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Transdermal iontophoresis of dopaminergic (pro) drugs : from formulation to in vivo application

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8

Summary, conclusions and future perspectives

1 Summary

For more than 45 years orally dosed levodopa (L-DOPA) has been regarded as the gold standard therapy for symptomatic treatment of Parkinson's disease (Pd) [1]. However its possible neurotoxicity and more importantly the induction of movement disorders after chronic use of L-DOPA, demand for alternative therapies [2]. One of the most important alternatives is the use of dopamine agonists, such as apomorphine, ropinorole, rotigotine and 5-OH-DPAT, which directly activate the post synaptic receptor [3]. Dopamine agonists are generally less associated with motor complications after long term use [4-5]. In addition continuous stimulation of the dopamine receptors is believed not only to reduce dyskinesia and motor complications, but also can be beneficial for other non-motor symptoms of Pd [6]. This emphasizes the need for controlled delivery that provides continuous dopaminergic stimulation. An attractive non-invasive delivery technique is transdermal iontophoresis.

By application of a low current across the skin it is possible to enhance the delivery of small charged molecules. At steady state conditions a constant delivery can be provided. In addition by modulation of the current density it is possible to adjust the administration rate and thus the dose to the demand of the therapy [7]. This can be of great benefit in early stage Pd, because initiation of therapy demands rapid dose adjustments. Therefore in the last decade the transdermal iontophoretic delivery of several dopamine agonists has been investigated: apomorphine, ropinirole, 5-OH-DPAT and rotigotine. These non-ergot dopamine agonists showed promising results *in vitro* and *in vivo*, demonstrating the feasibility of transdermal iontophoretic delivery of dopamine agonist for the symptomatic treatment of Parkinson's disease, as discussed in **CHAPTER 1**. But further optimization and improvement of the iontophoretic delivery of dopamine agonist is required to achieve a strong therapeutic effect. Higher transport efficiency can also lead to a reduction of the patch size and minimization of the applied current density. This can merely enhance the clinical applicability of transdermal iontophoresis.

The general objective of the presented research is the optimization of the transdermal iontophoretic delivery of dopamine agonists and its prodrugs for the symptomatic treatment of Parkinson's disease. To achieve this goal, the following specific items are considered:

1. Optimizing the transdermal iontophoretic delivery *in vitro* of a series of potent dopamine agonists, including rotigotine, 5-OH-DPAT and other potent

structural analogs. In addition the mechanisms driving the iontophoretic transport are addressed.

2. Synthesis of novel dopaminergic prodrugs of 5-OH-DPAT with an extra chargeable group for transdermal iontophoretic delivery. Subsequently the chemical and enzymatic stability are examined. This part of the investigations was performed by Jeroen De Graan and will result in a dissertation at the Rijks Universiteit Groningen, Groningen, The Netherlands
3. *In vitro* iontophoresis of selected prodrugs of 5-OH-DPAT to investigate the feasibility for transdermal iontophoretic delivery
4. Pharmacokinetic-pharmacodynamic (PK-PD) studies in an animal model with the most promising candidates with a special focus on controlling the plasma concentration and the pharmacodynamic effect with transdermal iontophoresis
5. Evaluation and optimization of the kinetic models, developed by Nugroho *et al.* for the characterization of transdermal iontophoretic transport *in vitro* and *in vivo* [8-10]

Part I-Optimizing the transdermal iontophoretic delivery of dopaminergic drugs *in vitro*

Previous studies showed the feasibility of transdermal iontophoretic delivery of 5-OH-DPAT *in vitro* [11] and *in vivo* [10]. But a thorough mechanistic characterization is still missing. Elucidating transport mechanisms driving the iontophoretic delivery is crucial to understand and optimize the iontophoretic delivery *in vivo*.

In **CHAPTER 3**, the transdermal iontophoretic delivery *in vitro* of 5-OH-DPAT was investigated from a mechanistic point of view. Especially the electroosmotic flow and the influence of the composition of the donor and acceptor phase were addressed. Acetaminophen as marker for the electroosmotic flow was also evaluated and approved in this chapter. The total flux is the sum of the passive, electroosmotic and electromigrative flux. The contribution of the passive flux was negligible. The main driving force for the iontophoretic delivery of 5-OH-DPAT is electromigration. Electromigration contributes for more than 85% to the total flux. Furthermore decreasing the pH of the donor phase from 6.0 to 5.0 did not affect the total flux at steady state (Flux_{ss}) situation. The Flux_{ss} increased 2.5- to 3-fold when the amount of Na^+ in the donor phase decreased from 78 mM to 10 mM. The Flux_{ss} showed a non-linear hyperbolic correlation with the donor concentration of 5-OH-DPAT and

reached a plateau towards the maximum solubility of 5-OH-DPAT in the donor phase. Together with the observed linear correlation between the Flux_{ss} and the current density, this shows that the administered dose can easily be adjusted with transdermal iontophoresis by changing the donor concentration and/or the current density.

