Symptomatic treatment of Parkinson’s disease:

The role of transdermal iontophoretic delivery of dopamine agonists
1 Introduction

Parkinson’s disease (Pd) is an age-related neurodegenerative disorder. Pharmacotherapy is the first line symptomatic treatment of this neurological disease [1]. Currently Levodopa is still considered the drug of first choice, but its suspected neurotoxicity and the induction of movement disability after chronic use demand for alternative therapies.

An attractive alternative is the use of (semi-)synthetic dopamine agonists. It has been suggested that continuous dopamine receptor stimulation is the best symptomatic treatment of Parkinson’s disease [2-4]. Therefore the administration of dopamine agonists in a continuous, well-controlled manner by transdermal iontophoresis is an attractive therapeutic strategy in the symptomatic treatment of Parkinson’s disease. In addition, by modulation of the current density and the donor concentration of the drug it will be possible to adjust the rate of administration to the requirements of the individual patient.

This thesis describes the investigation and optimization of the transdermal iontophoretic delivery of novel dopaminergic (pro)drugs for the symptomatic treatment of Parkinson’s disease. In this chapter, firstly the current strategy for the treatment of Pd is discussed. Secondly the role of transdermal iontophoretic delivery of dopamine agonists in the symptomatic treatment of Pd is addressed. Thirdly, the use of compartmental modeling in drug development for transdermal iontophoresis is reviewed.

2 Current strategies for the treatment of Parkinson’s disease

2.1 The condition

Parkinson’s disease, first described in 1817 as ‘shaking palsy’ by James Parkinson, is an age-related neurodegenerative movement disorder with a prevalence in Europe of 1.28-1.50 % in the age group above 60 [6]. This number will increase in the coming decades, since the life expectancy is augmenting and the ‘grey’ population is expanding. Pd has a world-wide distribution and has no gender preference [4]. The mean age of onset of Pd is about 60 years, but in case of young-onset Pd and juvenile-onset Pd, symptoms already may occur at the age of 21 to 40 years, and occasionally even earlier [2].

The clinical character of Pd is described by four cardinal features: resting tremor, rigidity, bradykinesia or slowness of movement, and gait disturbance with postural
instability. In addition Pd, mainly considered as a movement disorder, is often accompanied by nonmotor features including autonomic dysfunction, sleep disturbances, depression, addiction, dementia, speech problems, dysphagia, micrographia and hyposmia...[1, 3].

The causes of Pd are still not fully understood, but several studies on inherited parkinsonisms and studies with transgenic Pd-like animal models suggest that the aetiology of this disease lies within the complex interaction between multiple cellular factors, including genetic influences on cellular mechanism, together with exposure to environmental toxins and the unique vulnerability of the dopamine neurons in the substantia nigra [7]. The understanding of the involvement of genetic factors in the aetiology of Pd has been strengthened by the discovery of 13 genetic loci and 9 pathogenic genes. Their involvement in Parkinson’s disease, however, is not completely clear [8-10]. Moreover it is currently believed that only 5% of all cases of Parkinson’s disease have a genetic cause [1, 9]. Several environmental factors, especially long-term exposure to pesticides have been reported as risk factors for Pd. Furthermore an excess of vitamine E and viral and bacteriological infection have been associated with Pd, however proving evidence is still lacking [1]. Intriguingly smoking and intake of caffeine are believed to reduce the prevalence of the disease [11].

For many years it is known that the basal ganglia are involved in controlling and regulating voluntary movement. The basal ganglia, a group of subcortical nuclei, consist of the striatum (caudate and putamen), globus pallidus (interna and externa), substantia nigra (pars compacta and recticularis) and the subthalamic nucleus. Pathways from these nuclei form loops between the motor cortex and the motor thalamus. The first neurological hallmark of Pd is a degeneration of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNc) [12]. Therefore in patients with Pd the balance of the excitatory and inhibitory loops, which is modulated by the dopaminergic pathway from the substantia nigra, will be disturbed and will result via a pathway of overexcitation and excessive inhibition of the different basal nuclei in a reduced activity towards the motor cortex (Figure 1). As a consequence the conscious control of the skeletal muscles, controlled by the long axons of the neurons of the motor cortex, which project to the spinal cord, is disturbed. The second neurological feature of Pd is the presence of Lewy bodies, predominantly present in the brainstem. The exact role of these proteinaceous intracellular inclusions is currently under debate. It is unclear if Lewy bodies are a result of the disease or are involved in the cause of the pathology. It has been suggested that Lewy bodies are a results of a failed attempt to protect the damaged
neurons against certain toxins [13]. Furthermore excessive quantities of toxic α-synuclein inside Lewy bodies may disturb the normal cell physiology, resulting in cell death [3, 14-15]. Contrastingly Lewy bodies may act as simple biomarker for neuronal cell loss, not actively involved in the pathophysiology of Pd. Lewy bodies are also found in patients with other neurodegenerative disorders, including
Alzheimer disease, progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and dementia with Lewy Bodies (DLB).

