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

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Mechanistic studies of the transdermal iontophoretic delivery of 5-OH-DPAT *in vitro*

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Abstract

A characterization and optimization of the *in vitro* transdermal iontophoretic transport of 5-hydroxy-2-(N,N,-di-n-propylamino)tetralin (5-OH-DPAT) is presented. The utility of acetaminophen as a marker of electroosmotic flow was studied as well. The following parameters of iontophoretic transport of 5-OH-DPAT were examined: drug donor concentration, electroosmotic contribution, influence of co-ions, current density and composition of the acceptor phase. The steady-state flux (Flux_{ss}) of acetaminophen was linearly correlated with the donor concentration and co-iontophoresis of acetaminophen did not influence the iontophoretic flux of 5-OH-DPAT, indicating that acetaminophen is an excellent marker of electroosmotic flow. Lowering the Na^+ concentration from 78 mM to 10 mM in the donor phase, resulted in a 2.5 fold enhancement of the Flux_{ss} . The Flux_{ss} showed a non-linear relation with the drug donor concentration and an excellent linear correlation with the current density. Reducing the pH of the acceptor phase from 7.4 to 6.2 resulted in a dramatic decrease of the Flux_{ss} of 5-OH-DPAT, explained by a reduced electroosmotic flow and an increased counter-ion flow. Optimization of the conditions resulted in a maximum Flux_{ss} of 5-OH-DPAT of $1.0 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ demonstrating the potential of the iontophoretic delivery of this dopamine agonist for the symptomatic treatment of Parkinson's disease.

Key words: Controlled release; Skin; Transdermal drug delivery; Iontophoresis; Percutaneous; Electroosmosis; Acceptor phase; Dopamine agonist; Parkinson's disease

1 Introduction

Parkinson's disease is an age-related progressive neurological disorder with a prevalence of 1-2 % in people over the age of 50. Pharmacotherapy is the first line symptomatic treatment of this disease [1]. Currently orally administered levodopa is still considered the golden standard, but its possible neurotoxicity and the induction of movement disorders after chronic use demands for the development of alternative therapies [2]. One of the most important alternatives is the use of dopamine agonists, such as apomorphine, carbergoline, ropinirole and rotigotine which directly activate the postsynaptic dopamine receptor [3]. However when these dopamine agonists are administered orally, often fluctuations in plasma concentrations occur resulting in pulsatile dopaminergic stimulation. Therefore, the continuous dopamine stimulation may be the optimal symptomatic treatment of Parkinson's disease, reducing *off* periods and dyskinesias [2, 4].

Transdermal iontophoresis is one of the promising techniques for non-invasive continuous delivery of dopamine agonists. By applying an electric current across the skin it is possible to enhance the transdermal delivery of small charged molecules. In addition by modulation of the current and donor concentration it will be possible to adjust the rate of administration to the requirements of the individual patient, which will be an important advantage. In the last ten years several dopamine agonists have been studied for their ability to be administered by iontophoresis [5-10]. The potent dopamine agonist 5-hydroxy-2-(N,N,-di-n-propylamino)tetralin (5-OH-DPAT) [11-12], has already been proven to be suitable for iontophoretic delivery *in vitro* as well as *in vivo* [5, 13]. However characterization of the transport mechanisms driving the iontophoretic delivery *in vitro* is crucial to understand and optimize the iontophoretic delivery *in vivo* to patients.

Transdermal migration of chemicals during iontophoresis is influenced by several factors such as pH, the presence of ions having the same charge (co-ions) and ions having the opposite charge [14]. This implies that the iontophoretic transport of chemicals is influenced by the composition of the donor phase as well as the acceptor phase. In contrast to the donor phase, the effect of the composition in the acceptor phase has not been widely studied [14-16].

The aim of the present study was to fully characterize the transdermal iontophoretic transport of the dopamine agonist 5-OH-DPAT across the skin. Especially the electroosmotic contribution, the influence of ion composition in the donor and acceptor phase on the iontophoretic delivery of 5-OH-DPAT were studied.

Several marker molecules, like ^{14}C -mannitol and acetaminophen, are available to estimate the electroosmotic contribution during iontophoretic transport [17-21]. In the last few years acetaminophen has gained more interest over ^{14}C -mannitol, because of its practical advantages. Although acetaminophen has been widely used as a marker for electroosmosis, until now its transport has not been fully characterized. Therefore in the current study acetaminophen as a marker molecule for electroosmosis is evaluated as well.