The flux of 5-OH-DPAT decreased with decreasing pH of the acceptor phase from 7.4 to 6.2. This phenomenon was explained by an alteration of the permselective properties of the skin. At pH 6.2 the skin is less negatively charged, resulting in a reduced electroosmotic flow and increased competition of the counterions (mainly Cl^- ion) from acceptor to donor phase. This comprehensive study demonstrates the high transport efficiency of 5-OH-DPAT and elucidates different transport mechanisms during iontophoretic delivery.

A structurally related compound, rotigotine, is the first dopamine agonist in a passive patch (Neupro[®], UCB pharma) that is on the market in Europe for early and advanced Parkinson's disease [12-13]. *In vitro* investigations of rotigotine.HCl showed that with transdermal iontophoresis a higher transport rate with a shorter lag time can be achieved compared to passive delivery [14]. However in follow up studies, the maximum solubility in the donor phase was a limiting step to further enhance the iontophoretic transport [15].

One of the possibilities to change the physicochemical properties of a compound is the use of a different salt form. Therefore, in **CHAPTER 4**, passive and transdermal iontophoretic studies were conducted with a different salt form of rotigotine, rotigotine. H_3PO_4 . The results were compared to those obtained for rotigotine.HCl [14-15]. Prior to the transport studies, solubility studies showed that the solubility of rotigotine. H_3PO_4 was 2, 6 and 10-fold higher than the solubility of the HCl-salt at pH 6.0, 5.0 and 4.0, respectively. Moreover, because of the absence of a common ion effect, the amount of NaCl did not affect the maximum solubility in the donor phase. At low donor concentrations the transdermal passive and iontophoretic flux of rotigotine. H_3PO_4 and rotigotine.HCl were not significantly different. Due to the increase in maximum solubility of rotigotine the maximal iontophoretic transdermal transport of this compound however was almost twice when using rotigotine. H_3PO_4 ($135.8 \pm 12.5 \text{ nmol.cm}^{-2}.\text{h}^{-1}$) instead of rotigotine.HCl ($80.2 \pm 14.4 \text{ nmol.cm}^{-2}.\text{h}^{-1}$).

Subsequently, the potential of the iontophoretic delivery of rotigotine *in vivo* was evaluated in a series of simulations. In a first step the transport parameters of the iontophoretic delivery *in vitro* across human stratum corneum (HSC) were estimated using the compartmental kinetic models proposed by Nugroho *et al.* [8]. These

models were based on the assumption of a zero-order mass transfer from the patch to the skin and a first order release from the skin to the acceptor phase (*in vitro*) or the systemic circulation (*in vivo*). The transport parameters were the zero-order mass input (I_0), the skin release constant (K_R) and the lag time [16]. The kinetic model described the *in vitro* flux adequately. In the next step the apparent pharmacokinetic parameters, reported in literature [17-21], were combined with the estimated transport parameters to simulate the plasma levels *in vivo*. It was assumed that iontophoretic transport *in vivo* is the same as iontophoretic transport *in vitro* across HSC. The simulations demonstrated two important benefits of iontophoretic over passive transdermal delivery. Firstly, the onset time to achieve the desired level can be significantly reduced. Secondly, the plasma concentration can easily be titrated by modification of the current density to obtain a desired profile.

Next to 5-OH-DPAT and rotigotine, also other members of the 2-aminotetraline group and structural analogs are regarded as potent agonists at the D₁- and/or D₂-receptor in the striatum. 5 candidates were selected based on their potency and molecular structure: 8-OH-DPAC, 5-OH-EPAT, 5-OH-MPAT, 5,6-di-OH-MPAT and 5,6-di-OH-DPAT [22-28]. Except for 8-OH-DPAC, which is a chromanamine, all compounds are 2-aminotetralins. In **CHAPTER 5** the following properties of these 5 therapeutic agents were investigated: solubility, electrophoretic mobility, lipophilicity and *in vitro* transport across HSC and dermatomed human skin (DHS). The results of rotigotine and 5-OH-DPAT, adapted from literature, were added for comparison. Solubility studies ranked these molecules in the following order: rotigotine < 5,6-di-OH-DPAT < 5-OH-DPAT < 5-OH-MPAT < 5-OH-EPAT < 8-OH-DPAC. *In vitro* transport studies demonstrated that small structural changes can affect the iontophoretic transport significantly. Across HSC and DHS 5-OH-EPAT and 5-OH-MPAT showed a significant higher flux, compared to the other compounds investigated.

During current application the observed flux of all compounds did not reach steady state but gradually increased. Therefore to describe the *in vitro* iontophoretic transport a novel kinetic model was introduced. Instead of 1 first order release constant (K_R) to describe the release from the skin to the acceptor phase, as was proposed previously [8], 2 release constants (K_{R1} and K_{R2}) were introduced. This model confirms the presence of at least two transport routes as suggested in literature: (i) transport route across the appendageal structures and (ii) the transport route via the intercellular route in the skin [29-33]. Besides describing the flux profile during and after iontophoresis it is also important to identify the physicochemical properties that are related to the different transport parameters. Our

results demonstrate that the electrophoretic mobility and the molecular weight of the molecules can be used to predict the zero-order mass input into the skin (I_0) and the release constant K_{R2} , respectively. In addition K_{R1} is related to the lipophilicity, although further research is required to establish a clear relationship. With these parameters it will be possible to simulate the flux profile, using the proposed kinetic model, even when no steady state has reached yet. This approach can be of great interest for screening a series of new candidates.

