and for LECIG to be 36.5% lower. The associated inter-individual variability was 28% and 11%, respectively. Inter-individual variability was found to be significant on apparent clearance and central volume of distribution. The inter-individual variability on relative bioavailability was not retained in the model due to model instability and high uncertainty in the parameter estimate. The final model parameter estimates are given in (Table 8).

Table 8. Parameter estimates for the final population pharmacokinetic model of LCIG and LECIG, and results from the SIR evaluation.

Parameter	**LD (%RSE)[b]** **[%Shrinkage]**	**LD SIR (%RSE)[b]** **[95% CI]**
CL/F_{LCIG} (L/h/70 kg)	27.9 (7.31)	28.1 (5.82) [25.1; 31.5]
$CL/F_{LECIG,Shift}$[a]	-0.365 (5.24)	-0.364 (4.48) [-0.391;-0.328]
V_C/F (L/70 kg)	74.5 (7.60)	75.0 (8.60) [63.3; 87.8]
ka (hr^{-1})	50 FIX	-
ktr_{oral} (hr^{-1})	2.4 FIX	-
$F_{rel,LCIG/LECIG}$	1 FIX	-
$F_{rel,oral}$	1.03 FIX	-
$IIV_{CL/F,LCIG}$	27.9 (19.8) [1E-10]	28.6 (14.8) [21.2; 36.2]
$IIV_{CL/F,LECIG,Shift}$[a]	11.4 (23.5) [22.6]	12.0 (30.1) [4.49; 17.9]
IIV_{VC}	34.4 (17.0) [0.264]	35.6 (17.2) [24.2; 45.7]
Proportional error (%)	11.0 (27.4)	11.1 (8.96) [3.24; 13.1]
Additive error (µg/mL)	0.316 (10.2)	0.316 (6.14) [0.278; 0.354]

[a]Shift in CL/F for LECIG,

$$CL/F_i = TVCL/F_{LCIG} \times e^{\eta_{CL,LCIG}} \times \left(\frac{Weight}{70}\right)^{0.75} \times \left(1 + TVCL_{LECIG,Shift} \times e^{\eta_{CL,LECIG,Shift}}\right).$$

[b]Point estimate and the associated % relative standard error (% RSE, reported on the approximate standard deviation scale (SE/variance estimate)/2). LD, levodopa; IIV, inter-individual variability (CV%); SIR, sampling importance resampling; CI, confidence interval; LCIG, levodopa/carbidopa intestinal gel; LECIG, levodopa/entacapone/carbidopa intestinal gel.

The final observed and model predicted levodopa plasma concentration, normalized for the variability in the independent variables (pcVPC), stratified on treatment, is shown in (Figure 11).

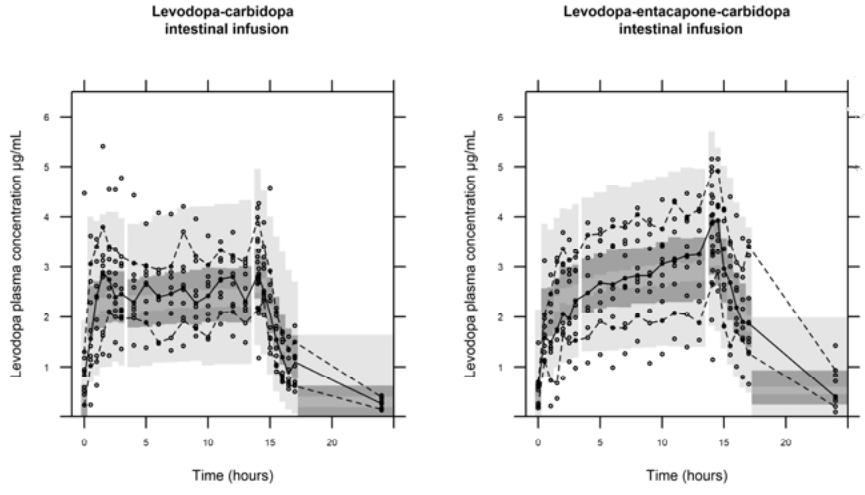

Figure 11. Prediction corrected visual predictive check (1000 replicates) of levodopa concentration-time data for LCIG and LECIG. The solid line is the median of the observed data. The dashed lines represent the observed 10th and 90th percentiles of the observations. The middle dark grey area is the 95% confidence interval for the median of the simulated data. The top and bottom light grey areas are the 95% confidence intervals for 10th and 90th percentiles of the simulated data.

A 14-hour infusion period was simulated, using the developed population levodopa pharmacokinetic model, with different morning bolus dose and continuous maintenance dose, compared to LCIG. The scenarios simulated included a 0% lower morning and maintenance dose; a 20% lower morning and maintenance dose and; a 0% lower morning dose with a 35% lower continuous maintenance dose. Figure 12 shows the simulated levodopa plasma concentration, displayed as the median and the 10th and 90th percentiles. The plasma concentration shows an increase during the infusion period when the same levodopa dose is administered with LECIG (lower left plot) as with LCIG. The 20% lower morning and maintenance dose, as the doses given in the original study, results in a slight increase in levodopa plasma concentrations over the infusion period. A similar drug exposure as with LCIG is observed when the continuous maintenance dose is decreased by 35%, indicating that, on a population level, this would be an appropriate dose adjustment.

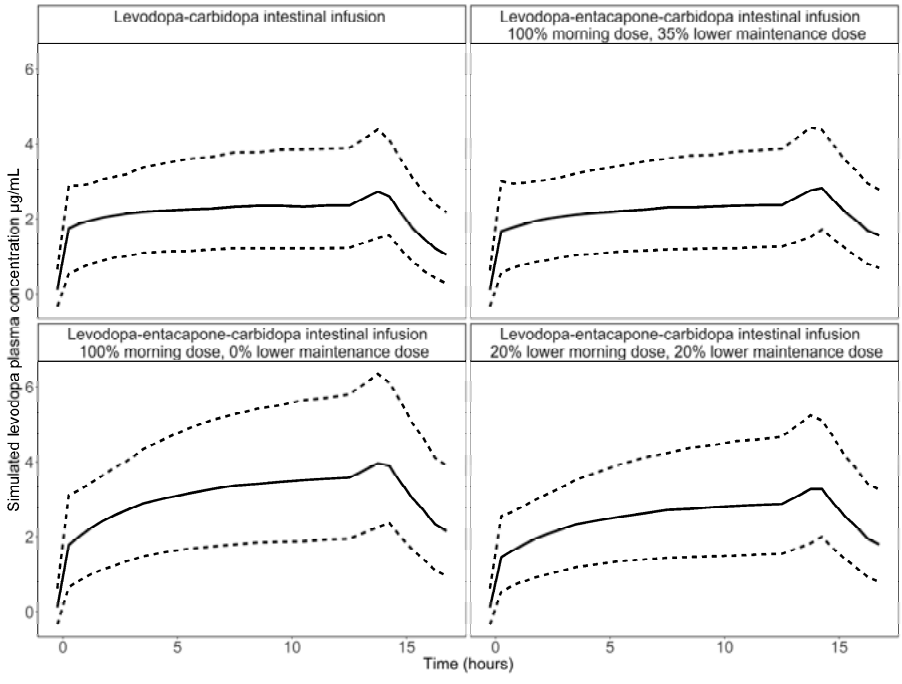

Figure 12. Predicted plasma concentration for the study population, with unchanged patient doses of LCIG (top left plot) and decreased continuous doses for LECIG treatment by 35% (top right plot), 0% decreased morning and maintenance dose (bottom left plot), and 20% decreased morning and maintenance dose (bottom right plot). The solid line represents the median of the simulated concentration and the dashed lines represent the 10th and 90th percentiles of the simulated data.

Clinical experience with levodopa-carbidopa microtablets treatment

Nine patients started the microtablet treatment due to fluctuation in motor function i.e. wearing-off symptoms and/or dyskinesia, or due to general difficulties of finding an appropriate dose. One patient did, in the patient records, not have a specific medical reason declared, and one patient initiated due to difficulties of swallowing tablets. The number of required dose adjustments varied greatly, between 0-15 adjustments, among the treated patients. (Table 9).

Table 9. Demographics and microtablet treatment overview, data collected from patient records.

					Daily dose of levodopa (mg)					Medication	
ID	**Sex**	**Age (y)**	**PD (y)**	**LD (y)**	**Prior dose**	**First dose**	**Latest/ Last dose**	**Treatment (days)**	**Dose adjustments**	**Prior (LEDD: Last Dose)**	**During (LEDD: Last dose)**
A[a,c]	F	70	10	10	600	555	860	629	13	605	865
B[a]	M	74	13	13	3700-4300[d]	3655	3900	93	1	4396.5	3896.5
C[a]	F	78	13	13	400-750	605	370	59	4	754.5	376.8
D[a]	M	69	10	10	775	1205	2140	388	15	763.1	2103.1
E[a,b,c]	M	64	24	NA	1700-2400	1980	1670	696	2	2610.6	1880.6
F[a,b]	F	51	10	NA	~1000	1150	945	640	3	981.5	926.5
G	M	68	7	7	800	1095	1250	63	2	800	1250
H	F	73	20	20	600-750	NA	NA	9	0	898.5	NA
I[a,b,c]	F	66	16	NA	700	820	890	610	1	939.3	898.3
J[a,b,c]	F	67	9	9	700	1080	1055	74	4	775	1130
K[a,b]	M	65	17	10	875-1000	875	930	74	3	1354.7	954.7
Mean ±SD		68 ±7	13 ±5	12 ±4	1077.3 (±931.7): 1252.3(±1127.2)	1302 (±917.7)	1401 (±1002.9)	Range 9-696	Range 0-15	1352.6 (±1151.8)	1428.1 (±1002.9)

[a]Answered the survey; [b]Ongoing treatment; [c]Dose dispenser reports available; [d]Clinically titrated doses that patient B responded well to without side effects; NA; Not available; LD, levodopa; LEDD, levodopa equivalent daily dose;

The total number of days (median (range)) treated with microtablets was for the patients who had discontinued 78 (9-629) days and for patients with ongoing treatment 610 (74-696) days. General progression resulting in insufficient symptom control of the disease was reported as a reason for termination for four patients. Other reasons for discontinuation was poor eyesight (one patient) and impaired cognition (one patient). Both conditions led to problems with using the dose dispenser.

The ability to perform daily activities was reported as improved by four patients. Four patients answered that it was unchanged and one patient could not recall (Table 10).