2 Materials and methods

2.1 Materials

5-OH-DPAT (HBr salt, purity>98%) was synthesized at the Department of Medicinal Chemistry of the University of Groningen, Groningen, the Netherlands. Silver, silver chloride (purity>99.99%), Trypsin (Type III from bovine pancreas) and trypsin inhibitor (Type II-S from soybean) were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Acetaminophen was purchased from Brocacef BV (Maarssen, the Netherlands) and D(-)Mannitol was obtained from BDH Laboratory supplies (Poole, UK). Bacteriological agar was purchased from Life Technologies (Paisley, Scotland). Spectra/Por[®] RC dialysis membrane disks (cut off value of 6000-8000 Da) were purchased from Spectrum laboratories, Inc (Rancho Dominguez, Ca, USA). Tetrahydrofuran (THF, stabilized, purity>99.8%) was obtained from Biosolve (Valkenswaard, the Netherlands). Triethylamine (TEA, purity>99%) was obtained from Acros Organics (Geel, Belgium). All other chemicals and solvents were of analytical grade. All solutions were prepared in Millipore water with a resistivity of more than 18 M Ω .cm.

2.2 Preparation of Human Stratum Corneum (HSC)

The preparation of human stratum corneum was performed according to the method described previously [13]. Briefly, within 24h after surgical removal of the human skin residual subcutaneous fat was removed. Dermatomed human skin (DHS) was obtained by dermatoming the skin to a thickness of about 300 μm . In order to obtain HSC, DHS was incubated with the dermal side on Whatman paper soaked in a solution of 0.1% trypsin in 150 mM phosphate buffered saline (PBS) pH 7.4 (NaCl: 8 g.L⁻¹, Na₂HPO₄: 2.86 g.L⁻¹, KH₂PO₄: 0.2 g.L⁻¹, KCl: 0.19 g.L⁻¹) overnight at 4 °C and subsequently for 1h at 37 °C after which HSC was peeled off from the underlying viable epidermis and dermis. HSC was subsequently washed in a 0.1%

trypsin inhibitor solution in Millipore water and several times in water and stored in a desiccator in a N₂ environment.

2.3 Preparation of salt bridge

The use of salt bridges enables to reduce the amount of competing cations in the donor phase, while the salt bridge still contains enough Cl⁻-ions, added as NaCl, to feed the anodal electrochemistry. An agar suspension was prepared as follows: 100 mL of PBS pH 7.4 was heated in an Erlenmeyer flask to boiling. Then 3.0 g agar was added, stirring was commenced and the solution was continuously heated until a uniform suspension was formed. The mixture was transferred to a silicon tube of appropriate length (total volume 1.0 mL). The tube was then left at room temperature until the mixture had gelled. Thereafter the anode was placed carefully in the salt bridge.

2.4 Solubility of 5-OH-DPAT

The solubility was determined according to the method described previously [6]. Briefly, 5-OH-DPAT was solubilized in 5 mM citrate buffer pH 5.0 and 6.0, containing 68 mM NaCl. After adjusting the pH of the solution with 0.05 M NaOH or HCl, each solution was shaken for 48h at 700 rpm (IKA-VIBRAX-VXR, Omnilabo International BV, Breda, The Netherlands) at room temperature. All saturated solutions were centrifuged at 3600 rpm (AllegraTM 6R centrifuge, Beckman Coulter, Fullerton, CA, USA) for 30 minutes. Each supernatant was then filtered by using a 0.2 µm porous membrane (PTFE Acrodisc[®], Pall, East Hills, NY, USA), after which the concentration was determined by HPLC.

2.5 *In vitro* transport studies

A 9-channel computer controlled power supply was used to provide a constant direct current (Electronics Department, Gorlaeus Laboratories, Leiden University, The Netherlands) during iontophoresis. The system was equipped with differential input channels per current source enabling on-line measurement of the electric resistance across HSC in each diffusion cell. Ag/AgCl was used as driver electrode pair. All transport studies were carried out, using a three chamber continuous flow through cell as described elsewhere [13]. In all experiments HSC of at least 2 different donors was used. The donor formulation was buffered with 5 mM citric acid and D-mannitol was added to the donor phase to obtain iso-osmolar conditions. Unless stated otherwise the cathodal chamber was filled with PBS pH 7.4 and the acceptor chamber was continuously perfused with PBS pH 7.4 at a flow rate of $\pm 6.5 \text{ ml.min}^{-1}$.