The diagnosis of PD is performed clinically and based on careful history taking and physical examination. Currently there are neither laboratory tests nor imaging techniques that can confirm with 100% certainty the diagnosis. The ultimate diagnosis can only be performed post mortem [16]. It can be very difficult to distinguish idiopathic Parkinson’s disease from other certain neurological disorders, making the diagnosis of PD very challenging. It has been estimated that around 10% of the patients are misdiagnosed [1, 17-18]. Especially in the early stages a lot of overlap can be observed between the features of PD and other forms of parkinsonism. Since the proper diagnosis is essential for the prognosis and therapeutic treatment, the identification of a possible secondary cause of parkinsonian symptoms is essential. In practice 3 secondary causes of parkinsonian symptoms are considered for differentiation from PD: essential tremor, drug induced parkinsonism and Parkinson’s plus syndromes.

### 2.2 Current treatment of Parkinson’s disease

The therapeutic strategy of Parkinson’s disease is to minimize the effect of depletion of dopamine in the striatum. The two general approaches used in the clinic today for the symptomatic treatment of PD are surgery and pharmacotherapy.

#### 2.2.1 Surgical therapy

Because of the limitations of levodopa, improvements in stereotactic operative techniques, the use of microelectrode recording to more accurately define the target site, improved imaging and insights into the anatomical and physiological organization of the basal ganglia, surgery in the treatment of PD has gained more interest in the last few years [4, 19]. Overactivity in the subthalamic nucleus, the globus pallidus and the thalamus results in inappropriate activity from the thalamus to the motor cortex and consequently development of the symptoms of PD [1]. Two surgical approaches are currently applied to inactivate the particular region in the basal ganglia. Firstly ablation, a structural lesion in the brain, can provide some anti-dyskinetic benefits. Because of the irreversibility and the side effects (e.g. hemorrhage, damage to neighboring structures), associated with these techniques, physicians now prefer the second surgical approach, i.e. high-frequency deep brain stimulation (DBS). With this technique an electrode is implanted into the brain target. It is associated with marked benefits in reducing drug-induced motor fluctuations and dyskinesias. Furthermore a minimal morbidity is observed in
Parkinson patients that undergo this surgical procedure [20]. The use of DBS in patients with depression, dementia or other mental health problems is however not recommended. These therapies are only designed to compensate for damaged neuronal circuits and therefore used in advanced Pd [21].

The most recent evolution in surgery is the possible neuroprotective effect of cell based therapies and gene therapies. Cell based therapies aim to stimulate the production of intrinsic dopamine by transplanting dopamine-producing cells. Firstly, although the implantation of embryonic dopaminergic neurons has been proven to be successful in animal studies, up to now no double-blind trial has proven the benefit of cell based therapies over placebo [22-24]. Secondly though the use of stem cells may look promising, the research is still in an initial stage since stem cell transplantation in animal models did not show beneficial effects compared to fetal nigral transplantation [25-26]. Gene delivery, using viral vectors to introduce the desired protein DNA in the target region, is also actively being investigated as possible treatment for Pd [27]. In analogy to cell based therapies, no placebo controlled double-blind clinical trial, considered as the gold standard for evaluating a new therapy, can clearly confirm the safety and efficacy of gene therapy for the treatment of Pd [23, 28-29].

2.2.2 Pharmacological approach

As discussed above, surgery still compels certain risks and mostly is used only in the treatment of advanced Pd. This makes pharmacotherapy the first line treatment of Parkinson’s disease. As no curative therapy has yet been discovered, the pharmacotherapeutic strategy is the symptomatic treatment of the disease. Usually pharmacotherapy is initiated with levodopa (combined with benserazide or cardidopa) as such or in combination with dopamine agonists. Although levodopa is most effective and still considered as the gold standard, it remains associated with serious motor complications after long term use [30-31]. This emphasizes the need for alternative therapies to treat the symptoms of Pd, certainly in the early stage of the disease. Up to date the most attractive alternative class of drugs are the dopamine agonists, which activate directly the dopamine receptor. Dopamine agonists can be divided into ergot derivatives (bromocriptine, pergolide, lisuride, cabergoline) and non-ergot derivatives (apomorphine, ropinirole, pramipexol, rotigotine) based on their molecular structure. Dopamine agonists have shown their efficacy as monotherapy and as adjunctive therapy for patients using levodopa. Several trials have shown that dopamine agonists can reduce the 'off' time and reduce or delay the use of levodopa [1]. In early stages of Pd, another class of drugs, monoamine oxidase
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type (MAO-B) inhibitors, that stimulate the dopamine receptor indirectly by inhibiting the breakdown of dopamine, is also used. MAO-B inhibitors, such as selegiline, rasagiline and safinamide, can provide modest anti-parkinsonian effects and delay the use of levodopa [32-33]. Moreover this class of drugs gained more interest because of their possible, however not clinically proven, neuroprotection [34-36]. Less frequently used as monotherapy are catechol-O-methyltransferase (COMT) inhibitors, such as tolcapone and entecapone with the same working principle as MAO-B inhibitors. Finally because of their lack of efficacy and serious side effects, anticholinergics and amantadine are sparingly prescribed for PD [1]. As PD progresses, the need for therapy tailoring increases and becomes more challenging. Besides an increase in the severity of the symptoms, the management of the long term motor complications, caused by levodopa will play a central role in pharmacotherapy. For patients in an advanced state of Parkinson’s disease a combination therapy of levodopa with dopamine agonists, MAO-B inhibitors or COMT inhibitors is used. Since levodopa and dopamine agonists are still the first choice in the treatment of early and advanced PD, these compounds will be discussed in more detail in the next paragraphs.