Part II-*In vivo* investigations of the controlled iontophoretic delivery of (S)-5-OH-DPAT

Optimization of the iontophoretic delivery of 5-OH-DPAT *in vitro* (**CHAPTER 3**) showed the great potential of this dopamine agonist and gave rise for investigating the controllability in an *in vivo* setting.

In **CHAPTER 6**, the controlled release following transdermal iontophoresis of (S)-5-OH-DPAT, the active enantiomer of 5-OH-DPAT, was investigated in a rat model under anesthetic conditions. Firstly, in analogy to the *in vitro* flux, the *in vivo* flux was linearly correlated with the applied current density. This confirms that the administered dose can easily be titrated by altering the current density. Next to a controlled plasma profile, the second aim of these studies was to investigate the PK-PD during a controlled and reversible pharmacological response following transdermal iontophoresis. Before conduction these PK-PD studies, an animal model under anesthetic conditions was developed. In a control experiment it was demonstrated that anesthesia (fentanyl, fluanisone and midazolam), current application with transdermal iontophoresis and blood sampling had no influence on the striatal dopamine release (pharmacodynamic end point). Thereafter the plasma profile and the striatal dopamine release were monitored simultaneously in the validated rat model following transdermal iontophoresis and intravenous (IV) infusion of (S)-5-OH-DPAT. For both administration routes a strong decrease in striatal dopamine release was observed, that returns to its initial state within 2h after the end of the administration.

The experimental data were extensively analyzed using non-linear mixed-effect modeling. To facilitate discrimination between delivery-specific parameters (I_0 , K_R) and drug specific parameters (Cl , Q , V_2 , and V_3), the data following transdermal

iontophoresis and intravenous (IV) infusion were analyzed simultaneously. The kinetic models to describe the plasma profile following transdermal iontophoresis, adapted from literature, were based on the zero-order mass input from the donor phase into the skin and the first order release from the skin to the systemic circulation [9]. In contrast to *in vitro* iontophoresis, one first order release constant (K_R) was sufficient to adequately describe the plasma profile following iontophoresis. To describe the striatal dopamine release, two models were compared: (i) indirect response model type I with inhibition of the production of response vs (ii) effect compartment model with sigmoidal $I_{\max}$ model. Based on the Akaike's information criterion (AIC) and the visual predictive check, it can be concluded that the effect compartment model describes more adequately the striatal dopamine release. Furthermore covariate analysis suggested that the delivery rate can be important for establishing a desired effect. The use of an integrated PK-PD model suggested that the steady state plasma concentrations can be translated to continuous dopaminergic stimulation. This can be of great benefit for symptomatic treatment of Parkinson's disease. It is generally believed that continuous stimulation of the dopamine receptor reduces motor fluctuations after chronic use and can be beneficial for non-motor complications, such as nocturnal disturbances [6].

Part III-Transdermal iontophoretic delivery of ester prodrugs of 5-OH-DPAT: from synthesis to *in vivo* studies

The promising results of the iontophoretic delivery *in vitro* and *in vivo* encouraged us to further improve the applicability of 5-OH-DPAT. Therefore 5-OH-DPAT, which has limited aqueous solubility, was esterified with four natural occurring amino acids: glycine-, proline-, valine- and β -alanine. The four resulting prodrugs all contain an extra chargeable amine group, compared to the parent drug.

In **CHAPTER 7** a thorough investigation is presented addressing the following properties of these prodrugs: chemical stability, solubility and transdermal iontophoretic transport. Stability studies investigated the influence of pH, temperature, current application and presence of skin on the stability of the prodrugs. Increasing the pH and temperature decreased the stability of all prodrugs, while current application and the presence of HSC did not affect the stability. Based on these results, valine- and β -alanine-5-OH-DPAT, with acceptable stability, were selected to conduct solubility and iontophoretic transport studies with. Compared to

the parent drug 5-OH-DPAT, valine- and β -alanine-5-OH-DPAT showed a 4- and more than 14-fold increase in maximum solubility in the donor phase (citric buffer, pH 5.0 with 68 mM NaCl), respectively. This enables to reduce significantly the patch size, without reducing the substantial amount of drug present in the patch. Iontophoretic transport studies *in vitro* across HSC and DHS, demonstrated that valine-5-OH-DPAT, a more lipophilic drug, was transported less efficiently than 5-OH-DPAT across both skin types. In contrast, β -alanine-5-OH-DPAT, a more hydrophilic prodrug, was transported with the same efficiency across HSC, but 40% more efficient across DHS compared to 5-OH-DPAT. It was also observed that both prodrugs during iontophoresis are substantially hydrolyzed to the parent drug 5-OH-DPAT, with a higher hydrolysis rate across DHS compared to HSC.