During the experiment the temperature of the acceptor phase was maintained at 32 °C, the skin temperature *in vivo*. Unless stated otherwise, the following protocol was used for *in vitro* iontophoretic transport studies: 6h passive diffusion, followed by 9h of iontophoresis at a current density of 500 $\mu\text{A}\cdot\text{cm}^{-2}$ and finishing with 5h passive diffusion. During the experiment the anodal and cathodal chamber were continuously stirred at 365 rpm. Samples were collected every hour with an automatic fraction collector (ISCO Retriever IV, Beun De Ronde BV, Abcoude, The Netherlands). The specific conditions for each individual series of experiments are described below.

2.5.1 Acetaminophen as marker molecule for the electroosmotic flow

To determine whether there is a linear correlation between the donor concentration acetaminophen and its iontophoretic transport, studies were performed with 3 different donor concentrations acetaminophen (7.5 mM, 15 mM and 30 mM) in a citric buffer pH 5.0, containing 68 mM NaCl.

The influence of co-iontophoresis of acetaminophen on the 5-OH-DPAT flux was also investigated. The donor formulation was at pH 5.0 in the presence of 68 mM NaCl and 3.9 mM 5-OH-DPAT. The iontophoretic transport of 5-OH-DPAT in presence (15 mM) and absence of acetaminophen was measured.

2.5.2 Electromigrative and Electroosmotic contribution to the transport of 5-OH-DPAT

The concentration of 5-OH-DPAT in the donor concentration was 1.5 or 3.9 mM. Acetaminophen (15 mM) was added to the donor solution as a marker molecule for the electroosmosis. The donor solution was buffered with citric buffer at a pH of either 5.0 or 6.0. All experiments were performed in the presence of 68 mM NaCl in the donor solution.

2.5.3 Influence of competing ions in the donor phase

The influence of competing co-ions in the donor phase was investigated, using different concentrations of 5-OH-DPAT varying from 0.15 to 35.1 mM. The concentration of NaCl in the donor phase was either 0 or 68 mM. In the absence of NaCl the only source of Na^+ was $\text{Na}_3\text{citrate}\cdot 2\text{H}_2\text{O}$, used to buffer the solution and the amount of Na^+ was calculated at 10 mM. Therefore the total Na^+ -concentration was either 10 mM or 78 mM. In the case of 10 mM Na^+ the Ag-electrode (anode) was placed in a salt bridge. The donor solution was buffered at pH 6.0.

2.5.4 Influence of the pH of the acceptor phase

The iontophoretic delivery of 5-OH-DPAT was investigated using PBS with a different pH as acceptor phase. The acceptor solution was either PBS pH 7.4 or PBS pH 6.2. (8 g.L⁻¹ NaCl, 0.19 g.L⁻¹ KCl, 0.43 g.L⁻¹ Na₂HPO₄.2H₂O, 0.97 g.L⁻¹ KH₂PO₄), both at iso-osmolar conditions. The concentration of 5-OH-DPAT was 1.5, 3.9 or 7.0 mM. All experiments were conducted in the presence of 68 mM NaCl in the donor solution, buffered at pH 5.0.

The electroosmotic or water flow across HSC was investigated using PBS as acceptor phase. The pH of the acceptor solution was either 7.4 or 6.2. The concentration of acetaminophen, used as a marker molecule for the electroosmotic flow, was 15 mM. The donor solution was buffered at pH 6.0 using 5 mM citrate buffer. All experiments were conducted in the presence of 68 mM NaCl in the donor phase.

The ion transport across HSC was investigated using PBS with different pH as acceptor phase. The pH of the acceptor solution was either 7.4 or 6.2. The pH of the donor solution was 6.0. A current of 500 $\mu\text{A.cm}^{-2}$ was applied during 19h. This experiment was performed in the absence of NaCl and the Ag-electrode (anode) was placed in a salt bridge. At regular time intervals (0, 2, 4, 6 and 19h) an aliquot of 100 μL of the donor solution was sampled to measure the osmolarity with an osmometer (Micro-osmometer type 13, Roebling, Berlin, Germany).