2.3 Limitations of current pharmacotherapy

Since its introduction in the early 60’s levodopa, a precursor for dopamine, remains the ‘gold standard’ for the treatment of PD [37]. To prevent side effects (such as nausea and vomiting), because of the peripheral metabolism of levodopa, levodopa is administered in combination with a peripheral decarboxylase inhibitor such as carbidopa or benserazide [38]. As mentioned earlier, levodopa is excellent in minimizing the symptoms of PD and improves the activities of daily living, independence, employability and survival of the patient. However the use of levodopa has been put in question, because of its possible neurotoxicity and the induction of movement disorders after chronic long term use. Firstly in vitro toxicity studies showed that levodopa is able to degenerate cultured dopaminergic neurons, but in humans or animals no clear proof is given so far that levodopa is toxic [23, 39-40]. In some reports neuroprotection has even been suggested [40]. Secondly and more importantly it is generally believed that chronic treatment with L-dopa can cause dyskinesias and motor fluctuations, including a wearing off and on-off phenomenon in the later stages of the disease. 50-80 % of PD patients, who have received this drug for 5 to 10 years, experience these complications following long term use [30-31]. A recent study Early vs Later Levodopa Therapy in PD (ELLDOPA) showed that indeed higher doses result in greater clinical benefits, but pose a greater risk for developing motor complications [1, 23, 41]. These L-DOPA
associated complications are a source of severe disability for patients with Pd. Management of these motor complications is very difficult and requires individualized therapy [42]. Current strategy to manage these symptoms can be a combination of low and multiple doses of L-DOPA with or without a combination with dopamine agonists (DA) [42] or functional surgery [43]. Although dopamine agonist (e.g. bromocriptine, pergolide, cabergoline, pramipexole, ropinorole, rotigotine) may be less effective, these drugs are still less associated with motor complications after long term use when used as the initial treatment in early Pd [44-45]. This reduction in motor complication is associated with their increased half-lives and L-DOPA sparing effect.

2.4 Continuous dopaminergic stimulation

Under normal physiological conditions nigral neurons fire continuously, providing a constant level of dopamine in the striatum, resulting in a constant stimulation of the dopaminergic neurons [46-47]. In addition a re-uptake mechanism into pre-synaptic terminals functions as a buffer to external stimuli and ensures a constant extracellular dopamine concentration [46-47]. In Pd, there is a loss of nigral neurons and their striatal terminals that normally store and regulate the release of dopamine. As the disease progresses, the buffer capacity reduces and pulsatile stimulation of the dopamine receptors increases [47]. In addition to the pulsatile stimulation, the exposure to fluctuating levels of dopaminergic agents, is also believed to be responsible for the development of motor fluctuations [47-49]. This emphasizes the need for dopaminergic treatments that provide non-pulsatile stimulation and thus mimics the normal physiological state more closely. This therapeutic strategy is believed not only to reduce the dyskinesia and motor response disturbances, but also can provide a beneficial effect for other symptoms, like nocturnal disturbances [47].

2.5 Strategies for continuous dopaminergic stimulation

A continuous stimulation of dopamine receptors can be achieved by prolonging the activity of levodopa by modifying the release or the delivery of this short-acting dopaminergic agent. For this purpose oral sustained release formulations of levodopa Madopar HBS® and Sinemet CR®, were developed. These long-acting controlled release formulations showed improvement in activities of daily living of the patient, however not in controlling motor fluctuations [47, 50-51]. In addition the intake of small doses of a liquid formulation of levodopa/carbidopa improved the ‘on’-time without worsening the dyskinesia, but did not affect the pulsatile levodopa plasma concentration or the motor response fluctuations [52]. In contrast clinical trials
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showed an improvement in motor dysfunction after chronic IV infusion of levodopa [53]. A more recent small open-label clinical trial, in which continuous intravenous levodopa [54] was compared to oral administration, also shows a reduced risk of motor complications in the infusion group [55]. Besides IV infusion, duodenal infusion via a portable pump of low doses of levodopa ameliorates plasma fluctuations and dyskinesia with a satisfactory therapeutic window in advanced PD patients [51, 56].