Table 10. Questions asked with answers (n=9)

Question	**Improved**	**Unchanged**	**Worsened**	**Don't know**
Experienced effect of microtablets on your disease symptoms?	6	2	1	-
Any change in ability to perform daily activities?	4	4	-	1
Does/did the dose dispenser facilitate remembering to take your tablets	7	2	-	-
Does/did the dose dispenser simplify or complicate your treatment in general?	8	-	1	-
	Well	**Unchanged**	**Not well**	**Don't know**
How does/did it go to see the screen?	8	-	1	-
How does/did it go to press the buttons?	8	-	1	-
How does/did it go to navigate through the menus?	8	-	1	-
How does/did it go to dispense the tablets?	8	-	1	-
How does/did it go to change the cartridge?	6	-	3	-
How was/is the portability of the dose dispenser in your everyday life?*	5	-	3	-

*n=8.

The dose dispenser facilitated the adherence for seven patients, according to their survey response, and eight patients stated that their treatment had become easier with the dose dispenser. Two patients reported no change in adherence, and one patient reported that the treatment had become more complicated, due to poor eyesight.

In the survey, six of nine patients declared that the treatment effect was improved with the microtablets, while two patients considered it to be unchanged and one patient stated that it had worsened.

Regarding the usability of the dose dispenser, eight out of nine patients responded "well", when asked about the ease of navigating through the menus, dispensing the tablets, seeing the instructions on the screen and pressing the buttons (dose dispenser has a touch screen). When asked about the ease of changing the cartridge, three of the patients answered "with some difficulty" and six patients answered "well", however, one of the six patients added that it too often had been difficult to replace the cartridge. Concerning the ease of bringing the dose dispenser with them in their everyday lives, five patients responded "well", while three patients responded "with some difficulty." One patient did not answer this question.

Four dose dispenser reports were obtained, because only four patients had dose dispensers with the software version 1.0.15. The reports showed that the total adherence ranged between 89% and 101% with a mean (SD) value of 97(±5)%. The timing adherence was found to be between 74% and 100% with a mean (SD) value of 89 (±12)%.

With regard to motor function, a third found their bradykinesia to be better and a third found it to be unchanged. A majority found their non-troublesome dyskinesia to be unchanged in general (Figure 13). A third found that the duration of troublesome dyskinesia was worsened and four patients found that the magnitude of the troublesome dyskinesia was worsened. A majority found the frequency of troublesome dyskinesia to be unchanged or better.

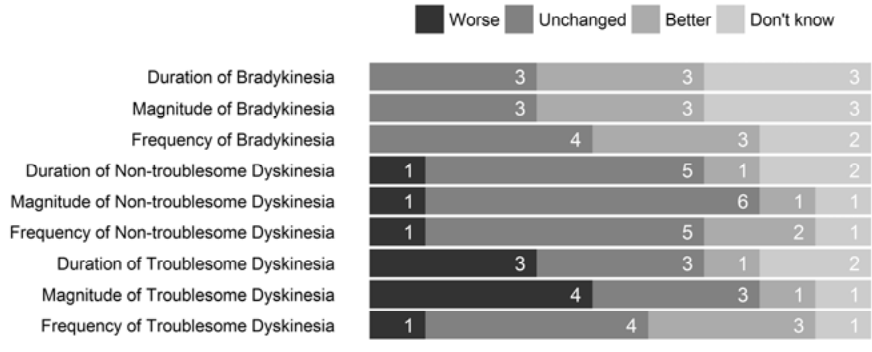

Figure 13. The experienced change in the duration, magnitude and frequency of bradykinesia, non-troublesome dyskinesia and troublesome dyskinesia during microtablet treatment. The response alternatives for "much worse" and "worse" are merged as "worse." The response alternatives for "better" and "much better" are merged as "better".

Pharmacodynamics

Levodopa/carbidopa microtablets

The mean TRS score was -1.4 at time 0, i.e. at dose administration, the mean UPDRS item score was 7.4 and the dyskinesia score was 0 (Figure 14). The mean time to maximum improvement was 79 (±60) minutes (n=16) when assessed with the UPDRS item score. The duration of effect, calculated as the mean for the patients that returned to a UPDRS score of less than 2 points from baseline, was 154 (±73) minutes (n=14).

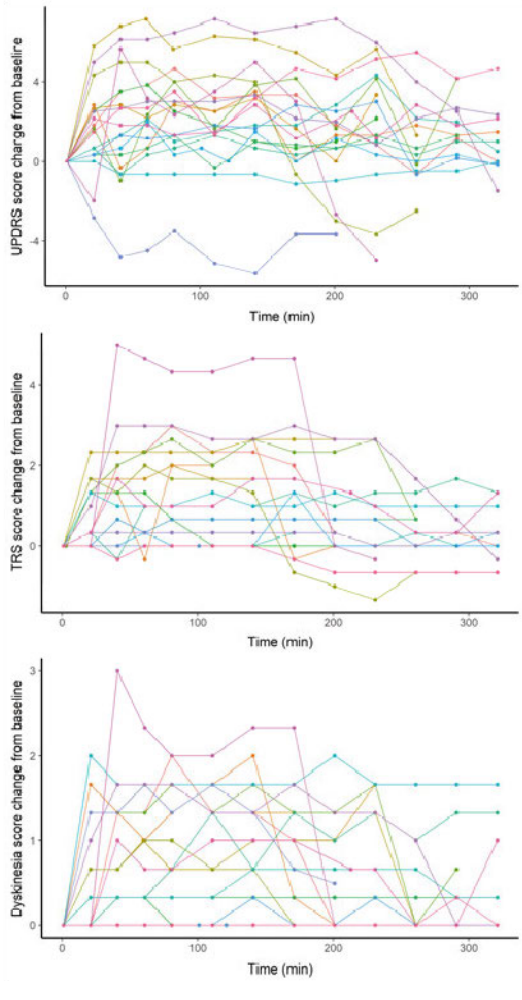

Figure 14. Individual scores as change from baseline for UPDRS, TRS and dyskinesia scores

The average time to onset of dyskinesia was 42 (±39) minutes (n=13) and maximum dyskinesia score was reached at 56 (±37) minutes. The mean duration of effect was 180 (±53) minutes (n=8), calculated for the patients that returned to a score of less than 0 on the TRS. The mean time to maximum

TRS score was 54 (±52) minutes (n=15). This was calculated on the patients that showed an improvement in TRS. The onset of dyskinesia is coinciding closely with improvement of motor function (Table 11).

Table 11. Time points (minutes) for improvement and deterioration assessed with selected UPDRS part III items score TRS and dyskinesia score for each patient.

ID	UPDRS III Improve-ment of ≥2[a] points	UPDRS III Return to baseline [a]	TRS Score ≥0	TRS Score <0	Choreatic dyskinesia score ≥1	Choreatic dyskinesia score <1	Last motor function test (min)
A	41	201	41	201	41	201	291
B	21	171	21	171	21	171	321
C	21	-	21	-	21	-	171
D	21	261	21	261	21	261	261
E	21	171	21	171	21	171	261
F	21	201	21	261	21	261	261
G	41	111	21	111	-	-	201
H	81	171	-	-	-	-	261
I	-	-	21	-	111	-	321
J	201	291	-	-	141	291	321
K	-	-	-	-	21	-	321
L	61	81	-	-	-	-	321
M	141	261	-	-	-	-	321
N	-	-	-	-	21	-	171
O	21	201	-	-	-	-	321
P	21	291	41	291	21	291	321
Q	41	201	41	201	41	201	231
R	21[b]	291[b]	21	-	41	261	321
S	-	-	-	-	-	-	321
Median[c]	21	201	21	201	21	261	280
Range	21-201	81-291	21-41	111-291	21-141	171-291	171-231
n	15	14	11	8	13	9	51.7

UPDRS part III; 6 items rated from 0 to 4,
TRS; Treatment response scale rating from -3 to +3, choreatic dyskinesia; rated from 0 to 4.
[a]At least two of the three raters had to agree, i.e. 1.5 points improvement from baseline or worsening.
[b]Within this range there were two occasions of temporary improvement/deterioration from baseline.
[c]For patients that improved and deteriorated according to the cutoff values.

One patient never reached therapeutic effect according to any of the motor function assessment scales, or developed dyskinesia, despite a higher than usual morning dose. Two patients developed only dyskinesia, which persisted throughout the study, but never reached “on”-state according to the TRS nor

the criteria set for improvement in UPDRS score. One patient reached "on"-state according to the TRS but not according to the UPDRS cutoff. Only one patient remained above the cutoff according to the UPDRS at study stop, however this patient experienced off-symptoms and had the last motor function test at 171 minutes, i.e. 2.5 hours prior to scheduled study stop. Ten patients completed the entire motor function assessment without additional medication. Three of the ten patients never reached "on" according to the TRS and UPDRS cutoff value, and the rest returned to "off"-state prior to the last motor function assessment.

Levodopa/entacapone/carbidopa intestinal gel

The mean TRS scores, during the LECIG and LCIG infusion treatments, are shown in Figure 15. The mean absolute deviation of TRS scores were compared between the two treatments, and were not found to significantly differ ($p = 0.84$). However, there is a tendency for a longer time to reach optimal motor function initially with LECIG compared to LCIG.

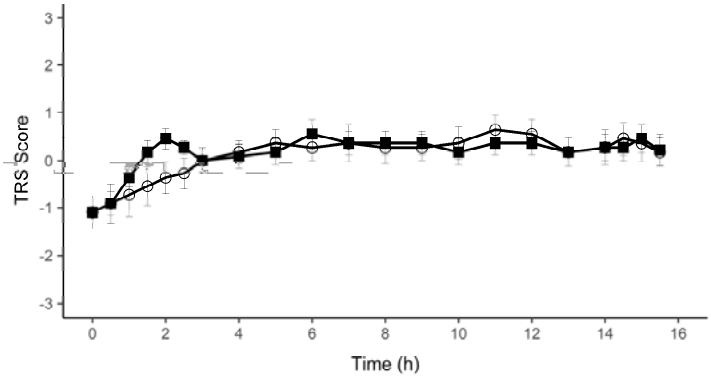

Figure 15. Mean (±SE) treatment response scale (TRS) score (0-15.5 h) during the two treatments (n=11). Filled squares represent levodopa/carbidopa infusion (LCIG), open circles represent levodopa/entacapone/carbidopa infusion (LECIG).

Genotyping of DDC and COMT

Among the eleven patients genotyped, the results from the $COMT_{SNP}$ (rs4680) showed that four patients had $COMT^{GG}$ (associated with high activity), four patients had $COMT^{AG}$ (associated with intermediate activity) and three patients had been genotyped with $COMT^{AA}$ (associated with low activity). The DDC_{SNP} genotyping showed that six patients had $DDC_{SNP}{}^{TT}$ (associated with high activity), four patients had $DDC_{SNP}{}^{CT}$ (associated with intermediate activity) and one patient had $DDC_{SNP}{}^{CC}$ (associated with low activity). The DDC_{INDEL} genotyping (rs3837091), revealed seven patients with

$DDC_{INDEL}^{AGAG/AGAG}$ (associated with high activity) and four patients with $DDC_{INDEL}^{AGAG/-}$ (associated with intermediate activity). No patient was homozygous for $DDC_{INDEL}^{-/-}$. The estimated apparent clearance for the different groups, stratified on treatment, and the individual shift in apparent clearance with the LECIG treatment, are shown in Figure 16.