Based on the *in vitro* results, β -alanine-5-OH-DPAT was selected for *in vivo* iontophoretic transport studies in the novel validated animal model, presented in **CHAPTER 6**. The results of the active enantiomer of the prodrug, β -alanine-(S)-5-OH-DPAT, were compared to the results of (S)-5-OH-DPAT, presented in the previous chapter. Despite a higher *in vitro* transport, lower plasma concentrations were observed following 1.5h current application ($250 \mu\text{A}\cdot\text{cm}^2$) of β -alanine-(S)-5-OH-DPAT in comparison to (S)-5-OH-DPAT. However the prodrug showed higher plasma concentrations post-iontophoresis, explained by a delayed release due to hydrolysis and skin depot formation. This resulted in a pharmacological effect with the same maximum as 5-OH-DPAT, but the effect lasted for a significant longer time. In analogy to (S)-5-OH-DPAT, the plasma profile and dopamine release following transdermal iontophoresis of β -alanine-(S)-5-OH-DPAT were analyzed using an integrated PK-PD model. To account for hydrolysis and depot formation, an extra compartment was integrated in the PK model for transdermal iontophoresis (**CHAPTER 6**) between the skin and the plasma concentration in parallel. Similar to (S)-5-OH-DPAT, an effect compartment model was used to describe the striatal dopamine release. The current findings show the possibility to tailor the physicochemical properties, the pharmacokinetic profile and pharmacological response with the use of prodrugs. The prodrug β -alanine-5-OH-DPAT with a balanced stability and high transport efficiency is a promising candidate for symptomatic treatment of Parkinson's disease.

2 Conclusions and future perspectives

Transdermal iontophoretic delivery of dopamine agonists has great potential for the symptomatic treatment of Parkinson's disease. It can be of benefit for reducing or replacing L-DOPA therapy, certainly in the initial stage of treatment, since continuous administration of dopamine agonists is less associated with motor complications [2]. The results of the studies presented in this thesis, together with previous studies, show that 2-aminotetralins (5-OH-MPAT, 5,6-di-OH-MPAT, 5-OH-EPAT, 5-OH-DPAT, 5,6-di-OH-DPAT, rotigotine) and 8-OH-DPAC can be transported across the skin with high efficiency [10-11, 15]. One of the advantages of transdermal iontophoresis is the possibility to achieve continuous plasma concentrations, leading to constant dopaminergic stimulation, as was demonstrated for (S)-5-OH-DPAT in **CHAPTER 6** and β -alanine-(S)-5-OH-DPAT in **CHAPTER 7**. These observations give rise for further developing an iontophoretic transdermal delivery device, with ultimately the possibility to design a feedback control system. This could result in a self-controlling delivery device, facilitating its therapeutic use. Such a self-controlling iontophoretic delivery device should have the following properties: (i) Drug: the device should contain a potent dopamine agonist which elicits a strong dopaminergic effect with a relatively small dose. Additionally, the dopamine agonist should have a good aqueous solubility, should be transported efficiently across the skin and should have excellent blood-brain barrier permeability. (ii) Delivery: besides an efficient delivery, that enables reducing the applied current density, the transdermal delivery should also be easily adjustable. As demonstrated for (S)-5-OH-DPAT in **CHAPTER 6**, the drug input and plasma concentration can easily be controlled by adjusting the current density. (iii) Biomarker: there should be a valid and sensitive surrogate endpoint available to monitor the drug effect. Preferably a pharmacodynamic biomarker is monitored instead of the drug plasma concentration. This poses quite a challenge for Parkinson's disease, since the biomarkers used up to today to monitor the dopaminergic effect in animals are measured intracranially. For humans, researchers are restricted to pharmacodynamic end-points such as tapping scores, walking time [34] and categorical rating scales [35-36]. All these end-points require active and conscious involvement of the patients. This underscores the need for novel biomarkers to monitor the dopaminergic effect in patients. (iv) monitoring: Such biomarkers are preferably monitored non-invasively, for instance with reverse iontophoresis. The utility of reverse iontophoresis has been demonstrated for the

treatment of diabetes: the Gluowatch[®] monitors with reverse transdermal iontophoresis the glucose level in plasma [37]. (v) Biosensor, that analyses the biomarker: should be small, preferably on nano or micro scale and therefore be very sensitive.

The development of such a self-controlling delivery device constitutes an enormous challenge, which requires the development and optimization of the chemical structure, the delivery and the detection. This involves an excellent understanding of