Dopamine agonists in general have significant longer half-lives compared to levodopa [57]. Several studies in patients have discovered that DA are less likely to induce motor fluctuations after chronic treatment. In addition some studies have suggested a neuroprotective effect of DA [50, 58-59]. Therefore this class of drugs has gained more popularity over the recent years as alternative to or in combination with levodopa. In accordance to the levodopa treatment, the current strategy is to achieve continuous stimulation of the dopamine receptors to reduce development of motor fluctuations. This led to the development of long-acting therapeutic formulations of DA. For instance the slow-release formulation of a dopamine agonist, bromocriptine, showed promising efficacy but has not been widely used in Parkinson’s disease [51]. Furthermore ropinirole, formulated as extended release tablets, requires only one dose intake per day. The incidence of motor complications is reduced, while the adverse effects are comparable to other dopamine agonists [50, 60]. Although promising results were obtained with oral modified release formulations, other delivery routes were explored to achieve continuous drug delivery. Various routes of administration of apomorphine, a potent short acting dopamine agonist, have been investigated, but currently only subcutaneous infusion is used in clinical practice [61]. Apomorphine is useful in the treatment of acute and long term treatment of “off”-periods and reduces the levodopa intake [50, 61]. Similar results were obtained with subcutaneous infusion of lisuride, an ergot dopamine agonist [62]. Skin nodules, however, were present in most patients after long term use, making this administration route for R-apomorphine and lisuride less attractive [63].

In summary there is a need for new delivery strategies for the symptomatic treatment of PD that fulfill following requirements. Firstly, the drug delivery is continuous, resulting in continuous stimulation of the dopamine receptor. Secondly, the administration is non-invasive, reducing costs and inconvenience for the patient. Thirdly, the dose is easily adjustable to the demand of the therapy. Rapid dose changes are often required, certainly when initiating therapy. Fourthly, ideally the delivery device also includes a feedback system. During delivery, such a device also
monitors a relevant end-point/biomarker, which via a feedback system controls the drug delivery. As discussed earlier with oral delivery it is difficult to achieve a continuous administration. Moreover the aforementioned non-oral strategies to achieve a continuous delivery are invasive, cumbersome and inconvenient for the patient. This emphasizes the need for alternative non-invasive delivery methods that ideally can fulfill all the requirements stated in the previous paragraph.

3 Transdermal drug delivery of dopamine agonists

Transdermal iontophoretic delivery is an attractive alternative delivery technique to provide continuous delivery. Therefore transdermal iontophoretic delivery of dopamine agonist can be an interesting therapeutic strategy for the symptomatic treatment of Parkinson’s disease.

3.1 Skin structure and skin barrier function

Transdermal delivery is the administration of the drug via the skin into the systemic circulation. Compared to oral and parental delivery, transdermal delivery has certain distinct advantages: 1) it offers the possibility for non-invasive delivery 2) it circumvents the first pass metabolism 3) this administration route can be beneficial for drugs that are sensitive to gastrointestinal degradation 4) transdermal delivery can be useful for therapeutics that are poorly absorbed via the GI tract, due to their physicochemical properties or due to disease progression 5) it can provide a sustained delivery during a longer period of time, increasing the applicability of the technique 6) it can deliver drugs with a zero order mass input, reducing plasma fluctuations.

However the barrier function of the skin challenges the design of transdermal therapeutic systems and limits the number of potential drug candidates for this administration route. The skin is the largest organ of the human body, comprises about 10% of the body mass and has a surface area of around 1.5 to 2.0 m² [64]. A typical square centimeter comprises 10 hair follicles, 12 nerves, 15 sebaceous glands, 100 sweat glands, 3 blood vessels with 92 cm total length, 360 cm of nerves and 3x10⁶ cells [65]. Two distinctive tissue layers can be found in human skin. The most upper layer is the stratified, avascular, cellular epidermis with the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum from the inner to the outermost layer. The epidermis is supported by a much thicker layer, the dermis. This layer consists of a matrix of connective tissue which is embedded in an amorphous substance of mucopolysaccharide. Blood vessels, nerves and
lymphatic vessels cross this matrix and skin appendages (sweat glands and pilosebaceous units) penetrate it. The lower side of the dermis is connected with the subcutaneous fat. Human skin displays two main types. The first type, hairy skin, encloses follicles and sebaceous glands, however no encapsulated sense organs. The second type, glabrous skin of the palms and the soles, consists mainly of thick epidermis with a compact and thick stratum corneum [65]. The stratum corneum, the outermost layer of the skin, is very lipophilic and forms the main barrier, protecting the body from water loss and penetration of exogenous agents. The stratum corneum or horny layer consists of corneocytes embedded in a highly organized lipid matrix.
in a brick and mortar like structure [66]. Both the corneocytes and the lipid matrix are important for the transport of drugs across the stratum corneum [67].

3.2 Passive diffusion

3.2.1 Basic concepts

Different transport routes have been proposed for passive penetration of molecules through the stratum corneum, a thin (10-20 μm), relative impermeable layer, which provides the rate limiting step for percutaneous penetration. Several macroroutes for penetration from the surface of the human skin into the subepidermal tissue are displayed in Figure 2 [5, 65, 68]: 1) via the sweat ducts 2) through the hair follicles with their associated sebaceous glands or 3) across the continuous stratum corneum between these appendages. It is generally believed that transport of most small lipophilic molecules occurs mostly via the latter pathway. This pathway can be subdivided into the transcellular route, though the corneocytes and lipid lamellae, and the intercellular pathway, solely through the lipid regions [65, 67]. For larger hydrophilic molecules and ions the appendegeal route may play also a significant role [68].