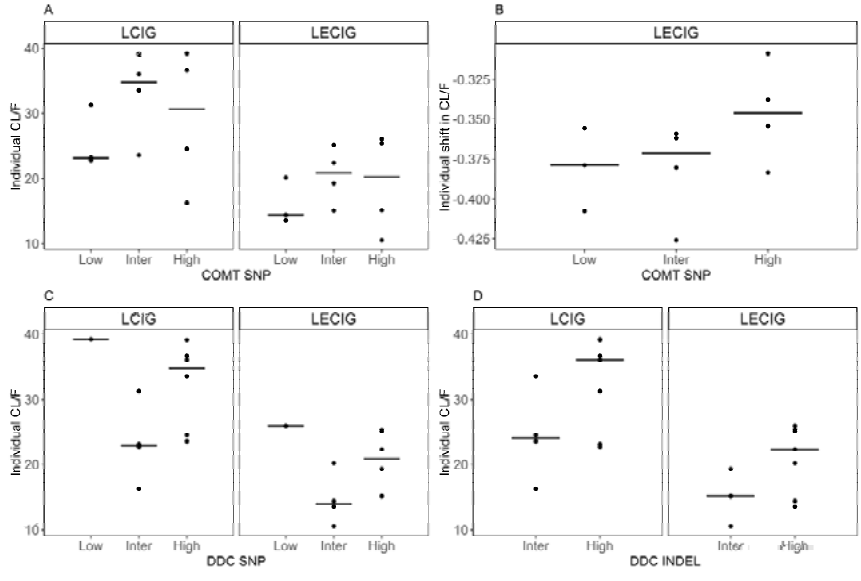

Figure 16. Graphical analysis of genotype results and individual estimated levodopa CL/F, with LCIG and LECIG treatment. $COMT_{SNP}$ (rs4680, top left, A) and individual shift in CL/F (top right, B), DDC_{SNP} (rs321451, bottom left, C), DDC_{INDEL} (rs3837091, bottom right, D). LCIG, levodopa/carbidopa intestinal gel; LECIG, levodopa/entacapone/carbidopa intestinal gel; COMT, catechol-O-methyl transferase; DDC, dopa decarboxylase; SNP, single-nucleotide polymorphism.

Because only few individuals were included in the study, a formal model based covariate analysis of the effect of genotype on apparent clearance was not performed. There is a very small tendency towards higher apparent clearance with $COMT_{SNP}$ (Figure 16 A). All $COMT_{SNP}$ genotypes display, with the addition of entacapone, a decrease in apparent clearance (Figure 16 B). The tendency from the graphical analysis indicate that the individuals with higher DDC_{SNP} enzyme activity have a slightly higher apparent clearance (Figure 16 C). The one patient with low activity in DDC_{SNP} is estimated to have a higher apparent clearance compared to the other groups. That patient, on the other hand, was found to have the polymorphisms $DDC_{INDEL}^{AGAG/AGAG}$ and $COMT_{SNP}^{GG}$, i.e. high activity. Patients with high activity according to the DDC_{INDEL} polymorphism show a tendency towards slightly higher median CL/F, compared to patient with intermediate activity (Figure 16 D).

Discussion

This thesis describes the first clinical studies with the levodopa/carbidopa microtablets and the levodopa/entacapone/carbidopa intestinal gel, both new levodopa therapies, in patients with advanced Parkinson's disease, and the development of population pharmacokinetic models for each treatment.

Pharmacokinetics

Levodopa-carbidopa microtablets

A clinical study was conducted to investigate the pharmacokinetics of the levodopa/carbidopa microtablets in advanced Parkinson's disease patients. The data were analyzed using non-compartmental analysis (**Paper I**) and non-linear mixed effects modeling approach to characterize the pharmacokinetics and investigate the influence of carbidopa and other possible factors that contribute to the large inter-individual variability (**Paper III**).

The population pharmacokinetic models for levodopa and carbidopa were developed separately, and were best described with a two and one-compartment model respectively. Double-peak profiles were observed in patients and healthy subjects, for both levodopa and carbidopa. The reason for presence of double-peaks following levodopa administration is yet to be entirely understood, but might be due to the metabolized levodopa in the gastro-intestinal tract causing an interruption in gastric emptying.[58,138] To describe the double-peak phenomenon, the models included parallel absorption compartments and transit compartments describing the absorption delay. A previously published paper[59] suggested a semi-mechanistic model where the levodopa plasma concentration acts as a feedback on the gastric emptying, however, it was developed with the support of both scintigraphy and paracetamol data. The model proved too complex for our data, i.e. it resulted in unidentifiable parameters when implemented.

In the non-compartmental analysis, both levodopa peak concentration and systemic exposure ($AUC_{0\text{-}4/dose}$, n=14) were significantly higher in patients compared to the healthy subjects. Five subjects were excluded from the non-compartmental analysis due to an early drop-out or a high drug concentration prior to dose administration.

Because the microtablets were given in the fasting state[139], the mean (±SD) time to maximum plasma concentration (T_{max}) for levodopa was short (32 ±

23 minutes), and did not differ significantly between the populations. The mean levodopa T_{max} is slightly shorter compared to the previously reported values of T_{max} from levodopa/carbidopa immediate release tablets administered to patients in individual doses, where subjects were also in fasting state and blood sampling was done as frequently as in our study.[140,141]

The results from the AALASSO, revealed that the HY score (most significant among the tested covariates) and carbidopa dose have a significant impact on levodopa's apparent clearance. It is possible that the HY score in our analysis is a representation of a combination of covariates (e.g. years on levodopa treatment and age) all contributing to the total effect observed. Differences in levodopa maximum concentration and AUC have been reported to be related to long-term levodopa therapy.[142,143] Higher age and levodopa dose, and decreased creatinine clearance, have also been reported to decrease levodopa apparent clearance.[144,145] In the covariate analysis, age was found to have a significant effect on levodopa relative bioavailability, possibly due to the age-related decreased enzymatic activity, but the increase was very modest and is not expected to be clinically meaningful. An age-related difference in levodopa bioavailability has been previously observed, but this effect was reported to be abolished by the addition of a high carbidopa dose (100 mg) that was administered one hour prior to levodopa administration.[37]

Carbidopa dose was found to have a significant effect in the initial covariate analysis on levodopa apparent clearance. The relationship was found to be non-linear, and to decrease with increasing carbidopa dose, by approximately 15%, when comparing a dose of 75 mg carbidopa to 50 mg carbidopa. When the individual, model-predicted carbidopa concentration was investigated as a covariate on levodopa's apparent clearance, the relationship was not found to be significant. A reason for this finding could be because levodopa metabolism mainly occurs during the first-pass metabolism.[34,36] Increasing levodopa dose has been reported to significantly decrease levodopa apparent clearance (levodopa administered in 4:1 ratio with either benserazide or carbidopa) by Jorga et al., (2000).[145] Because the combination treatment is usually (always in Sweden) administered in 4:1 ratio (also in our study), it is not possible to identify if the effect is only from carbidopa, levodopa, or a combination of both.

The carbidopa half-life was, in the non-compartmental analysis, found to be longer in patients (n=13) compared with healthy subjects. In the population pharmacokinetic analysis, we identified age as a significant covariate on carbidopa's apparent clearance, which is in agreement with the findings. The model-estimated half-life for the mean age of the patient population (71.4 years), was 155 minutes, which is somewhat lower than the mean half-life estimated with non-compartmental analysis (171 ± 37 minutes).[146]

For carbidopa, sex on mean transit time for the second depot compartment (MTT_2) was found as a significant covariate, suggesting a longer interruption in gastric emptying for women compared to men. This is in agreement with

studies where sex-related differences in gastric emptying have been specifically investigated.[147,148] The second plasma concentration-time peak of carbidopa appears 22 minutes later for women. In the graphical analysis, in contrast to carbidopa, no sex-related difference was observed for levodopa, however, it has faster absorption and may not be as sensitive to gastric emptying. The impact of gastric emptying could have a larger influence on the absorption of levodopa when administered as a regular tablet. A study[149] including 619 patients, found that wearing-off symptoms were more common in women compared to men. Perhaps one of the contributing factors for this could be the longer cessation in gastric-emptying.

The HY stage had a significant effect on carbidopa apparent clearance, with a higher stage associated with a modest increase, which is probably not clinically significant. Interestingly, study association (included as a covariate on all parameters but MTT_2) was not found to improve the model further.

In this dataset, some covariates were highly correlated, and an initial run using SCM showed that several of the covariate-parameter relationships tested produced a similar drop in OFV. The AALASSO method was therefore chosen, since it has been shown to perform better with highly correlated covariates and small datasets. Unlike the SCM method, the AALASSO tests all relationships simultaneously, and has been suggested to perform better with small datasets. To note is that in this case several of the covariates included in the final model had only a modest effect on the parameter value, such as HY on carbidopa apparent clearance and age on levodopa relative bioavailability.

The data from an external dataset was adequately predicted by the levodopa model, except the initial plasma concentration which was over predicted. As was done in the final levodopa model, the levodopa apparent clearance was adjusted with the total carbidopa dose administered, instead of a cumulative amount over time. The initial over-prediction of the observed levodopa concentrations could indicate time-dependent changes in levodopa PK, that the model does not capture optimally.

Levodopa-entacapone-carbidopa intestinal infusion

To compare the new levodopa/entacapone/carbidopa intestinal gel treatment to the conventional levodopa/carbidopa intestinal gel treatment, a clinical trial, including eleven patients, was conducted (**Paper IV**). The results suggested that, with the addition of entacapone, the levodopa/carbidopa dose could be decreased without decreasing the systemic exposure of levodopa. However, the plasma concentration was observed to increase during the day, which indicated that the dose could be decreased more than 20%.

By allowing a lower dose of levodopa, the volume gel needed can be decreased. The treatment is administered with a smaller pump, compared to the LCIG cassette with pump system, which is often reported to be a burden in

frail patients.[150,151] The lighter pump system and container, used for the first time for intestinal infusion, may facilitate daily living.