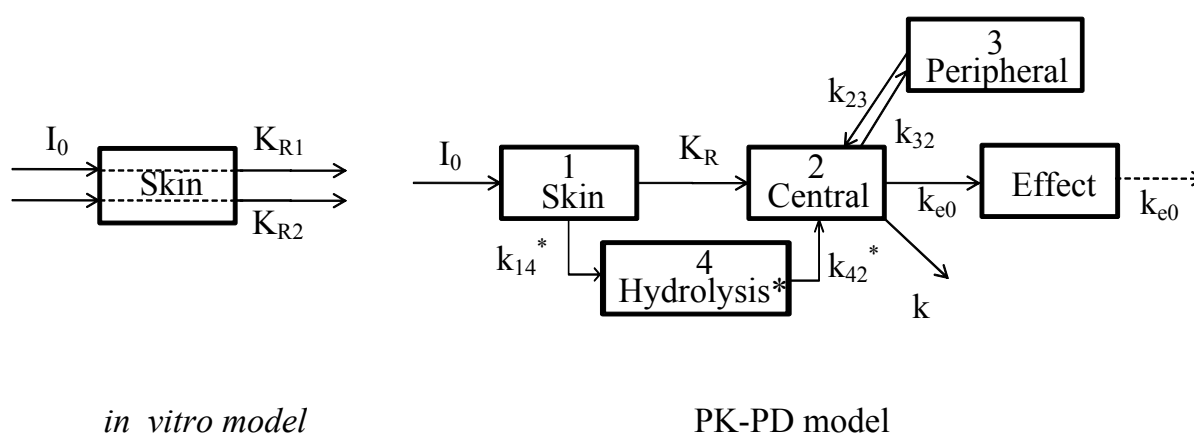


Figure 1: Schematic representation of the compartmental model of the iontophoresis *in vitro*, the pharmacokinetic and pharmacodynamic model following transdermal iontophoresis.

I_0 : zero order drug input during time of current application

K_{R1} and K_{R2} : first order release constants from the skin to the acceptor phase (*in vitro*)

K_R : first order release constants from the skin to the plasma (*in vivo*)

k : elimination rate constant

k_{14} : first order skin-hydrolysis compartment distribution rate constant

k_{42} : first order hydrolysis compartment-plasma distribution rate constant

k_{23} : first order plasma-peripheral distribution rate constant

k_{32} : first order peripheral-plasma distribution rate constant

k_{e0} : first order distribution rate constant from plasma to effect compartment and elimination rate constant from effect compartment

*The inclusion of a hydrolysis compartment in the model is only used following transdermal iontophoresis of the prodrug β -ala- (S)-5-OH-DPAT.

the relationships between the molecular properties, the iontophoretic transport, the pharmacokinetics and the pharmacodynamics. To obtain a better understanding of these relationships, a powerful analytical tool or method is required that can describe and predict the time course of the iontophoretic transport, the plasma concentration and the drug effect. The compartmental models, as used in **CHAPTER 5-7**, allow such type of analysis. The models describe in a comprehensive manner the time dependent transport, plasma and drug effect profiles. With regard to the characterization of the iontophoretic transport this offers major advantages over the use of methods based on the estimation of single parameters (e.g. permeation-lag time method). In addition compartmental modeling is able to connect the rate of iontophoretic delivery to the pharmacokinetic and pharmacodynamic properties and ultimately system behavior.

In this thesis the iontophoretic transport of a series of molecules, with small structural differences, were investigated in **CHAPTER 5**. This allowed investigating the influence of the physicochemical properties on the transdermal iontophoretic transport. The transport parameter I_0 , which is the zero-order mass input during iontophoresis from donor to skin, could be related not only to the donor concentration and applied current density, but also to the electrophoretic mobility of the compound. Furthermore the two first order release constants from skin to acceptor phase, K_{R1} and K_{R2} are related to the lipophilicity and molecular weight, respectively. Based on these property-transport relationships, simulations were conducted with the kinetic models presented in **CHAPTER 5** to investigate the impact of the different transport parameters on the transport profile (Figure 1). The results of the simulations are displayed in Figure 2. As expected, I_0 affects tremendously the value of the steady state flux, but it does not affect the shape of the flux profile (Figure 2A). The value of the zero-order mass input is influenced by three factors, the donor concentration, the current density and the electrophoretic mobility. To diminish skin irritation, the current density should be kept as low as possible. Therefore electrophoretic mobility and aqueous solubility for a high donor concentration are the key parameters to increase the iontophoretic transport. Screening different compounds with capillary electrophoresis to determine the electrophoretic mobility is useful to select appropriate candidates for transdermal iontophoresis. In addition improving solubility with the use of prodrugs (**CHAPTER 7**) or by selecting an alternative salt form (**CHAPTER 4**) can merely help to reduce the patch size, without reducing the amount of drug in the patch.

Figure 2B shows the influence of the release constant K_{R1} on the transport profiles. As can be observed, K_{R1} affects the time to steady state during the current