2.5.5 Influence of current density

The iontophoretic transport of 5-OH-DPAT (3.9 mM), buffered at pH 6.0, across HSC was investigated at different current densities. The protocol used for these experiments is: 2h passive diffusion + 6h 125 $\mu\text{A.cm}^{-2}$ + 6h 250 $\mu\text{A.cm}^{-2}$ + 4h 500 $\mu\text{A.cm}^{-2}$ + 6h passive diffusion. The Na⁺-concentration in the donor phase was 10 mM and the Ag-electrode (anode) was placed in a salt bridge.

2.6 Analytical methods

Prior to and at the end of a transport study pH and osmolarity of donor and acceptor compartment were measured. All samples of the iontophoretic transport studies were analyzed by RP-HPLC using a Superspher[®] 60 RP-select B, 75 mm-4 mm column (Merck KGaA, Darmstadt, Germany). 5-OH-DPAT was detected using a scanning fluorescence detector (Waters[™] 474, Millipore, Milford, MA, USA) at excitation and emission wavelengths of 275 and 302 nm. Acetaminophen was detected using a UV detector (Dual λ Absorbance Detector 2487, Waters, Milford, USA) at a

wavelength of 254 nm. Filtered and degassed mobile phase contained 100 mM acetate buffer (pH 3.6)/THF (95/5 v/v) with the addition of 30 mM TEA to prevent peak tailing. The injection volume was 50 μL and the flow rate was set to 1.5 $\text{mL}\cdot\text{min}^{-1}$. Calibration curves showed a linear response when using concentrations of compounds between 0.1 and 40 $\mu\text{g}\cdot\text{mL}^{-1}$ ($R^2>0.9999$). The limit of detection (LOD) and limit of quantification (LOQ) were experimentally determined for all compounds of interest: 5-OH-DPAT has a LOD and LOQ of 3.5 and 5.8 $\text{ng}\cdot\text{mL}^{-1}$ respectively. Acetaminophen has a LOD and LOQ of 5.8 and 9.6 $\text{ng}\cdot\text{mL}^{-1}$, respectively.

2.7 Determination of electromigrative and electroosmotic contribution

The total iontophoretic flux consists of the passive flux (J_{pass}), the electroosmotic flux (J_{EO}) and the electromigrative flux (J_{EM}). The passive flux is calculated during 6h prior to the iontophoretic phase. The electroosmotic flow is calculated as described below. The electromigrative flux is calculated by subtracting the passive and electroosmotic flux from the total flux.

The water flow during iontophoresis can be derived from the flux of the neutral permeant acetaminophen by the following Equation [22]:

$$V_w = \frac{J_{ace}}{C_{ace}} \quad (1)$$

where V_w is the water flow, J_{ace} is the steady state flux (Flux_{ss}) and C_{ace} the donor concentration of acetaminophen. When plotting the J_{ace} as function of the donor concentration of acetaminophen C_{ace} , the water flow can be calculated from the slope of the regression line. The electroosmotic flow of 5-OH-DPAT (J_{EO}) is proportional to the volume flow (V_w) and the molar concentration of the solute, 5-OH-DPAT ($C_{5\text{-OH-DPAT}}$) [22] and can be calculated as follows:

$$J_{EO} = V_w \times C_{5\text{-OH-DPAT}} \quad (2)$$

2.8 Data analysis

The cumulative flux of 5-OH-DPAT and acetaminophen during iontophoretic transport was plotted as a function of time. The Flux_{ss} was estimated from the linear part of the slope of this plot according to the permeation lag-time method [13]. All data are presented as mean $\pm$ standard deviations (SD). When the effect of two factors was investigated simultaneously two-way ANOVA was used for statistical analysis. When a statistical analysis was performed for only 2 groups, a Students t-test was used. For all statistical analysis a significance level of $p<0.05$ was used.