The systemic exposure of carbidopa was found to be lower compared to the LCIG treatment. The dose adjusted systemic exposure was however found to be higher for carbidopa. Carbidopa has in vitro been found to be a COMT substrate, which could be a reason for the higher dose adjusted exposure.[152]

The addition of entacapone resulted in a decrease of 3-OMD plasma concentration, however this was not compared statistically. The development of neuropathy has been hypothesized to occur due to requirement of methyl groups during the metabolism levodopa to 3-OMD, leading to dysfunction of Hcy metabolism and vitamin B6, B12, and/or folate deficiencies. A COMT-inhibition may hypothetically, reduce the risk of this side effect.[71,153]

A population pharmacokinetic model was developed for the intestinal infusion treatments to investigate the impact of entacapone on levodopa (**Paper V**). The developed model showed that the apparent clearance was significantly lower (37%) when entacapone was simultaneously infused. The conclusion from this analysis was that the continuous maintenance dose needs to be reduced corresponding to the decrease in apparent clearance, i.e. on a population level by approximately 35%. This is in contrast to the previously suggested 20% following LCIG infusion administered with oral entacapone.[123] A reason for this could be that the oral doses were administered every five hours, while here we have continuous infusion, meaning that the plasma concentration, and thereby inhibition, does not fluctuate. The bioavailability of entacapone is approximately 30-46%.[154] The infusion could perhaps also result in an increased bioavailability of entacapone, through immediate delivery to the small intestine and thereby a shorter intestinal residence time.

The model shows a tendency towards initially over predicting the plasma concentration, as observed from the plasma concentration-time curve, following LECIG administration. The reason for this is not clear, but has been observed with orally administered levodopa/carbidopa/entacapone treatments.[155,156] One theory is that, with the addition of COMT inhibitor, more levodopa is available. It may thereby be competing with itself for the saturable transporters across the intestinal membrane. Another theory was that higher levodopa concentrations caused a delay in gastric emptying, however in this study, this would not be an influencing factor since the infusion treatment is bypassing the stomach. Entacapone may compete with levodopa, due to molecular similarities, for transport across the intestinal membrane, thereby potentially affecting the absorption rate. Conversely, this was not observed for one of the transporters investigated with respect to this.[157]

Decreased absorption of levodopa, causing fluctuations in plasma concentration, may happen due to competition with dietary protein in the gastro-intestinal tract.[56] Protein was investigated as a covariate on the absorption or relative bioavailability, because trends in the levodopa plasma concentration were observed around meal times. The effect was explored both as a binary

variable (food intake yes/no) and as a continuous variable where the amount of protein at each meal (in gram) was used. However, according to the model implementations tested, no statistical benefit was observed by incorporating the food intake in the model. A reason for this could be the high inter-individual variability, and few individuals. Additionally, it was not an objective in the study to investigate the food effects, so the sampling times were not optimized with respect to this. Differences in e.g. gastro-intestinal mobility, and patients' overall mobility could also be a reason for the fluctuation in plasma concentration.

Comparison of the results from the two population pharmacokinetic models

The apparent clearance for levodopa with the levodopa/carbidopa intestinal infusion was estimated to 28 L/h (95% SIR CI 25-32 L). Since the doses of carbidopa are higher with the intestinal infusion (mean 275 mg, range 101-396 mg) a comparison of levodopa apparent clearance with the levodopa/carbidopa microtablets is not straight forward. A daily dose of 75 to 100 mg of carbidopa is believed to be necessary for DDC inhibition.[43] Assuming a full inhibition with 100 mg carbidopa, and a HY stage of 3, the estimated apparent clearance with the levodopa/carbidopa microtablets is 33 L/h, which is close to the estimated apparent clearance during the infusion treatment. The small difference could be due to innate differences between the relatively small populations that the models are based on. The apparent clearance values estimated are in line with the previously been reported values (25-37 L/h) for levodopa co-administered with carbidopa in individual doses.[128,145,158]

The total volume of distribution was estimated to 75 L (95% SIR CI 63-88 L) for levodopa with the infusion model, and 90 L in the microtablet model. The reported estimates of total apparent volume of distribution vary widely, between 43 to 131 L.[128,145,158] This is probably a reflection of variation in both study population and analysis technique.

The bioavailability of levodopa co-administered with carbidopa (100 mg 1 hour prior and additional 50 mg after 6 hours) is reported to be approximately 85%.[37] The levodopa relative bioavailability of levodopa/carbidopa intestinal infusion was reported to be slightly lower (by 3%) compared to levodopa/carbidopa immediate release tablets.[128]

Clinical experience with levodopa-carbidopa microtablets treatment

The microtablet treatment was initially available in Sweden on licensed prescription, and to investigate how the treatment was perceived in a real-life setting by the patients, an observation study was conducted (**Paper II**).

The need for fine-tuned doses and inability to improve motor function with other oral treatments was the main reason for treatment initiation. Insufficient symptom control was the main reason for discontinuation. Two of the patients that discontinued were considered for more advanced treatments (subcutaneous apomorphine and deep brain stimulation), indicating that oral therapy in general was insufficient. Some patients had higher daily levodopa doses with the microtablets. A possible explanation could be an increase in dose fractionation, resulting in a higher daily dose[159], or that some doses were increased at time points where the effect was insufficient.[160]

The patient perceived ability to perform daily activities was reported as improved by four patients, and unchanged by four. Seven patients perceived that the dose dispenser had facilitated their adherence, which could be a reason to the improvement in daily activities. A majority also stated that their treatment had become easier with the dose dispenser. A study by Stepien and Nyholm (2014)[161] reported that dose fractionation was common with other levodopa/DDC-inhibitor treatments. With time, the number of doses increased and the median dosing interval decreased. Patients with advanced PD usually also have other anti-parkinsonian medications prescribed. A complex dosing schedule has been shown to decrease adherence,[103] and the reminder by the dose dispenser could be a reason for the reported improvement in adherence. The screen on the dose dispenser also indicates if a dose has been missed, which can also make it easier for patients to follow their adherence during the day.

The four dose dispenser reports collected indicate that total adherence is high (97%). This is consistent with previous reports, where electronic tools were used to assess patient adherence to medication.[102,118] In contrast we report a higher timing adherence (89%), compared to a European multicenter study where the timing adherence was only 24%, but the total adherence was 98%.[102] A recently published 4-week study (2+2 weeks, n=24) where the microtablet and dose dispenser treatment was optimized with an objective motor function monitoring tool also reported a high timing adherence for the two observation periods (91% and 96% respectively) and a high total adherence (88% and 91% respectively).[160]

With regard to motor function, a majority found their bradykinesia and non-troublesome dyskinesia to be improved or unchanged. Three and four patients respectively experienced that the duration and magnitude of troublesome dyskinesia worsened. The worsening may be a cause of increased adherence. However, these results should be cautiously interpreted due to the limitations of the retrospective design and the low number of patients.

The results from the survey concerning usability of the dose dispenser, were positive according to the majority of the patients. Difficulties changing the cartridge and ease of bringing the dose dispenser during the everyday life was reported more problematic by three and four patients respectively. The latter could be related to the size of the dispenser or the need for a glass of

water to disperse the tablets. Despite this, eight out of nine patients thought that the dose dispenser made their therapy easier.

Pharmacodynamics

To investigate the effect from the newly developed treatments, the response in motor function was assessed (**Paper I and IV**). The complexity behind motor response, and the high individual variability is well-known.[82] This was also observed in Paper I, where the variability in response and duration of improvement was high. The inclusion criteria were that the patients had to experience wearing-off symptoms and/or dyskinesia. This made the patient population heterogeneous regarding start of levodopa treatment and years since symptom onset. Not all patients improved according to the cutoff that was set. A reason for this could be due to removal of concomitant medication, or due to sleep benefit, a phenomenon where some patients experience good motor function in the morning.[162] The development of dyskinesia was coinciding with improvement of motor function. A population pharmacokinetic-pharmacodynamic model, with similar study design, found that the motor response and dyskinesia have close onsets and duration effects, and that maximum response was inevitably associated with dyskinesia.[158] When mixed symptoms are present, the rating according to the TRS should be based on the walking ability. This could be one reason to why some patients did not reach the TRS cutoff value. The duration of effect, calculated for the patients that improved and returned to baseline was 154 (± 73) minutes according to the UPDRS and 180 (± 53) minutes according to the TRS, which suggest that the improvement does not remain long after the plasma concentration of levodopa starts to decline. The results highlight the importance of individualized dosing for optimal treatment outcome.

In **Paper IV**, the effect from the LECIG treatment was compared with the LCIG treatment. The TRS score was used for the assessment. It was not found to be significantly different between the treatments, however the mean curve suggests that some patients needed longer time to reach optimal effect with LECIG.

Effect of genotype on levodopa pharmacokinetics

The effect of genotype was explored on the levodopa apparent clearance, because it was observed in the clinical study that not all patients had the expected increase in levodopa systemic exposure with the addition of entacapone. Due to the few number of included subjects, a formal covariate analysis of the impact of genotype on CL/F was not performed, and the results are primarily exploratory.

The apparent clearance was found to decrease for all individuals with the levodopa/entacapone/carbidopa intestinal treatment, regardless or $COMT_{SNP}$ genotype (95% SIR CI 34-40%). Corvol., et al (2011)[73] also reported a significant decrease in apparent clearance (oral levodopa/carbidopa co-administered with placebo and 200 mg of entacapone) for the low and high activity groups. However, in contrast to our findings, their results indicated that the reduction is significantly greater for the high (40%) activity group compared to low (25%) activity group (according to $COMT_{SNP}$ rs4680). This could be due to higher administered doses of entacapone with the infusion treatment (median entacapone dose was 955 mg, range 320 to 1140 mg), or due to the small population size in our study. No clear trend was seen between dose and apparent clearance and all patients received high doses of entacapone, therefore a dose dependent decrease was not explored.

An effect of the DDC polymorphism has not been observed on levodopa pharmacokinetics.[76] Our results indicate a small tendency towards higher CL/F with the different DDC polymorphisms, however the possible clinical impact needs to be investigated in larger populations.

Conclusions

This thesis has described the pharmacokinetics of two novel treatments, the levodopa/carbidopa microtablets and the levodopa/entacapone/carbidopa intestinal gel, as well as the pharmacodynamics in advanced Parkinson's disease patients, the clinical experience with the microtablet treatment and a suggestion for dosing for the levodopa/entacapone/carbidopa intestinal infusion treatment.

- The results from the microtablet trial revealed differences in maximum plasma concentration and systemic exposure between patients and healthy subjects. High inter-individual variability in improvement and deterioration of motor function was observed, highlighting the need for individualized treatment.

- The evaluation of the clinical experience of the microtablet treatment concept included patients that had the treatment prescribed by their physician. The patients perceived that the microtablets along with the dose dispenser improved treatment adherence and that the treatment had become easier. The activities of daily living and symptoms of bradykinesia and non-troublesome dyskinesia were mainly perceived as improved or unchanged. High adherence was observed from the four dose dispenser reports obtained.

- The pharmacokinetic population model developed for the levodopa/carbidopa microtablets described the data well, and revealed that carbidopa dose and HY stage are factors that have an impact on levodopa apparent clearance. Age was found to have an impact on carbidopa apparent clearance. Women were found to have a longer carbidopa mean transit time for the second peak, compared to men.