3.2.2 Dopamine agonists

Due to the difficult permeability properties of the skin, the choice of potential candidates of dopamine agonists for passive transdermal delivery is limited. In order to penetrate the skin, the permeant should be potent, since the relative bioavailability is low and should have specific physicochemical properties: moderate hydrophilicity and low molecular weight (<500 Mw). Despite these restrictions several molecules have been investigated for the symptomatic treatment of Parkinson’s disease with transdermal passive delivery. All the DA of which the feasibility of (passive and/or iontophoretic) transdermal delivery has been investigated together with the structure of levodopa are depicted in Figure 3.

In the 1980’s naxagolide was identified as a very potent dopamine agonist and a potential candidate for transdermal delivery due to its moderate lipophilic nature. Despite the very promising results that were obtained in vitro and later in vivo in primates and humans [69-70], the further development was discontinued because of the lack of efficacy as monotherapy and concerns about the toxicity [71]. These results encouraged further investigations with other potential candidates.

A number of gel formulations for bromocriptine, an ergot derived dopamine agonist, were developed and transdermal delivery was compared with oral delivery in rabbits.
It was observed that a gel, formulated with chitosan showed similar plasma concentration profile as with a commercially available tablet [72]. An enhancement in transdermal transport in vitro of pergolide, another ergot derived dopamine agonist, was obtained using elastic vesicles, but further investigation in vivo is necessary [73]. Transdermal delivery of a third ergot derivative dopamine agonist, lisuride showed promising efficacy as add-on therapy for PD. Axxonis Pharma, a pharmaceutical company in Germany, recently submitted a European marketing authorization application for the transdermal patch and subcutaneous infusion of lisuride [50, 74]. The non-ergot derivatives, such as piribedil, apomorphine and rotigotine do not cause serious fibrotic reactions, associated with the use of the older ergot-derived compounds, described above. For this reason the non-ergot derivatives are the preferred dopamine agonists to commence therapy, especially in younger patients [1]. A randomized double blind clinical study, including 72 patients with PD, failed however to show clinical efficacy of transdermal delivery of piribedil, explained by low plasma concentrations [75]. However one year later a study was published on transdermal piribedil that demonstrated, a long-lasting effect (reversal of motor deficits) in MPTP-treated common marmosets [76].

Apomorphine is regarded as a potent dopamine agonist, since its introduction for the treatment for PD in 1951. Different administration routes have been explored for this D1/D2 dopamine agonist, including transdermal delivery [1]. The transdermal delivery of apomorphine, formulated as a hydroxyl-propyl-methyl-cellulose gel and formulated in microemulsions was investigated in rabbits and mice, respectively. Both studies reported sufficient bioavailability and suggested potential use in humans [77-78]. One of the two microemulsions, used for the in vivo study in mice, was applied transdermally in 21 patients with PD. The transdermal delivery of this micro-emulsion, containing apomorphine, provided a sustained release of apomorphine, resulting in stable therapeutic plasma levels and reduction of off periods [79]. However the clinical efficacy and tolerability has not been investigated in a placebo controlled double blind study.

Another potent dopamine agonist rotigotine, the (-)-enantiomer of the aminotetralin derivative, 2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin, is the first transdermal delivery system (Neupro®) approved by the regulatory authorities for the symptomatic treatment of all stages of PD in Europe and of early-stage PD in USA [80]. Due to drug stability (crystallization inside the patch), compromising the bioavailability, the EMEA limited the supply of rotigotine and the FDA asked professionals and patients for a recall of Neupro® [80]. After adjustments concerning storage conditions, Neupro® is since June 2009 available in Europe for symptomatic
Figure 3: The chemical structures and Mw of levodopa and ergot and non-ergot dopamine agonists, that were investigated for administration with passive and/or iontophoretic transdermal delivery for the symptomatic treatment of PD.
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treatment of Pd. In the USA up to the present time Neupro is not yet available on the market. In addition to approval for the symptomatic treatment of Pd, Neupro® has been approved for the treatment of moderate to severe restless leg syndrome in adult patients [81]. Patches releasing 1, 2, 4, 6, 8 mg of rotigotine during 24 h are available [82]. In 63 patients with early Pd after repeated daily administration of 8 mg/24h stable steady-state plasma concentrations were observed [83] and animal studies demonstrated that continuous plasma concentrations translate into continuous dopamine stimulation [47, 84]. Three large-scale placebo-controlled phase III trials investigated the efficacy of transdermal delivery of rotigotine as mono-therapy for early stage Pd. All three trials showed an improvement in UPDRS (unified Parkinson’s disease rating scale)-ADL (activities daily living)-Motor subscores in baseline vs placebo [80, 85-87]. In addition the first phase III clinical trial reported that response reached a plateau when delivering between 6 and 8 mg/24h. This was the base to set the maximum approved dose as monotherapy in the USA and Europe to 6 and 8 mg rotigotine per 24h, respectively [80]. Furthermore a subgroup analysis in the second trial showed that the efficacy is independent of gender, age, disease severity and disease duration [80]. Finally the third trial, which compared transdermal rotigotine vs ropinirole over a 37-week period, reported that ropinirole resulted in a better symptomatic effect than rotigotine, which could be explained by possible under-dosing of the patients receiving rotigotine, compared to ropinirole [87]. Two large phase III trials (PREFER and CLEOPATRA PD-study) investigated the efficacy of transdermal rotigotine as adjunct therapy to L-dopa in advanced Pd [80, 88-89]. The phase III trials showed a significant improvement in the UPDRS-Motor score and one phase III trial did not show any reduction in the required L-dopa dose. However both phase III trials showed a significant reduction in daily off time and an increase in on time without dyskinesia after waking for rotigotine vs placebo [88-89]. Thus as monotherapy for early stage Pd and as adjunct therapy for advanced stage Pd, transdermal rotigotine has been proven to be efficacious and useful. In addition, continuous dopamine stimulation has been suggested to contribute to the prevention of motor complications later in the disease course. Moreover dopamine agonists have been less associated with motor complication than levodopa, emphasizing the potential of transdermal rotigotine to play an important role in the symptomatic treatment of Pd.