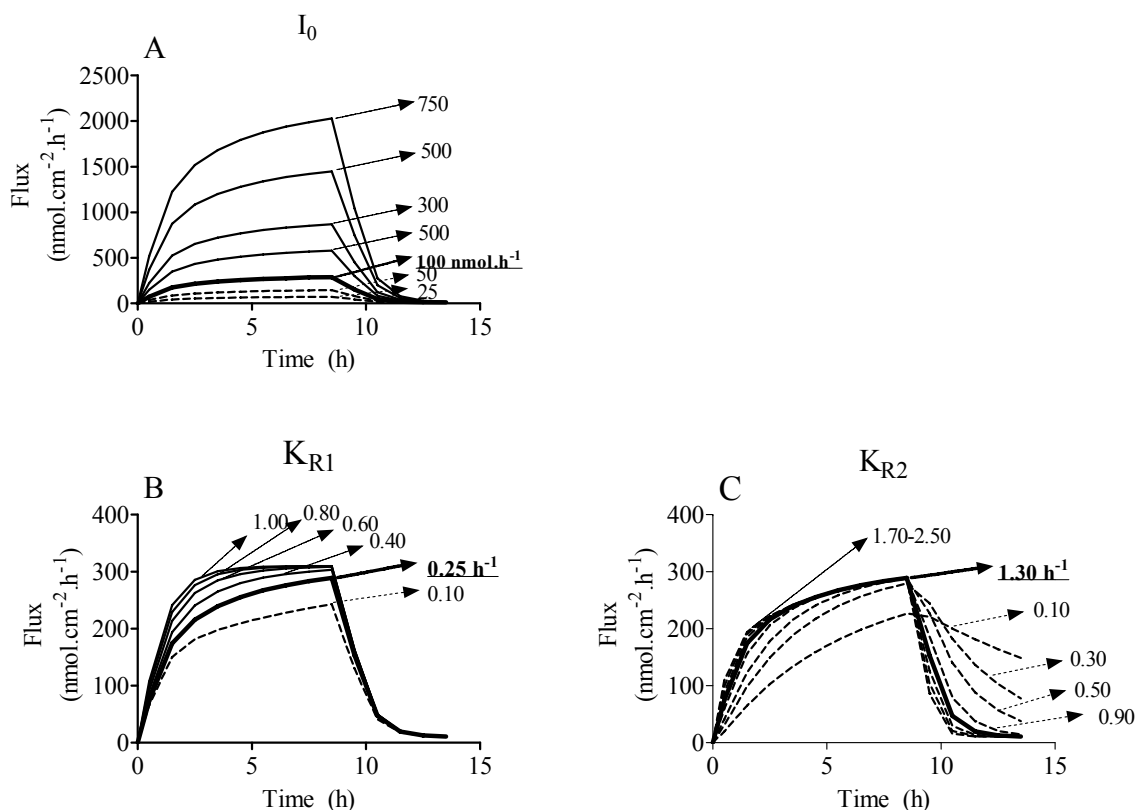


Figure 2: Simulations of iontophoretic transport profiles *in vitro*, changing the zero-order mass input I_0 (A), the first order release constants, K_{R1} (B) and K_{R2} (C). The full thick line is the simulated profile with the transport parameters of 5-OH-DPAT, the intermittent lines and the full lines are the profiles with lower and higher values than of 5-OH-DPAT, respectively. Depicted in every graph are also the values of the parameter used for the simulations. Underlined are the values of transport parameters of 5-OH-DPAT. These values were kept constant when simulations were conducted, changing another transport parameter.

application. The higher K_{R1} , the faster steady state is reached. Therefore a high K_{R1} could be favorable, but K_{R1} is related to the lipophilicity of the compound. Compounds with a higher lipophilicity are less soluble in an aqueous environment and are therefore transported less efficiently with transdermal iontophoresis, as was observed for instance for rotigotine (CHAPTER 4). This should be taken into account when screening new compounds. The value of K_{R2} affects the time to steady state and the post-iontophoretic decline of the transport rate (Figure 2C). A lower K_{R2} , attributed to a higher molecular weight, results in a slower release from the skin

into the acceptor phase. Figure 2C, shows also that for K_{R2} values, higher than 1.30, no large differences in transport profiles are observed. This value can be considered as a minimum threshold value for a relatively rapid transport through and a rapid release from the skin. These simulations show that multiple parameters should be taken into account to optimize the iontophoretic transport profile.

A next step is to evaluate the influence of transport parameters K_R and I_0 on the drug profile *in vivo*. Based on the obtained results and using the PK-PD models (Figure 1), as presented in **CHAPTER 6** and **7**, *in vivo* simulations were performed. The results are depicted in Figure 3. The intrinsic zero-order mass driving force I_0 tremendously affects the PK profile as shown in Figure 3A. In a linearly dependent manner the steady-state plasma concentration is augmented with increasing I_0 . The corresponding simulations performed to investigate the PD effect with changing I_0 are shown in Figure 3C. From an I_0 value of $50 \mu\text{g}\cdot\text{h}^{-1}$ the maximum response remained unchanged when increasing the intrinsic driving force I_0 . With increasing dose the duration of maximum effect after patch removal increases. This was also observed for β -ala-(S)-5-OH-DPAT in **CHAPTER 7**, where a higher value of I_0 , compared to its parent drug resulted in a prolonged effect. This means that the time to recover to basal dopamine levels increases with increasing dose. On the one hand the prolonged effect can be beneficial for patients who suffer from nocturnal disturbances. Nocturnal disturbances are one of the most common non-motor complications in patients with PD [38]. It is believed that continuous stimulation of the DA receptor during the night can reduce the occurrence of nocturnal disturbances [6]. On the other hand this prolonged effect can be a drawback to initiate therapy to treat Parkinson's disease, since rapid changes in delivery schemes may be required. In addition one should also take into account that with increasing plasma concentrations there is an increasing risk of side effects, such as nausea, vomiting and hallucinations [2]. Therefore it is imperative to achieve constant dopaminergic stimulation with optimized plasma concentrations, based on an understanding of the PK-PD relationship and driven by an acceptable current density. The delivery rate also affects the pharmacodynamic efficiency (EFF), as was observed in **CHAPTER 6**. EFF is defined as the pharmacological response per unit of concentration and calculated with the following equation:

$$EFF = \frac{AAEC}{AUC} \quad (1)$$

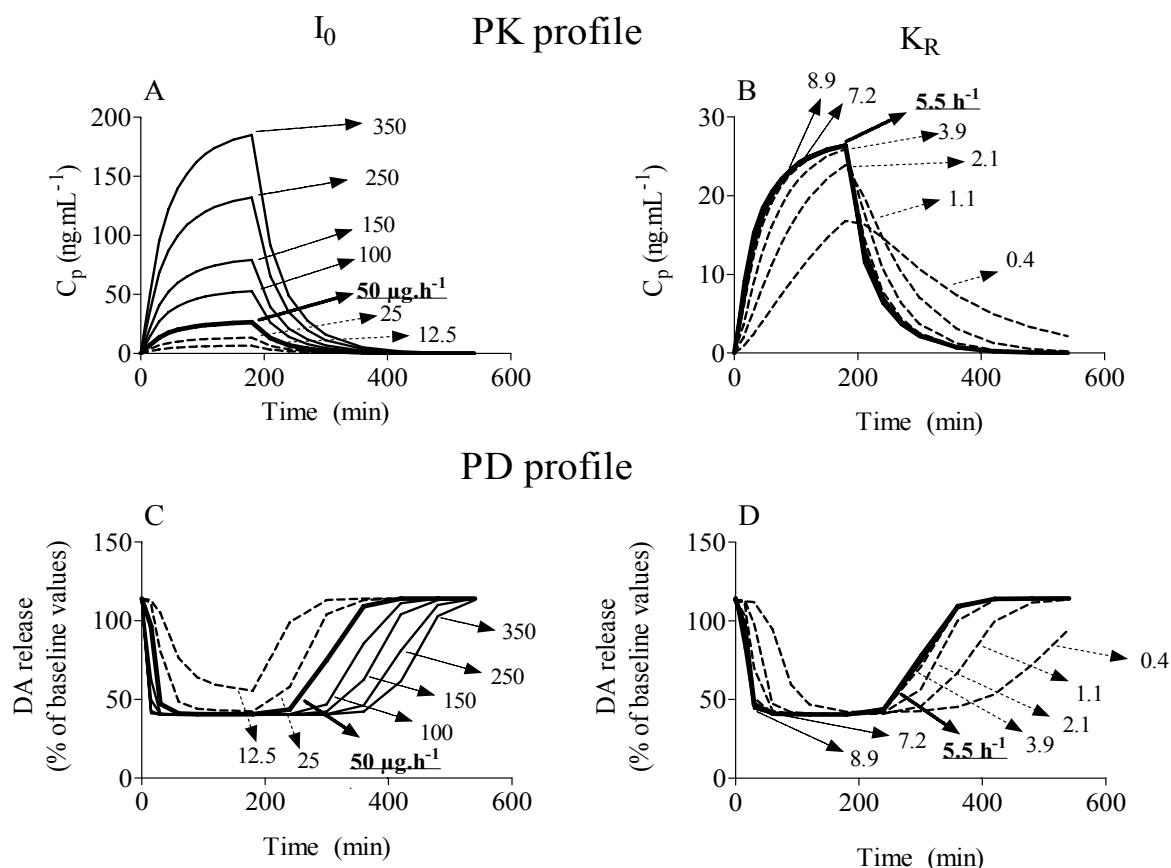


Figure 3: Simulations of transport profiles *in vivo*, changing the different transport parameters. **A:** the plasma concentration profile, simulated with different values of the zero-order mass input I_0 . **B:** the plasma concentration profile, simulated with different values of the first order release constants K_R . **C-D:** the corresponding dopamine release (PD endpoint) profile, simulated with different values of I_0 and K_R . The full thick line is the simulated profile with the transport parameters of 5-OH-DPAT, the intermittent lines and the full lines are the profiles with lower and higher values then of 5-OH-DPAT, respectively. Underlined are the values of transport parameters of 5-OH-DPAT. These values were kept constant when simulations were conducted, changing another transport parameter.

Where AUC ($\text{ng}\cdot\text{ml}^{-1}\cdot\text{min}$) is the area under the plasma concentration curve and $AAEC$ ($\text{DA}\%\cdot\text{min}$) is the area above the effect curve (normalized for baseline dopamine levels). Within the range of delivery rates, investigated in **CHAPTER 6**, it was suggested that a higher delivery rate is favorable. By adjusting the current density, the delivery rate can easily be titrated. Therefore with transdermal iontophoresis the design of complicated dosing regimens is possible. For instance, a high initial dose, obtained with a high current density, can be followed by a lower maintenance dose by gradually decreasing the current density. Next to I_0 , the release constant K_R also affects the PK-PD profiles. Figure 3B shows that K_R affects the entire plasma profile during and after iontophoresis. K_R values of 3.9 and higher do not change the profile dramatically. If K_R drops below a value of 2.1 h^{-1} however, the PK profile starts to show a slower time to steady state and also a slower decline of the transport post iontophoresis. These profile changes are also found in the PD profile as shown in Figure 3D. Again a K_R value of 3.9 h^{-1} can be considered as the threshold K_R value. 5-OH-DPAT has a K_R of 5.5 and small structural changes, resulting in relative small differences in K_R would not affect the PK and PD profile.