- The clinical trial with levodopa/entacapone/carbidopa intestinal gel confirmed that the addition of entacapone allowed for a lower amount of levodopa administration, without resulting in a lower systemic exposure, or causing a significant worsening of the treatment outcome. The treatment was well tolerated. Slowly increasing plasma concentration over time indicated that there was room for improved dose translation from the con-

ventional levodopa/carbidopa intestinal gel treatment. A population pharmacokinetic model was developed investigating the impact of simultaneous entacapone infusion. It was found that the continuous maintenance dose could be decreased by approximately 35%, on a population level, due to a significantly lower apparent clearance. An effect from entacapone was identified in all individuals, regardless of $COMT_{SNP}$ polymorphism.

Future prospects

Levodopa seems to remain the gold standard of anti-Parkinson's disease treatment.[91,163] The development of new levodopa-based therapies has increased over the last years, with the aim to overcome levodopa's limitations as a drug.

The microtablet treatment concept may fill a gap in the therapy for patient with advanced Parkinson's disease. The low-dose tablets allow for an individualized dosing with small increments in both dose and dose interval, while the dose dispenser can facilitate the therapy for patients. It may improve treatment adherence and the diary function enables easy reporting of symptoms in connection to dose intake. The dose dispenser offers a good platform for algorithm-based treatment optimization, and the developed pharmacokinetic microtablet model could be a first step towards a model based individualized treatment.

- Because the treatment offers a flexibility for dose adjustment, it would be interesting to evaluate if it would be beneficial to optimize the treatment in an early phase of the disease, e.g. when wearing-off symptoms start to occur. This could perhaps prevent or delay the onset of severe fluctuations and the need for advanced treatments.

Although the model describes the single-dose administration well, it was observed from the external validation that the developed model over predicted the levodopa concentrations for the initial doses following the administration of low doses of levodopa/carbidopa microtablets to healthy volunteers.

- A multiple dose study could be the next step, to validate the model in the patient population, and further elucidate the levodopa-carbidopa interaction.

- Ultimately the model should be expanded with a pharmacodynamics model to describe the patients pharmacokinetic-pharmacodynamic relationship. This could assist clinicians in objective assessment of clinical needs and optimization of dosing. The collected pharmacodynamic data can be used as a first step towards describing the pharmacokinetic-pharmacodynamic relationship. The model could be used for initial dosage finding in the clinic, because the motor function assessed with the scales needs to be done by trained personnel. For continued monitoring over

time, during at home use, the patient assessed efficacy from the dose dispenser diary should be coupled with the pharmacokinetic-pharmacodynamic model, to capture if dose adjustments may be needed. An alternative to patient self-assessment is by the use of objective measurements. However, while this would be the optimal scenario, and has shown promising results[160], there is an economic limit to the usability of this. With the use of the diary-function, non-motor symptoms could also be assessed and taken into account.

The infusion treatment incorporating entacapone showed promise and may be an alternative to the current conventional treatment for some patients. In this thesis, long-term safety was not addressed, nor could we with the data assess the relative bioavailability or volume of distribution to find how morning doses should be translated from the conventional treatment.

- Larger, long-term studies need to be conducted to further evaluate possible side effects, development of peripheral neuropathy, and to confirm the dosage suggestion. To investigate the adjustments in morning dose, administration of bolus doses only, and/or repeated sampling for a longer time period post dosing could be informative.

- Interestingly, the motor function according to the treatment response scale, despite increasing plasma concentration, was not found to differ significantly between the treatments. This raises the question of if the patients may need a higher dose later in the evening. It could be something worth investigating, possibly by assessing patients use of the possibility to take extra bolus doses during the day.

- The infusion treatment, that bypasses the stomach and is not influenced by the gastric emptying, could be a good way of investigating the impact of protein intake on the absorption of levodopa, as well as on the treatment effect.

Populärvetenskaplig sammanfattning

Parkinsons sjukdom kännetecknas av förlust av nervceller som producerar dopamin, vilket leder till dopaminbrist i hjärnan. Symtom som då uppstår är dels av motorisk karaktär som långsamma rörelser, stelhet, och skakningar, men även icke-motoriska symtom som ångest och depression.

Farmakokinetik beskriver hur ett läkemedel tas upp i kroppen, hur det fördelas och hur kroppen gör sig av med läkemedlet. Farmakodynamik beskriver effekten av läkemedlet. I en populationsfarmakokinetisk modell används matematiska modeller för att beskriva läkemedlets farmakokinetik. Dessa kan användas för att öka förståelsen för hur man bör dosera ett läkemedel samt undersöka hur faktorer, som vikt och ålder, påverkar läkemedlets omsättning. Modellerna kan även kopplas samman med modeller som beskriver effekten för att öka förståelsen för hur effekten förändras med dos.

Levodopa är ett läkemedel som kan ta sig in i hjärnan och omvandlas till dopamin. Det är den mest effektiva behandlingen mot symtomen, men med tiden blir sjukdomen svårare att behandla på grund av att sjukdomen fortskrider (ökad förlust av nervceller som producerar dopamin). Det terapeutiska fönstret – intervallet inom vilket blodkoncentrationer av läkemedlet behöver vara i kroppen för att ge tillräcklig effekt, utan att orsaka biverkningar – minskar. Dosen behöver då anpassas för varje patient, och läkemedlet kan behöva tas oftare.

Denna avhandling fokuserar på två nya tillvägagångssätt för levodopa-behandlingar; levodopa/karbidopa mikrotabletter och levodopa/entakapon/karbidopa intestinal gel, som är framtagna för patienter med avancerad Parkinsons sjukdom. Karbidopa och entakapon är läkemedel som kan ges tillsammans med levodopa för att öka koncentrationen av läkemedlet i kroppen, genom att minska dess nedbrytning.

Levodopa/karbidopa mikrotabletter

Mikrotabletterna är små tabletter som man kan lösa i vatten. De innehåller en låg dos av levodopa och karbidopa och kan därför användas för att finjustera behandlingen för varje patient. Eftersom tabletterna är mycket små i storlek, och många tabletter måste tas för att komma upp i en lämplig dos, används behandlingen tillsammans med en dosautomat. Dosautomaten kan påminna patienten om vilken dos som ska tas vid vilken tidpunkt. Den har en larmfunktion som larmar när det är dags att ta en dos, samt en dagboksfunktion där patienten kan anteckna hur de mår under behandlingen.

En klinisk studie genomfördes för att utvärdera farmakokinetiken och farmakodynamiken av levodopa och karbidopa när de ges som mikrotabletter. Skillnader i farmakokinetiken observerades mellan patienterna och tidigare behandlade friska individer. En stor skillnad i hur stor effekt patienterna fick och hur länge den varade observerades mellan patienterna. Detta betonar vikten av individuell bedömning av effekten för att kunna ge en så bra behandling som möjligt.

En populationsfarmakokinetisk modell för levodopa och karbidopa utvecklades och effekten av olika faktorer undersöktes. Svårighetsgraden av sjukdomen och ökad dos av karbidopa visade sig öka patienternas exponering för levodopa. Ökad ålder är en faktor som ökar patienters exponering för karbidopa.

En observationsstudie genomfördes också för att utvärdera behandlingen i klinisk praxis. Patienter som någon gång förskrivits behandling med mikrotabletterna inkluderades i denna studie. En majoritet av patienterna rapporterade att dosautomaten hade förenklat deras behandling och att de upplevde att de var bättre på att ta sin medicin när de skulle. Symtomen, med avseende på långsamma rörelser och biverkning i form av ofrivillig dansliknande rörlighet som inte var besvärande, uppfattades i huvudsak som förbättrad eller oförändrad.

Levodopa/entakapon/karbidopa intestinal gel

Levodopa/entakapon/karbidopa intestinal gel utvecklades för att undersöka om det är möjligt att sänka dosen levodopa, utan att försämra effekten. En konventionell behandling med levodopa/karbidopa intestinal gel finns redan på marknaden. Gelen administreras direkt in i tarmen via en slang som går från magen, och den ges kontinuerligt under dagen med en pump. Denna behandling brukar förskrivas till patienter som inte längre får tillräcklig kontroll av sina symtom med tabletter.

För att undersöka farmakokinetiken och farmakodynamiken av den nyutvecklade gelen, genomfördes en klinisk prövning där den jämfördes med den konventionella gel-behandlingen. Med den nya behandlingen kunde man sänka dosen levodopa som gavs, utan att försämra effekten. En ökande blodkoncentration observerades, och en populationsmodell utvecklades för att undersöka dosjusteringar. Slutsatsen var att den kontinuerliga underhållsdosen kan minskas med cirka 35%. Genetiska variationer av ett enzym (ett protein som bryter ner levodopa i kroppen så det elimineras snabbare) som entakapon hämmar undersöktes också. Detta då det finns en teori om att de som har en viss variant eventuellt gynnas mer av ett tillägg av entakapon. I denna studie identifierades en effekt av entakapon hos samtliga individer, oberoende av genetisk variant av enzymet.

Båda nya behandlingarna är lovande alternativ till nuvarande behandlingsstrategier och de utvecklade modellerna kan i framtiden användas för optimering av behandling hos patienter med Parkinsons sjukdom.

Acknowledgments

This thesis was carried out at the Department of Neuroscience, Faculty of Medicine and the Department of Pharmaceutical Biosciences, Faculty of Pharmacy, Uppsala University.

I would like to express my sincere gratitude to all who have contributed directly or indirectly, to the completion of this thesis,

My supervisors, it has been a true honor and pleasure working with you and I will really miss our discussions,

Dag Nyholm, my main supervisor, thank you for giving me the opportunity to conduct this work, and guiding me through it with continuous enthusiasm and great encouragement, for sharing your great expertise and for always involving me in new projects,

Elisabet Nielsen, my co-supervisor, thank you for invaluable guidance, support and patience during the model development process, for always being encouraging and keeping your door open when I had "just a quick question",

all the patients who volunteered to participate in the studies,

all my co-authors for the great collaboration, invaluable input and advice. Special thanks to ***Sten-Magnus Aquilonius, Håkan Askmark*** and ***Radu Constantinescu*** for looking through and rating the many patient videos,

all the colleagues, doctors and nurses at the Department of Neurology and Neuromottagningen for sharing your knowledge, nice discussions, helping out with my study and the pleasant atmosphere, especially ***Prof. Ann-Marie Landtblom,*** for giving me the opportunity to improve my teaching skills, ***Sari*** for all the administrative (and other) help, ***Andreas*** for good times organizing and attending the Neuropub, ***Johan*** *and* ***Kalle*** it was a great fun (and very educational) to teach with you,

all the past and present colleagues, teachers, professors and researchers at the Department of Pharmaceutical Biosciences for the pleasant discussions, the travel companionship, and for making these years very enjoyable. A special thanks to ***Prof. Mats Karlsson*** and ***Prof. Margareta Hammarlund-Udenaes*** for the great work environment, ***Brendan*** for all the good times with fun discussions that would go on for "just one more beer", ***Henrik***, for great talks, both scientific and non-scientific, when breaks were needed, ***Ida,*** for not only being a good colleague but a very good friend, ***Eva***, for the "girls-night-out", sincere conversations and many laughs, ***Joao***, for always checking in with a smile, ***Gunnar,*** for answering all my FREM (and other) questions and sharing all your chili-knowledge, ***Rikard***, for the invaluable help through all the cluster problems towards the end, ***Irena***, for sharing your genotyping expertise,

I would like to thank ***all my friends*** and ***my family*** for all their support and encouragement throughout the years,
Mia, för du är en underbar vän, vänner som du är en sällsynt gåva,
Mamma *och mina syskon* ***Michaela*** *och* ***Aleksandar,*** för all er kärlek och för ert kontinuerliga stöd,
Baka i deda, uvijek ste vjerovali i bili tu za nas. Jako sam zahvalna da sam imala vašu podršku. Nema riječi kojima bih mogla opisati koliko mi puno značite. Volim vas do neba,
Robin, det går inte att beskriva i ord hur tacksam jag är för att få ha dig i mitt liv – tack för att du finns här för mig med din kärlek och omtanke.