Although the passive delivery of dopamine agonist has great potential, investigations to improve the transdermal delivery are required to increase the delivery rate and to expand the number of potential candidates for continuous delivery via the skin. The first major approach to overcome the skin barrier and to improve transdermal
delivery is the use of chemical enhancers, such as azone, glycols, terpenes etc. They facilitate stratum corneum transport by interaction with the lipids in the skin and/or increase partitioning [90]. A second approach is the use of particulate systems (e.g. nano-particles, vesicles, liposomes). These systems can increase the skin transport by improving drug solubilization in the formulation, drug partitioning into the skin and skin permeability [91]. The use of chemical enhancers and particulate systems can cause skin irritation and therefore limit the number and concentration (in formulation) of enhancers used [90]. Finally a third approach for permeation increase is the use of physical enhancement methods, such as sonophoresis (ultrasound), electroporation, microneedles thermal ablation, microdermabrasion and iontophoresis [91]. Whereas ultrasound, microneedles and thermal ablation show potential for delivery of macromolecules, such as vaccines and therapeutic proteins [91], iontophoresis is promising method for the delivery of small and moderate hydrophilic molecules, such as dopamine agonists.

3.3 Iontophoresis

3.3.1 Basic concepts

Transdermal iontophoresis is one of the promising alternative techniques for non-invasive continuous delivery of dopamine agonists. By applying a small electrical current (≤ 50 μA.cm⁻²) across the skin it is possible to enhance the transdermal delivery of small ionized therapeutic agents as illustrated in Figure 4. Besides a continuous administration and increased bioavailability, a particular advantage of iontophoresis is the possibility to adjust the delivery rate to the demand of the therapy. Especially in symptomatic treatment of PD this can be very important, since individual titration is often required when administering dopaminergic agents. Three transport mechanisms are involved in transdermal iontophoretic delivery: 1) passive diffusion, resulting from a concentration gradient between the patch and the systemic circulation 2) the electromigration which is the result from the current flow from the anode (+ electrode) to the cathode (- electrode) across the skin and 3) electroosmosis, which can be attributed to the current induced solvent flow across the skin in the counter direction of the skin charge [92]. For small charged molecules the contribution of the passive flow is often negligible, making electromigration and electroosmosis the principle transport mechanisms with a dominating transport due to electromigration. With increasing molecular size, however, the contribution of the electroosmosis increases and may be really important for large peptides and proteins [92].
In addition for uncharged molecules electroosmosis is considered to be the only transporting mechanism involved in transdermal iontophoresis. Therefore marker molecules, such as mannitol and acetaminophen, are used to quantify the electroosmotic contribution during iontophoretic transport of various molecules. In the last few years acetaminophen has gained more interest over $^{14}$C-mannitol, because of its practical advantages [93-97].

Current application across the skin will drive the molecules through the pathway of least electrical resistance. In case of iontophoresis of the three suggested penetration routes (transcellular, intercellular and transappendageal), transport is reported to occur primarily via the intercellular and transappendageal route (Figure 2). The major contribution is via the appendages in the skin, including hair follicles and sweat glands [98]. In addition ions can also migrate via the damaged structures in the skin [90]. These penetration pathways can exist in parallel [65], as was suggested for the percutaneous penetration of methothrexate [99]. The relative contribution of the different pathways is presumably dependent on the physicochemical properties of the permeant. For instance Jadoul et al. showed that fentanyl, a relatively lipophilic molecule, was distributed across the whole human stratum corneum (HSC), while the more hydrophilic thyrotropin releasing hormone was mainly localized in the pores after iontophoresis [100]. Confocal laser scanning microscopy studies with calcein, a

![Figure 4: Iontophoresis set-up in vivo with the transport of positively charged ions across the skin during current application](image-url)
charged hydrophilic dye and nile red, a neutral lipophilic dye, confirmed that the more lipophilic compound is transported via the lipid-filled intercellular regions of the stratum corneum, while iontophoresis enhanced the transcutaneous transport of calcein mainly in the follicular structures [101].

Up to today 2 iontophoretic delivery systems made it to the market. In 2007 EMEA approved IONSYS®, manufactured by ALZA corporation, a credit card system delivering fentanyl.HCl for analgesia [102]. However because of safety issues Janssen-Cilag withdrew IONSYS® voluntary from the market and as a consequence the EMEA suspended its registration [103]. Currently the only commercialized iontophoresis system on the market is Lidosite® from Vyteris, delivering the anesthetic lidocaine.HCl. Lidosite® can be applied for local analgesia prior to blood sampling, venipunctures or small dermatological procedures [104].