The similarity of the compartmental modeling of the *in vitro* and *in vivo* transport allowed us to examine the *in vitro* – *in vivo* correlation specifically. For both the analysis of the *in vitro* transport and the *in vivo* plasma profiles kinetic models were used, based on the zero-order mass input from donor patch to skin (I_0) and the first order release constant(s) (K_R) from skin to acceptor (*in vitro*) or plasma (*in vivo*). However, to describe *in vitro* transport 2 release constants were required for all the compounds investigated. In contrast, to describe *in vivo* iontophoretic transport of 5-OH-DPAT 1 release constant was sufficient. In addition, an extra (hydrolysis) compartment was included in the *in vivo* PK model to adequately describe the transport of β -ala-(S)-5-OH-DPAT. This suggests that different transport mechanisms govern the transdermal iontophoretic transport *in vitro* and *in vivo*. These differences can at least partially be attributed to the following factors. Firstly, the structure of human skin differs from the structure of rats. Especially the density of hair follicles will contribute to the difference. Previous studies showed that the appendageal route is believed to be the predominant route during iontophoresis and post-iontophoresis [33]. Therefore it is suggested that the higher density of hair follicles in rat skin results in a much higher contribution of the appendageal route, reflected by a higher K_{R2} . *In vivo* across rat skin the contribution of K_{R1} might therefore become negligible. This could explain why one K_R is sufficient to describe the skin release *in vivo*. Secondly the clearance of the penetrant by the acceptor flow may be different from the clearance *in vivo* by the microcirculation in the skin [39].

Thirdly, concerning the hydrolysis of β -ala-5-OH-DPAT, the activity of the esterase *in vivo* may be different from the esterase activity in freshly excised skin. Despite these differences the transport parameters *in vitro* and *in vivo* corresponded relatively well.

The estimated *in vivo* steady state flux ($J_{ss} vivo$) is compared with the estimated *in vitro* steady state flux ($J_{ss} vitro$) (Table 1). The absolute value of $J_{ss} vivo$ in rats corresponds most closely with the $J_{ss} vitro$ across dermatomed human skin for 5-OH-DPAT and to the $J_{ss} vitro$ across human stratum corneum for β -ala-5-OH-DPAT. Since K_{R2} describes the release constant post iontophoresis *in vitro*, this

Table 1: Comparison of the transdermal iontophoretic steady state flux (J_{ss}) of 5-OH-DPAT and β -ala-5-OH-DPAT across rat skin *in vivo* and human skin *in vitro*.

			5-OH-DPAT		β -ala-5-OH-DPAT	
	Parameter	unit	mean	RSE%	mean	RSE%
Rat skin <i>in vivo</i>	$J_{ss} vivo$	nmol.cm ⁻² .h ⁻¹	174.6	8	221.2	8
	K_R	h ⁻¹	2.2 ^a -5.5 ^b	21-26	0.31 ^c -4.8 ^d	23-21
HSC <i>in vitro</i>	$J_{ss} vitro$	nmol.cm ⁻² .h ⁻¹	239.4	9	272.5	4
	K_{R2}	h ⁻¹	1.39	10	1.69	8
DHS <i>in vitro</i>	$J_{ss} vitro$	nmol.cm ⁻² .h ⁻¹	190.3	1	381.3	7
	K_{R2}	h ⁻¹	0.56	11	0.588	5

Note: The transport rates *in vivo* are normalized to a current density of 500 μ A.cm⁻². The donor concentration was 3.9 mM and pH was 5.0. ^aThis value was obtained from the PK study; ^bThis value was obtained from the PK-PD study; ^cThis value is the first order release constant K_R value from the skin to central compartment; ^dThis value is the first order release constant k_{42} value from the hydrolysis to central compartment

release constant was compared to the *in vivo* release constant. As can be observed, the *in vivo* release constant was more closely related to the *in vivo* release constant across HSC. For both 5-OH-DPAT and the prodrug β -ala-5-OH-DPAT these differences between the *in vitro* and *in vivo* transport parameters are relatively small, certainly considering the interspecies differences. Nugroho *et al.* showed comparable differences between the *in vitro* and *in vivo* transport parameters using the same species [10]. Furthermore in the same study it was demonstrated that by combining the *in vitro* transport parameters with the *in vivo* PK-PD parameters, it was possible to predict the pharmacokinetic transport profile and even the pharmacodynamic effect [10]. In addition, previous studies showed that iontophoresis diminishes the interspecies variations [40]. This emphasizes that *in vitro* transport studies across human skin can provide valuable information for extrapolation towards the *in vivo* situation, even in other species like rats. Modeling and simulations can therefore be very helpful in designing novel experiments.

In conclusion, this thesis presents various potential candidates for the symptomatic treatment of Parkinson's disease with the use of transdermal iontophoresis. Thereby a large framework is constructed to screen new potential candidates for transdermal iontophoretic delivery from physicochemical properties via *in vitro* transport studies towards *in vivo* application. This framework is supported by the application of novel kinetic models to describe the transport profiles during and after iontophoresis. These models can be used to simulate and design new *in vivo* studies and to get a better comprehension about the *in vitro-in vivo* and the PK-PD relationships. Ultimately these models constitute a scientific basis to design a closed-loop self controlled delivery system.

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