The work was performed with financial support from Vinnova, Sweden's innovation agency "Innovations for Future Health", the Swedish Parkinson's Disease Association, the Swedish Parkinson's disease foundation and The Swedish State Support for Clinical Research (ALF). I would also like to thank Apotekarsocieteten and Smålands Nation for travel grants for various courses and conferences around the world, and the Clinical Trial Consultants AB (CTC) personnel for their professional assistance with the study described in Paper I and for conducting the study described in Paper IV.

References

1. Goetz CG. The History of Parkinson's Disease: Early Clinical Descriptions and Neurological Therapies. Cold Spring Harb Perspect Med [online serial]. 2011;1:a008862.
2. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol. 2006;5:525–535.
3. Aquilonius S-M, Mouradian MM, editors. Parkinson's Disease: Role of Continuous Dopaminergic Stimulation. Crowthorne: ESP Bioscience Ltd; 2012.
4. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci. 1986;9:357–381.
5. Obeso JA, Rodriguez-Oroz MC, Stamelou M, Bhatia KP, Burn DJ. The expanding universe of disorders of the basal ganglia. Lancet Lond Engl. 2014;384:523–531.
6. Hornykiewicz O. Dopamine (3-Hydroxytyramine) and Brain Function. Pharmacol Rev. 1966;18:925–964.
7. Fahn S, Oakes D, Shoulson I, et al. Levodopa and the progression of Parkinson's disease. N Engl J Med. 2004;351:2498–2508.
8. Parent M, Parent A. Substantia nigra and Parkinson's disease: a brief history of their long and intimate relationship. Can J Neurol Sci J Can Sci Neurol. 2010;37:313–319.
9. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24:197–211.
10. Lionnet A, Leclair-Visonneau L, Neunlist M, et al. Does Parkinson's disease start in the gut? Acta Neuropathol (Berl). 2018;135:1–12.
11. Burke RE, O'Malley K. Axon degeneration in Parkinson's disease. Exp Neurol. 2013;246:72–83.
12. Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry. 2008;79:368–376.
13. Berardelli A, Sabra AF, Hallett M. Physiological mechanisms of rigidity in Parkinson's disease. J Neurol Neurosurg Psychiatry. 1983;46:45–53.
14. Kerr GK, Worringham CJ, Cole MH, Lacherez PF, Wood JM, Silburn PA. Predictors of future falls in Parkinson disease. Neurology. 2010;75:116–124.
15. Postuma RB, Aarsland D, Barone P, et al. Identifying prodromal Parkinson's disease: Pre-Motor disorders in Parkinson's disease. Mov Disord. 2012;27:617–626.
16. Müller B, Assmus J, Herlofson K, Larsen JP, Tysnes O-B. Importance of motor vs. non-motor symptoms for health-related quality of life in early Parkinson's disease. Parkinsonism Relat Disord. 2013;19:1027–1032.

17. Chaudhuri KR, Prieto-Jurcynska C, Naidu Y, et al. The nondeclaration of nonmotor symptoms of Parkinson's disease to health care professionals: an international study using the nonmotor symptoms questionnaire. Mov Disord. 2010;25:704–709.
18. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord. 2007;22:1689–1707.
19. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30:1591–1601.
20. Rizzo G, Copetti M, Arcuti S, Martino D, Fontana A, Logroscino G. Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis. Neurology. 2016;86:566–576.
21. Antonini A, DeNotaris R. PET and SPECT functional imaging in Parkinson's disease. Sleep Med. 2004;5:201–206.
22. Appel L, Jonasson M, Danfors T, et al. Use of 11C-PE2I PET in differential diagnosis of parkinsonian disorders. J Nucl Med. 2015;56:234–242.
23. Nationella riktlinjer för vård vid multipel skleros och Parkinsons sjukdom – Stöd för styrning och ledning [online]. Accessed at: http://www.socialstyrelsen.se/publikationer2016/2016-12-1.
24. Clarke CE, Davies P. Systematic review of acute levodopa and apomorphine challenge tests in the diagnosis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry. 2000;69:590–594.
25. de Roos P, Bloem BR, Kelley TA, et al. A Consensus Set of Outcomes for Parkinson's Disease from the International Consortium for Health Outcomes Measurement. J Park Dis. 2017;7:533–543.
26. Ebersbach G, Baas H, Csoti I, Müngersdorf M, Deuschl G. Scales in Parkinson's disease. J Neurol. 2006;253 Suppl 4:IV32-35.
27. Nyholm D, Nilsson Remahl AIM, Dizdar N, et al. Duodenal levodopa infusion monotherapy vs oral polypharmacy in advanced Parkinson disease. Neurology. 2005;64:216–223.
28. Goetz CG, Poewe W, Rascol O, et al. Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. Mov Disord. 2004;19:1020–1028.
29. Carlsson A, Lindqvist M, Magnusson T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature. 1957;180:1200.
30. Nutt JG, Fellman JH. Pharmacokinetics of levodopa. Clin Neuropharmacol. 1984;7:35–49.
31. Lennernäs H, Nilsson D, Aquilonius SM, Ahrenstedt O, Knutson L, Paalzow LK. The effect of L-leucine on the absorption of levodopa, studied by regional jejunal perfusion in man. Br J Clin Pharmacol. 1993;35:243–250.
32. Frankel JP, Kempster PA, Bovingdon M, Webster R, Lees AJ, Stern GM. The effects of oral protein on the absorption of intraduodenal levodopa and motor performance. J Neurol Neurosurg Psychiatry. 1989;52:1063–1067.
33. Leenders KL, Poewe WH, Palmer AJ, Brenton DP, Frackowiak RSJ. Inhibition of L-{18F}fluorodopa uptake into human brain by amino acids demonstrated by positron emission tomography. Ann Neurol. 1986;20:258–262.
34. Granerus AK, Jagenburg R, Svanborg A. Intestinal decarboxylation of L-Dopa in relation to dose requirement in Parkinson's disease. Naunyn Schmiedebergs Arch Pharmacol. 1973;280:429–439.

35. Goodall M, Alton H. Metabolism of 3,4-dihydroxyphenylalanine (L-dopa) in human subjects. Biochem Pharmacol. 1972;21:2401–2408.
36. Sasahara K, Nitanai T, Habara T, et al. Dosage form design for improvement of bioavailability of levodopa IV: Possible causes of low bioavailability of oral levodopa in dogs. J Pharm Sci. 1981;70:730–733.
37. Robertson DR, Wood ND, Everest H, et al. The effect of age on the pharmacokinetics of levodopa administered alone and in the presence of carbidopa. Br J Clin Pharmacol. 1989;28:61–69.
38. Gershanik OS. Improving l-dopa therapy: The development of enzyme inhibitors. Mov Disord. 2015;30:103–113.
39. Bartholini G, Pletscher A. Decarboxylase inhibitors. Pharmacol Ther [B]. 1975;1:407–421.
40. Nyholm D, Lewander T, Gomes-Trolin C, et al. Pharmacokinetics of levodopa/carbidopa microtablets versus levodopa/benserazide and levodopa/carbidopa in healthy volunteers. Clin Neuropharmacol. 2012;35:111–117.
41. Grahnén A, Eckernäs SA, Collin C, Ling-Andersson A, Tiger G, Nilsson M. Comparative multiple-dose pharmacokinetics of controlled-release levodopa products. Eur Neurol. 1992;32:343–348.
42. Jaffe ME. Clinical Studies of Carbidopa and L-dopa in the Treatment of Parkinson's Disease. Yahr MD Ed Adv Neurol. New York: Raven Press; 1973. p. 161–172.
43. Pinder RM, Brogden RN, Sawyer PR, Speight TM, Avery GS. Levodopa and decarboxylase inhibitors: a review of their clinical pharmacology and use in the treatment of parkinsonism. Drugs. 1976;11:329–377.
44. Hoehn MM. Increased dosage of carbidopa in patients with Parkinson's disease receiving low doses of levodopa. A pilot study. Arch Neurol. 1980;37:146–149.
45. Cilia R, Laguna J, Cassani E, et al. Daily intake of Mucuna pruriens in advanced Parkinson's disease: A 16-week, noninferiority, randomized, crossover, pilot study. Parkinsonism Relat Disord. Epub 2018 Jan 11.
46. Cilia R, Laguna J, Cassani E, et al. Mucuna pruriens in Parkinson disease: A double-blind, randomized, controlled, crossover study. Neurology. 2017;89:432–438.
47. Männistö PT, Kaakkola S. New selective COMT inhibitors: useful adjuncts for Parkinson's disease? Trends Pharmacol Sci. 1989;10:54–56.
48. Keränen T, Gordin A, Karlsson M, et al. Inhibition of soluble catechol-O-methyltransferase and single-dose pharmacokinetics after oral and intravenous administration of entacapone. Eur J Clin Pharmacol. 1994;46:151–157.
49. Bet L, Bareggi SR, Pacei F, Bondiolotti G, Meola G, Schapira AHV. Bimodal administration of entacapone in Parkinson's disease patients improves motor control. Eur J Neurol. 2008;15:268–273.
50. Kuoppamäki M, Korpela K, Marttila R, et al. Comparison of pharmacokinetic profile of levodopa throughout the day between levodopa/carbidopa/entacapone and levodopa/carbidopa when administered four or five times daily. Eur J Clin Pharmacol. 2009;65:443–455.
51. Stocchi F, Rascol O, Kieburtz K, et al. Initiating levodopa/carbidopa therapy with and without entacapone in early Parkinson disease: The STRIDE-PD study. Ann Neurol. 2010;68:18–27.