3 Results

3.1 Acetaminophen as marker molecule for the electroosmotic flow

First the proportionality of the flux with the donor concentration was investigated. As depicted in Figure 1A a variation in donor concentration of acetaminophen of 7.5, 15.0 and 30.0 mM resulted in steady state fluxes (Flux_{ss}) of 30.6 ± 7.3 , 48.6 ± 7.8 and $103.5 \pm 26.5 \text{ nmol.cm}^{-2}.\text{h}^{-1}$, respectively. The acetaminophen concentration and Flux_{ss} are linearly related. The linear regression line was forced through zero ($R^2=0.995$). According to Equation 1 the slope of the regression line represents the water flow during iontophoretic transport and is estimated at $3.39 \pm 0.17 \text{ ml.cm}^{-2}.\text{h}^{-1}$. Furthermore as depicted in Figure 1B a change from pH 5.0 to 6.0 in the donor phase results in a significant increase in acetaminophen flux from $50.0 \pm 11.0 \text{ nmol.cm}^{-2}.\text{h}^{-1}$ to $68.0 \pm 5.9 \text{ nmol.cm}^{-2}.\text{h}^{-1}$ (t-test; $p<0.05$). Secondly the influence of co-iontophoresis of acetaminophen on the Flux_{ss} of 5-OH-DPAT is presented in Table 1. The steady state flux of 5-OH-DPAT (3.9 mM), buffered at pH 5.0, remained

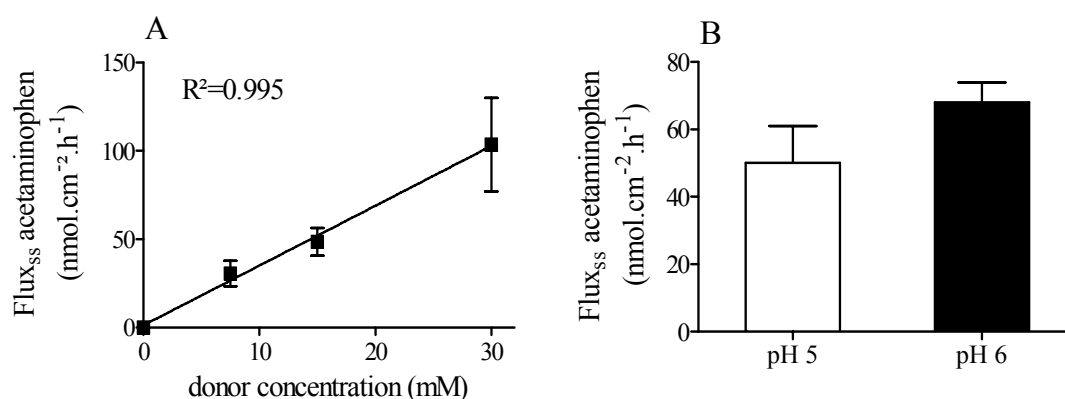


Figure 1 A: The linear correlation between the steady state flux (Flux_{ss}) vs the donor concentration during iontophoresis ($500 \mu\text{A.cm}^{-2}$) of acetaminophen at donor pH 5.0. Data are presented as mean $\pm$ SD ($n=5-7$). The acceptor phase consisted of PBS pH 7.4.

B: bar plot of the Flux_{ss} of acetaminophen (15 mM) at donor pH 5.0 (white bar) and pH 6 (black bar). Data are presented as mean + SD ($n\geq 4$). A current density of $500 \mu\text{A.cm}^{-2}$ was applied and PBS pH 7.4 was used as acceptor phase. A significant difference is observed between the Flux_{ss} at pH 5.0 and 6.0 (t-test; $p<0.05$).

unaffected when acetaminophen (15mM) was added to the donor phase (t-test; $p>0.05$). Moreover the iontophoretic flux profile of 5-OH-DPAT remained also unchanged (data not shown).

3.2 Solubility of 5-OH-DPAT

The solubility of 5-OH-DPAT was determined in a citric buffer (5 mM), containing 68 mM NaCl at pH 5.0 and 6.0. The maximum solubility decreases with increasing pH from 56.7 ± 1.4 mM at pH 5.0 to 47.4 ± 1.4 mM at pH 6.0.

3.3 Iontophoretic delivery of 5-OH-DPAT

3.3.1 Electromigrative and electroosmotic contribution

The passive flux appeared to be negligible, contributing only a maximum of 0.02% to the total flux. The results of the determination of the electromigrative and electroosmotic contribution are provided in Table 1. The electroosmosis flux has a minor but significant contribution to the 5-OH-DPAT iontophoretic flux. No

Table 1: The steady state flux of 5-OH-DPAT after iontophoresis ($500 \mu\text{A} \cdot \text{cm}^{-2}$) by the difference in pH, acetaminophen concentration and Na^+ concentration. The acceptor phase consisted of PBS pH 7.4. Results are presented as mean $\pm$ SD ($n \geq 3$).