Instead of delivery of molecules, it is also possible with iontophoresis to extract molecules from the skin. Therefore so-called ‘reverse iontophoresis’ has been explored in the last two decades to monitor subdermal levels of several molecules like lithium [105], phenytoin [106], valproate [107], lactate [108], urea [109], amino acids [110] and glucose [111]. The GlucoWatch® Biographer (Johnson & Johnson, New Brunswick, NJ) was approved in 2002 by the FDA to monitor the glycaemia for up to 12h [111]. These results show the potential of reverse iontophoresis to monitor different molecules subdermally. By monitoring a relevant biomarker it may even be possible to design a feedback system that can control the delivery of the therapeutic drug.

3.3.2 Dopamine agonists

Transdermal iontophoresis of various non-ergot dopamine agonists have been studied in vitro and in vivo for symptomatic treatment of Pd. Several studies on the transdermal iontophoretic delivery of R-apomorphine have been reported. After promising results in vitro across human skin [112], in small scale clinical studies apomorphine was applied using transdermal iontophoresis in patients with Pd. After application of 2 different current densities (250 and 375 μA.cm⁻²) for 1 hour, in all patients measurable plasma concentrations were observed. However these plasma levels were subtherapeutic in all patients, except one [113]. Li et al. showed an improvement of the transdermal iontophoretic delivery of this dopamine agonist after non-occlusive pretreatment of the skin with a surfactant formulations [114]. A 2-fold and 1.4 fold increase in flux was observed in studies across HSC and dermatomed human skin (DHS), respectively [115]. These findings led to further exploration of the feasibility of the transdermal iontophoretic delivery of apomorphine in a clinical
study including 16 patients. 2 groups of patients were compared receiving R-
apomorphine using transdermal iontophoresis (250 μA.cm⁻², 3 hours) with or without
pretreatment of with the surfactant formulation, respectively. With a similar
enhancement ratio (1.3 times) as was observed for transport across DHS, the
surfactant pretreatment increased the iontophoretic delivery. In this study 62.5% and
37.5% respectively, of the surfactant pretreated and control group, reported clinical
improvement [116].

The feasibility of transdermal iontophoretic administration of ropinirole was
explored, because of its suitable fysicochemical properties (Mw: 260.4 g.mol⁻¹; pKa:
9.68 and 12.43; logP=3.32) [117]. Studies, investigating in vitro transport across full
piglet skin in the absence of competing co-ions, showed that the flux of ropinirole
was independent of the donor concentration, however linearly dependent of the
current density [118]. In a follow-up study with hairless rats, similar plasma profiles
were observed, despite the 10-fold difference in donor concentration. The absence of
competing co-ions results in a constant flux, independent of the donor concentration
and dependent on the mobility of the drug, the skin diffusivity and the counterion in
the skin (mainly Cl⁻). The authors concluded that, based on the in vitro flux and in vivo
plasma concentrations, therapeutic levels of ropinirole in humans can be
achieved with transdermal iontophoresis, providing an alternative administration
route for the drug, as a tablet available [117].

Finally several studies were conducted to investigate the iontophoretic delivery of 2
dopamine agonist of the 2-aminotetralin group. Most dopamine agonists of this
group are shown to be very potent [119-125]. In addition the low molecular weight,
the chargeability of the nitrogengroup and the moderate lipophilicity, make several
members of the 2-aminotetralingroup interesting candidates for transdermal
iontophoresis. Therefore Nugroho et al. studied the transdermal iontophoretic
delivery of rotigotine.HCl (Mw: 351.9 g.mol⁻¹; pKa (nitrogen): 7.9; logP: 4.03) and
of 5-OH-DPAT.HBr (Mw: 328.3 g.mol⁻¹; pKa (nitrogen): 8.12; logP: 2.19) [126-
127]. In vitro transdermal iontophoresis studies of the potent dopamine receptor
agonist rotigotine, currently on the market as passive delivery system (cf. supra),
were performed. Increasing the pH of the donor phase increases the flux, doubling
the NaCl concentration reduces the flux and lowering the pH of the acceptor phase
does not affect the flux. Furthermore electroosmosis contributed only 2% to the total
flux [128]. In a follow up assay it was shown that in the concentration range tested,
the flux was linearly dependent on the donor concentration and the current density.
Replacing HSC by DHS reduced, while higher temperatures increased it [126].
Although the maximum flux of 80 nmol.cm⁻².h⁻¹ is considered to be sufficient for
therapeutic levels, the limited solubility of this compound prevented the researchers to further increase the iontophoretic delivery.