52. Nyholm D, Johansson A, Lennernäs H, Askmark H. Levodopa infusion combined with entacapone or tolcapone in Parkinson disease: a pilot trial. Eur J Neurol. 2012;19:820–826.
53. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord. 2010;25:2649–2653.
54. Wade LA, Katzman R. 3-O-methyldopa uptake and inhibition of L-dopa at the blood-brain barrier. Life Sci. 1975;17:131–136.
55. Nutt JG, Woodward WR, Gancher ST, Merrick D. 3-O-Methyldopa and the response to levodopa in Parkinson's disease. Ann Neurol. 1987;21:584–588.
56. Nyholm D, Lennernäs H. Irregular gastrointestinal drug absorption in Parkinson's disease. Expert Opin Drug Metab Toxicol. 2008;4:193–203.
57. Kurlan R, Nutt JG, Woodward WR, et al. Duodenal and gastric delivery of levodopa in parkinsonism. Ann Neurol. 1988;23:589–595.
58. Robertson DR, Renwick AG, Wood ND, et al. The influence of levodopa on gastric emptying in man. Br J Clin Pharmacol. 1990;29:47–53.
59. Ogungbenro K, Pertinez H, Aarons L. Empirical and Semi-Mechanistic Modelling of Double-Peaked Pharmacokinetic Profile Phenomenon Due to Gastric Emptying. AAPS J. 2014;17:227–236.
60. Evans MA, Triggs EJ, Broe GA, Saines N. Systemic availability of orally administered L-dopa in the elderly Parkinsonian patient. Eur J Clin Pharmacol. 1980;17:215–221.
61. Nyholm D, Lennernäs H, Gomes-Trolin C, Aquilonius S-M. Levodopa pharmacokinetics and motor performance during activities of daily living in patients with Parkinson's disease on individual drug combinations. Clin Neuropharmacol. 2002;25:89–96.
62. Santos-García D, de la Fuente-Fernández R, Valldeoriola F, et al. Polyneuropathy while on duodenal levodopa infusion in Parkinson's disease patients: we must be alert. J Neurol. 2012;259:1668–1672.
63. Mancini F, Comi C, Oggioni GD, et al. Prevalence and features of peripheral neuropathy in Parkinson's disease patients under different therapeutic regimens. Parkinsonism Relat Disord. 2014;20:27–31.
64. Ceravolo R, Cossu G, Bandettini di Poggio M, et al. Neuropathy and levodopa in Parkinson's disease: evidence from a multicenter study. Mov Disord. 2013;28:1391–1397.
65. Müller T, Renger K, Kuhn W. Levodopa-associated increase of homocysteine levels and sural axonal neurodegeneration. Arch Neurol. 2004;61:657–660.
66. Hughes RAC. Peripheral neuropathy. BMJ. 2002;324:466–469.
67. Müller T, van Laar T, Cornblath DR, et al. Peripheral neuropathy in Parkinson's disease: levodopa exposure and implications for duodenal delivery. Parkinsonism Relat Disord. 2013;19:501–507 ; discussion 501.
68. Zis P, Grünewald RA, Chaudhuri RK, Hadjivassiliou M. Peripheral neuropathy in idiopathic Parkinson's disease: A systematic review. J Neurol Sci. 2017;378:204–209.
69. 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;86:490–495.

70. Toth C, Breithaupt K, Ge S, et al. Levodopa, methylmalonic acid, and neuropathy in idiopathic Parkinson disease. Ann Neurol. 2010;68:28–36.
71. Cossu G, Ceravolo R, Zibetti M, et al. Levodopa and neuropathy risk in patients with Parkinson disease: Effect of COMT inhibition. Parkinsonism Relat Disord. 2016;27:81–84.
72. Weinshilboum RM, Otterness DM, Szumlanski CL. Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. Annu Rev Pharmacol Toxicol. 1999;39:19–52.
73. Corvol J-C, Bonnet C, Charbonnier-Beaupel F, et al. The COMT Val158Met polymorphism affects the response to entacapone in Parkinson's disease: A randomized crossover clinical trial. Ann Neurol. 2011;69:111–118.
74. de Lau LML, Verbaan D, Marinus J, Heutink P, van Hilten JJ. Catechol-O-methyltransferase Val158Met and the risk of dyskinesias in Parkinson's disease. Mov Disord. 2012;27:132–135.
75. Contin M, Martinelli P, Mochi M, Riva R, Albani F, Baruzzi A. Genetic polymorphism of catechol-O-methyltransferase and levodopa pharmacokinetic-pharmacodynamic pattern in patients with Parkinson's disease. Mov Disord. 2005;20:734–739.
76. Devos D, Lejeune S, Cormier-Dequaire F, et al. Dopa-decarboxylase gene polymorphisms affect the motor response to L-dopa in Parkinson's disease. Parkinsonism Relat Disord. 2014;20:170–175.
77. Obeso JA, Olanow CW, Nutt JG. Levodopa motor complications in Parkinson's disease. Trends Neurosci. 2000;23, Supplement 1:S2–S7.
78. Contin M, Riva R, Martinelli P, et al. Response to a standard oral levodopa test in parkinsonian patients with and without motor fluctuations. Clin Neuropharmacol. 1990;13:19–28.
79. Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov Disord. 2001;16:448–458.
80. Senek M, Nyholm D. Continuous drug delivery in Parkinson's disease. CNS Drugs. 2014;28:19–27.
81. Espay AJ, Lang AE. Common Myths in the Use of Levodopa in Parkinson Disease: When Clinical Trials Misinform Clinical Practice. JAMA Neurol. 2017;74:633–634.
82. Nyholm D, Johansson A, Aquilonius S-M, Hellquist E, Lennernäs H, Askmark H. Complexity of motor response to different doses of duodenal levodopa infusion in Parkinson disease. Clin Neuropharmacol. 2012;35:6–14.
83. de la Fuente-Fernández R, Sossi V, Huang Z, et al. Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. Brain J Neurol. 2004;127:2747–2754.
84. Pavese N, Evans AH, Tai YF, et al. Clinical correlates of levodopa-induced dopamine release in Parkinson disease: a PET study. Neurology. 2006;67:1612–1617.

85. Tedroff J, Aquilonius SM, Hartvig P, Bredberg E, Bjurling P, Långström B. Cerebral uptake and utilization of therapeutic [beta-11C]-L-DOPA in Parkinson's disease measured by positron emission tomography. Relations to motor response. Acta Neurol Scand. 1992;85:95–102.
86. Leenders KL, Palmer AJ, Quinn N, et al. Brain dopamine metabolism in patients with Parkinson's disease measured with positron emission tomography. J Neurol Neurosurg Psychiatry. 1986;49:853–860.
87. Mouradian MM, Chase TN. Central mechanisms and levodopa response fluctuations in Parkinson's disease. Clin Neuropharmacol. 1988;11:378–385.
88. Fabbrini G, Mouradian MM, Juncos JL, Schlegel J, Mohr E, Chase TN. Motor fluctuations in Parkinson's disease: central pathophysiological mechanisms, Part I. Ann Neurol. 1988;24:366–371.
89. Mouradian MM, Juncos JL, Fabbrini G, Schlegel J, Bartko JJ, Chase TN. Motor fluctuations in Parkinson's disease: central pathophysiological mechanisms, Part II. Ann Neurol. 1988;24:372–378.
90. Rascol O, Brooks DJ, Korczyn AD, et al. Development of dyskinesias in a 5-year trial of ropinirole and L-dopa. Mov Disord. 2006;21:1844–1850.
91. LeWitt PA. Levodopa therapy for Parkinson's disease: Pharmacokinetics and pharmacodynamics. Mov Disord. 2015;30:64–72.
92. Olanow CW. Levodopa: effect on cell death and the natural history of Parkinson's disease. Mov Disord. 2015;30:37–44.
93. Dezsi L, Vecsei L. Monoamine Oxidase B Inhibitors in Parkinson's Disease. CNS Neurol Disord Drug Targets. 2017;16:425–439.
94. Nutt JG. On-off phenomenon: relation to levodopa pharmacokinetics and pharmacodynamics. Ann Neurol. 1987;22:535–540.
95. Rocha J-F, Falcão A, Santos A, et al. Effect of opicapone and entacapone upon levodopa pharmacokinetics during three daily levodopa administrations. Eur J Clin Pharmacol. 2014;70:1059–1071.
96. Ferreira JJ, Lees A, Rocha J-F, et al. Opicapone as an adjunct to levodopa in patients with Parkinson's disease and end-of-dose motor fluctuations: a randomised, double-blind, controlled trial. The Lancet Neurology, 2016, 15.2: 154-165.
97. Freitas ME, Ruiz-Lopez M, Fox SH. Novel Levodopa Formulations for Parkinson's Disease. CNS Drugs. 2016;30:1079–1095.
98. Bredberg E, Tedroff J, Aquilonius SM, Paalzow L. Pharmacokinetics and effects of levodopa in advanced Parkinson's disease. Eur J Clin Pharmacol. 1990;39:385–389.
99. Nyholm D, Askmark H, Gomes-Trolin C, et al. Optimizing levodopa pharmacokinetics: intestinal infusion versus oral sustained-release tablets. Clin Neuropharmacol. 2003;26:156–163.
100. Volkmann J, Albanese A, Antonini A, et al. Selecting deep brain stimulation or infusion therapies in advanced Parkinson's disease: an evidence-based review. J Neurol. 2013;260:2701–2714.
101. Malek N, Grosset DG. Medication adherence in patients with Parkinson's disease. CNS Drugs. 2015;29:47–53.
102. Grosset D, Antonini A, Canesi M, et al. Adherence to antiparkinson medication in a multicenter European study. Mov Disord. 2009;24:826–832.