Donor phase				Results			
5-OH-DPAT conc.	Ace conc.	Na^+ conc.	pH	Flux _{ss} of 5-OH-DPAT	J_{EO}	Relative contribution	J_{EM}
(mM)	(mM)	(mM)		($\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	($\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)	(% J_{EO})	($\text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$)
3.9	0	78	5	188.9 ± 25.5	n.a.	n.a.	n.a.
3.9	15	78	5	210.4 ± 13.0	13.7 ± 3.4	6.5 ± 1.7	196.7 ± 13.4
3.9	15	78	6	214.3 ± 6.0	14.5 ± 1.0	6.8 ± 0.5	199.8 ± 6.0
3.9	15	10	6	482.0 ± 36.8	14.7 ± 4.4	3.0 ± 0.9	467.3 ± 37.0

n.a.: not applicable

Ace: acetaminophen

significant change in the contribution of J_{EO} and J_{EM} to the total flux of 5-OH-DPAT is observed when changing the pH of the donor solution from pH 5.0 to 6.0 ($p>0.05$).

Also depicted in Table 1, at a donor concentration of 3.9 mM 5-OH-DPAT a pH change from 5.0 to 6.0 does not significantly change the 5-OH-DPAT Flux_{ss} ($p>0.05$). When focusing on the influence of 5-OH-DPAT on the acetaminophen flux, 3.9 mM 5-OH-DPAT buffered at either pH 5.0 or 6.0 does not change the acetaminophen flux demonstrating that 5-OH-DPAT does not change the negative charge of the HSC and thus does not change the permselectivity of the HSC (data not shown).

3.3.2 Influence of competing ions in the donor phase on the iontophoretic flux of 5-OH-DPAT

The effect of NaCl in the donor compartment was studied. The total concentration of Na^+ in the donor phase was either 10 mM (absence of NaCl) or 78 mM (addition of 68 mM NaCl). The transport studies were conducted with 5 different donor concentrations of 5-OH-DPAT varying between 0.15 and 35.1 mM 5-OH-DPAT. Figure 2A shows the flux profile of 5-OH-DPAT (1.5 mM) when 10 mM or 78 mM

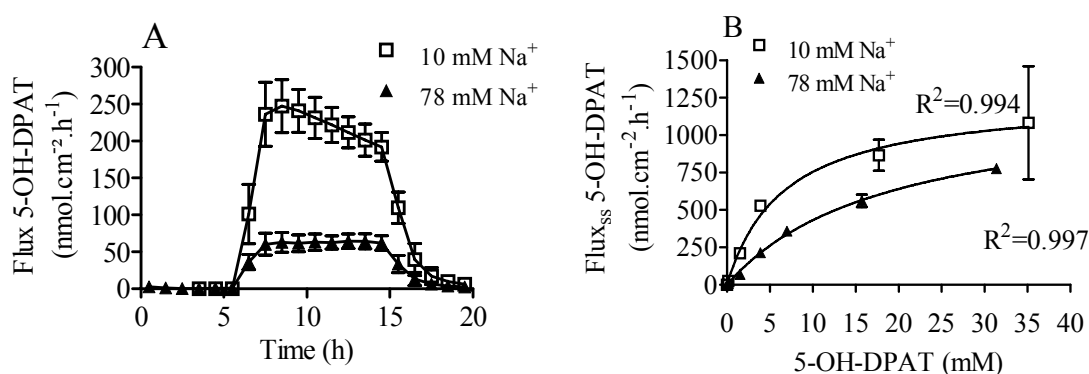


Figure 2 A: Iontophoretic flux ($500 \mu\text{A.cm}^{-2}$) vs time profile of 5-OH-DPAT solution (1.5 mM) buffered at pH 6.0 in the presence of 10 mM (open square) and 78 mM Na^+ (closed triangle). PBS pH 7.4 was used as acceptor phase. Data are presented as mean $\pm$ SD ($n=3$). **B:** The non-linear correlation of the Flux_{ss} vs the donor concentration in the presence of 10 mM (open square) and 78 mM Na^+ (closed triangle) in the donor phase, buffered at pH 6.0. A current density of $500 \mu\text{A.cm}^{-2}$ was applied and PBS pH 7.4 was used as acceptor phase. Data are presented as mean $\pm$ SD ($n=3-6$).