The second 2-aminotetralin investigated was 5-hydroxy-2-(N,N-di-n-propylamino)tetralin (5-OH-DPAT). This dopamine agonist has similar structure and potency as rotigotine [127]. The absence of the thienylgroup in the side chain of 5-OH-DPAT makes this compound less lipophilic, which could lead to increased solubility and a faster transdermal transport because of less retardation in the lipophilic environment of the skin. In vitro studies showed a higher flux for 5-OH-DPAT, compared to rotigotine. The flux was linearly dependent on the concentration in the given concentration range (0-7 mM) and on the current density. Increasing the salt concentration from 0.07 M to 0.14 M dramatically decreased the flux with almost 10 fold. In analogy to rotigotine, transport across DHS was less compared to transport across HSC [127]. Although the in vitro transport of this potent dopamine agonist has not yet been fully characterized, these results showed an improved transport compared to rotigotine. Therefore the authors continued with 5-OH-DPAT to explore the potential of transdermal iontophoresis in a series of in vivo studies. The pharmacodynamic effect of 5-OH-DPAT was investigated in an anaesthetized rat model with the dopamine level as pharmacodynamic end-point, measured by online microdialysis. Administration of 5-OH-DPAT with transdermal iontophoresis during 3 hours using a current density of 250 μA.cm⁻² resulted in a strong and long lasting effect, which was suggested to persist for 24 hours post-iontophoresis [129].

In conclusion transdermal iontophoresis has great potential as novel delivery strategy for symptomatic treatment of Pd. Firstly, the results presented in this section show that this non-invasive delivery technique can provide continuous delivery that can be controlled mainly by donor concentration and current density. This makes iontophoresis a flexible delivery technique, enabling rapid titration of drug delivery. Secondly, several dopamine agonists are successfully administered with transdermal iontophoresis in vitro, in animals and even in patients. Thirdly, monitoring biomarkers with reverse iontophoresis opens the door to design a feedback system to control drug delivery. Despite these promising results there is still a need for improvement in delivery efficiency. In addition a better understanding of the different transport mechanisms involved in iontophoretic delivery will also contribute to improve drug delivery. This demands for further optimization of the iontophoretic delivery of previously investigated DA and for further exploration of
novel potent dopaminergic drugs with the desired physicochemical properties for transdermal iontophoresis.

4 Modeling transdermal iontophoretic delivery in vitro and in vivo

Describing the profile of a drug in different parts of the body (e.g. plasma, urine, brain, skin,…) during and after administration with various delivery techniques has been a challenge in drug development for many years. The complexity of the skin as biological membrane makes mathematical modeling of transdermal (iontophoretic) delivery difficult. Most of the models found in literature focus on the transport mechanisms during transport of the molecules [93, 130-141]. In some of these reports a correlation was suggested between the physicochemical properties (pKa, electrophoretic mobility, lipophilicity, Mw,…) of the solutes and the corresponding transport efficiency [93, 130, 132, 134-135, 139-141]. One of the limitations of these physicochemical property-transport relationships is that they are based on a single parameter to describe the transdermal iontophoretic transport. With this approach the information on the shape of the iontophoretic flux curve is neglected, which makes extrapolation from in vitro experiments towards the in vivo situation more difficult. However, very little research has been performed on development of models describing the whole transport profile during iontophoresis in vitro and in vivo. Most of the models used to describe the plasma concentration following transdermal iontophoresis were based on a zero order mass input from the donor solution into the systemic circulation in analogy to intravenous infusion [142-144]. However this assumes that immediate steady state conditions would apply after turning on the current. But the development of a steady state takes time, because of the barrier function of this biological membrane. This is clearly demonstrated by the profiles that have been published [112-113, 116, 129, 145-148].

To account for the change of input rate during iontophoresis before reaching steady state more complex kinetic models have been developed by Nugroho et al. [147-148]. In these models the rate of mass transfer (I₀) from the patch into the skin during iontophoresis is assumed to be constant due to the iontophoretic driving force. Furthermore it is believed that the release from the skin into the acceptor solution (in vitro) and in the systemic circulation (in vivo) is according to a first order process with a release constant Kᵣ. This approach was successfully applied to describe the iontophoretic transport in vitro [147] and in vivo [148] during and also after current application. The proposed in vivo pharmacokinetic (PK) models were combined with a pharmacological model (indirect response type I) to analyze the pharmacokinetics
and pharmacodynamics (PD) of 5-OH-DPAT following transdermal iontophoresis. Besides adequately describing the plasma concentration and the pharmacodynamic effect in rats, it was shown that based on results from in vitro transport studies across rat stratum corneum and dermatomed rat skin it was possible to predict the plasma profile as well as the dopaminergic effect [129].

These results illustrate several strong benefits of compartmental modeling. Firstly an integrated approach makes it possible to combine data from several experiments, facilitating analysis of different experiments. Secondly compartmental modeling can be helpful in understanding the different mechanisms involved in iontophoretic transport. In addition the establishment of a PK-PD relationship gives more insight in the causal path from blood to the pharmacodynamic effect. Thirdly a robust in vitro-in vivo correlation can be very useful for screening newly developed compounds and can be a powerful tool to design new assays at various stages of drug development. To improve screening a next step would be the identification of the relationships between the molecular and physicochemical properties and the different transport parameters, which can offer a base for selecting potential candidates.

In conclusion the compartmental modeling approach can reduce, refine, and maybe even replace in vivo studies, which are often very costly and time consuming certainly at later stages of drug development.
Role of transdermal iontophoretic delivery of dopamine agonists

References

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