103. Daley DJ, Myint PK, Gray RJ, Deane KHO. Systematic review on factors associated with medication non-adherence in Parkinson's disease. Parkinsonism Relat Disord. 2012;18:1053–1061.
104. 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;122:71–77.
105. Nyholm D, Odin P, Johansson A, et al. 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;15:316–323.
106. Othman AA, Dutta S. Population pharmacokinetics of levodopa in subjects with advanced Parkinson's disease: levodopa-carbidopa intestinal gel infusion vs. oral tablets. Br J Clin Pharmacol. 2014;78:94–105.
107. Westin J, Nyholm D, Pålhagen S, et al. A pharmacokinetic-pharmacodynamic model for duodenal levodopa infusion. Clin Neuropharmacol. 2011;34:61–65.
108. Nyholm D, Lewander T, Johansson A, Lewitt PA, Lundqvist C, Aquilonius S-M. Enteral levodopa/carbidopa infusion in advanced Parkinson disease: long-term exposure. Clin Neuropharmacol. 2008;31:63–73.
109. Olanow CW, Kieburtz K, Odin P, et al. 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;13:141–149.
110. Wirdefeldt K, Odin P, Nyholm D. Levodopa-Carbidopa Intestinal Gel in Patients with Parkinson's Disease: A Systematic Review. CNS Drugs. 2016;30:381–404.
111. Klostermann F, Jugel C, Müller T, Marzinzik F. Malnutritional neuropathy under intestinal levodopa infusion. J Neural Transm. 2012;119:369–372.
112. Jugel C, Ehlen F, Taskin B, Marzinzik F, Müller T, Klostermann F. Neuropathy in Parkinson's Disease Patients with Intestinal Levodopa Infusion versus Oral Drugs. PLoS ONE. 2013;8:e66639.
113. Kurth MC, Tetrud JW, Irwin I, Lyness WH, Langston JW. Oral levodopa/carbidopa solution versus tablets in Parkinson's patients with severe fluctuations: a pilot study. Neurology. 1993;43:1036–1039.
114. Bennett JP, Turk M, Landow E. Continuous oral administration of L-dihydroxyphenylalanine (L-DOPA) solution to patients with advanced Parkinson's disease. Clin Neuropharmacol. 1989;12:285–292.
115. Pappert EJ, Goetz CG, Niederman F, Ling ZD, Stebbins GT, Carvey PM. Liquid levodopa/carbidopa produces significant improvement in motor function without dyskinesia exacerbation. Neurology. 1996;47:1493–1495.
116. Nyholm D, Ehrnebo M, Lewander T, et al. Frequent administration of levodopa/carbidopa microtablets vs levodopa/carbidopa/entacapone in healthy volunteers. Acta Neurol Scand. 2013;127:124–132.
117. Bredenberg S, Nyholm D, Aquilonius SM, Nyström C. An automatic dose dispenser for microtablets--a new concept for individual dosage of drugs in tablet form. Int J Pharm. 2003;261:137–146.
118. Grosset KA, Bone I, Reid JL, Grosset D. Measuring therapy adherence in Parkinson's disease: a comparison of methods. J Neurol Neurosurg Psychiatry. 2006;77:249–251.

119. Wright DFB, Winter HR, Duffull SB. Understanding the time course of pharmacological effect: a PKPD approach. Br J Clin Pharmacol. 2011;71:815–823.
120. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development. CPT Pharmacomet Syst Pharmacol. 2012;1:e6.
121. Bonate PL. Pharmacokinetic-pharmacodynamic modeling and simulation. Springer, New York; 2011.
122. Beal S, Sheiner LB, Boeckmann A, Bauer RJ. NONMEM User's Guides. (1989-2009), Icon Development Solutions, Ellicott City, MD, USA, 2009.
123. Nyholm D, Johansson A, Lennernäs H, Askmark H. Levodopa infusion combined with entacapone or tolcapone in Parkinson disease: a pilot trial. Eur J Neurol. 2012;19:820–826.
124. ICH Topic Q 2 B, Validation of Analytical Procedures: Methodology, Note for guidance on validation of analytical procedures (CPMP/ICH/281/95).
125. EUROPEAN MEDICINES AGENCY. Guideline on bioanalytical method validation. *Committee for Medicinal Products for Human Use (CHMP).* 2011.
126. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2015.
127. Acharya C, Hooker AC, Türkyılmaz GY, Jönsson S, Karlsson MO. A diagnostic tool for population models using non-compartmental analysis: The ncappc package for R. Comput Methods Programs Biomed. 2016;127:83–93.
128. Othman AA, Dutta S. Population pharmacokinetics of levodopa in subjects with advanced Parkinson's disease: levodopa-carbidopa intestinal gel infusion vs. oral tablets. Br J Clin Pharmacol. 2014;78:94–105.
129. Anderson BJ, Holford NHG. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol. 2008;48:303–332.
130. Ribbing J, Nyberg J, Caster O, Jonsson EN. The lasso--a novel method for predictive covariate model building in nonlinear mixed effects models. J Pharmacokinet Pharmacodyn. 2007;34:485–517.
131. Haem E, Harling K, Ayatollahi SMT, Zare N, Karlsson MO. Adjusted adaptive Lasso for covariate model-building in nonlinear mixed-effect pharmacokinetic models. J Pharmacokinet Pharmacodyn. 2017;44:55–66.
132. Keizer RJ, Karlsson MO, Hooker A. Modeling and Simulation Workbench for NONMEM: Tutorial on Pirana, PsN, and Xpose. CPT Pharmacomet Syst Pharmacol. 2013;2:6:1-9.
133. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J. 2011;13:143–151.
134. Dosne A-G, Bergstrand M, Harling K, Karlsson MO. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. J Pharmacokinet Pharmacodyn. 2016;43:583–596.
135. The Unified Parkinson's Disease Rating Scale (UPDRS): Status and recommendations. Mov Disord. 2003;18:738–750.

136. Eisenberg DP, Kohn PD, Hegarty CE, et al. Common Variation in the DOPA Decarboxylase (DDC) Gene and Human Striatal DDC Activity In Vivo. Neuropsychopharmacol. 2016;41:2303–2308.
137. Stacy MA, Murphy JM, Greeley DR, Stewart RM, Murck H, Meng X. The sensitivity and specificity of the 9-item Wearing-off Questionnaire. Parkinsonism Relat Disord. 2008;14:205–212.
138. Robertson DR, Renwick AG, Macklin B, et al. The influence of levodopa on gastric emptying in healthy elderly volunteers. Eur J Clin Pharmacol. 1992;42:409–412.
139. Baruzzi A, Contin M, Riva R, et al. Influence of meal ingestion time on pharmacokinetics of orally administered levodopa in parkinsonian patients. Clin Neuropharmacol. 1987;10:527–537.
140. Heikkinen H, Nutt JG, LeWitt PA, Koller WC, Gordin A. The effects of different repeated doses of entacapone on the pharmacokinetics of L-Dopa and on the clinical response to L-Dopa in Parkinson's disease. Clin Neuropharmacol. 2001;24:150–157.
141. Djaldetti R, Giladi N, Hassin-Baer S, Shabtai H, Melamed E. Pharmacokinetics of etilevodopa compared to levodopa in patients with Parkinson's disease: an open-label, randomized, crossover study. Clin Neuropharmacol. 2003;26:322–326.
142. Murata M, Kanazawa I. Effects of chronic levodopa therapy on dopa pharmacokinetics. Eur Neurol. 1997;38 Suppl 2:50–55.
143. Adamiak U, Kaldonska M, Klodowska-Duda G, et al. Pharmacokinetic-pharmacodynamic modeling of levodopa in patients with advanced Parkinson disease. Clin Neuropharmacol. 2010;33:135–141.
144. Contin M, Riva R, Martinelli P, Albani F, Baruzzi A. Effect of age on the pharmacokinetics of oral levodopa in patients with Parkinson's disease. Eur J Clin Pharmacol. 1991;41:463–466.
145. Jorga K, Banken L, Fotteler B, Snell P, Steimer JL. Population pharmacokinetics of levodopa in patients with Parkinson's disease treated with tolcapone. Clin Pharmacol Ther. 2000;67:610–620.
146. Senek M, Aquilonius S-M, Askmark H, et al. Levodopa/carbidopa microtablets in Parkinson's disease: a study of pharmacokinetics and blinded motor assessment. Eur J Clin Pharmacol. 2017;73:563–571.
147. Bennink R, Peeters M, Van den Maegdenbergh V, et al. Comparison of total and compartmental gastric emptying and antral motility between healthy men and women. Eur J Nucl Med. 1998;25:1293–1299.
148. Datz FL, Christian PE, Moore J. Gender-related differences in gastric emptying. J Nucl Med. 1987;28:1204–1207.
149. Colombo D, Abbruzzese G, Antonini A, et al. The "Gender Factor" in Wearing-Off among Patients with Parkinson's Disease: A Post Hoc Analysis of DEEP Study. Sci World J. 2015.
150. Volkmann J, Albanese A, Antonini A, et al. Selecting deep brain stimulation or infusion therapies in advanced Parkinson's disease: an evidence-based review. J Neurol. 2013, 260.11: 2701-2714.
151. Nyholm D, Klangemo K, Johansson A. Levodopa/carbidopa intestinal gel infusion long-term therapy in advanced Parkinson's disease. Eur J Neurol. 2012;19:1079–1085.

152. Hagan RM, Raxworthy MJ, Gulliver PA. Benserazide and carbidopa as substrates of catechol-O-methyltransferase: new mechanism of action in Parkinson's disease. Biochem Pharmacol. 1980;29:3123–3126.
153. Klostermann F. Intestinal levodopa infusion and COMT inhibition - a promising link. Eur J Neurol. 2012;19:795–796.
154. Alqahtani S, Kaddoumi A. Development of a physiologically based pharmacokinetic/pharmacodynamic model to identify mechanisms contributing to entacapone low bioavailability. Biopharm Drug Dispos. 2015;36:587–602.
155. Ingman K, Naukkarinen T, Vahteristo M, Korpela I, Kuoppamäki M, Ellmén J. The effect of different dosing regimens of levodopa/carbidopa/entacapone on plasma levodopa concentrations. Eur J Clin Pharmacol. 2012;68:281–289.
156. Müller T, Erdmann C, Muhlack S, et al. Pharmacokinetic behaviour of levodopa and 3-O-methyldopa after repeat administration of levodopa/carbidopa with and without entacapone in patients with Parkinson's disease. J Neural Transm Vienna Austria 1996. 2006;113:1441–1448.
157. Camargo SMR, Vuille-dit-Bille RN, Mariotta L, et al. The molecular mechanism of intestinal levodopa absorption and its possible implications for the treatment of Parkinson's disease. J Pharmacol Exp Ther. 2014;351:114–123.
158. Simon N, Viallet F, Boulamery A, Eusebio A, Gayraud D, Azulay J-P. A combined pharmacokinetic/pharmacodynamic model of levodopa motor response and dyskinesia in Parkinson's disease patients. Eur J Clin Pharmacol. 2016;72:423–430.
159. Destée A, Rérat K, Bourdeix I. Is there a difference between levodopa/dopa-decarboxylase inhibitor and entacapone and levodopa/dopa-decarboxylase inhibitor dose fractionation strategies in Parkinson's disease patients experiencing symptom re-emergence due to wearing-off? The Honeymoon Study. Eur Neurol. 2009;61:69–75.
160. Johansson D, Ericsson A, Johansson A, et al. Individualization of levodopa treatment using a microtablet dispenser and ambulatory accelerometry. CNS Neurosci Ther. 2018;1-9.
161. Nyholm D, Stepien V. Levodopa fractionation in Parkinson's disease. J Park Dis. 2014;4:89–96.
162. van Gilst MM, Bloem BR, Overeem S. "Sleep benefit" in Parkinson's disease: a systematic review. Parkinsonism Relat Disord. 2013;19:654–659.
163. Lotia M, Jankovic J. New and emerging medical therapies in Parkinson's disease. Expert Opin Pharmacother. 2016;17:895–909.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1437

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-343036

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2018