Na^+ was present in the donor phase. Reduction of the amount Na^+ in the donor phase augments the flux of 5-OH-DPAT dramatically. These results show a clear steady state flux when 78 mM Na^+ is present in the donor phase. However when the amount Na^+ present in the donor phase is 10 mM the flux is reduced gradually during the iontophoretic period.

As shown in Figure 2B non-linear regression analysis showed a hyperbolic correlation between the Flux_{ss} vs donor concentration when the Na^+ content in the donor phase was 10 mM ($R^2=0.997$) and 78 mM ($R^2=0.994$). In the presence of NaCl the Flux_{ss} of 5-OH-DPAT gradually increased from 9.4 ± 0.3 (0.15 mM 5-OH-DPAT) to 775.0 ± 28.4 nmol.cm⁻².h⁻¹ (31.4 mM 5-OH-DPAT). In the absence of NaCl there was an increase in Flux_{ss} of 5-OH-DPAT from 25.7 ± 2.8 (0.15 mM 5-OH-DPAT) to 1081.9 ± 377.8 nmol.cm⁻².h⁻¹ (35.1 mM 5-OH-DPAT). Comparing the 5-OH-DPAT Flux_{ss} at equal donor concentrations, an enhancement factor varying between 2.5 and 3.0 is observed when the amount of competing co-ions in the donor phase was reduced. As presented in Table 1 the addition of 68 mM NaCl to the donor solution has no influence on the absolute value of the electroosmotic flux of 3.9 mM 5-OH-DPAT in a donor solution, buffered at pH 6.0 ($p>0.05$).

Table 2: Comparison of the steady state flux of 5-OH-DPAT when using PBS pH 7.4 or PBS pH 6.2 as acceptor phase at different donor concentrations. The donor phase consisted of 5-OH-DPAT buffered at pH 5.0, containing 78 mM Na^+ and a current density of 500 $\mu\text{A.cm}^{-2}$ was applied. Results are presented as mean $\pm$ SD (n=4-9).

	PBS pH 7.4	PBS pH 6.2
Donor concentration	Flux_{ss}	Flux_{ss}
(mM)	(nmol.cm ⁻² .h ⁻¹)	(nmol.cm ⁻² .h ⁻¹)
1.5	69.8 ± 7.2	68.2 ± 8.2
3.9	211.9 ± 11.1^a	137.7 ± 8.6^a
7.0	358.9 ± 28.5^b	208.9 ± 10.2^b

a, b: t-test: $p<0.05$

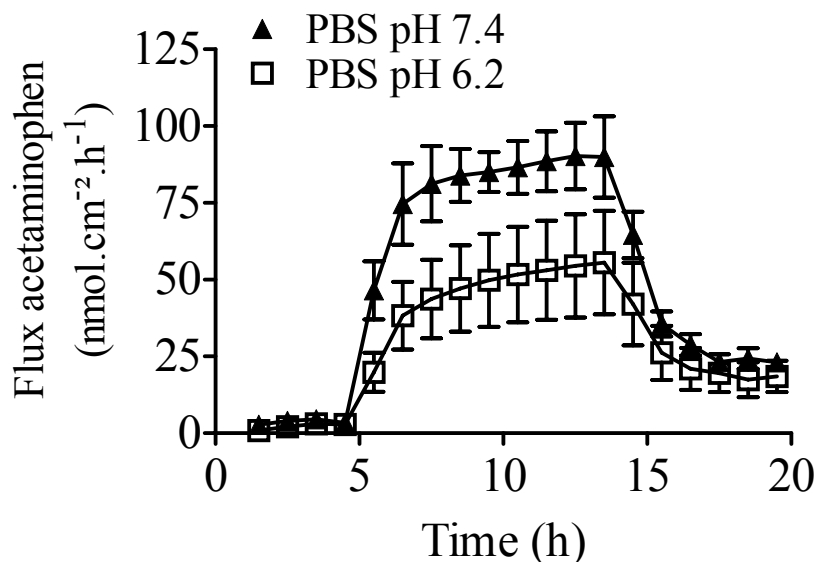


Figure 3: The acetaminophen flux vs time profiles, during iontophoresis using PBS pH 7.4 (closed triangle) and PBS pH 6.2 (open square) as acceptor phase. A buffered donor solution (pH 6.0) containing 15 mM acetaminophen was used, applying a current density of 500 $\mu\text{A}.\text{cm}^{-2}$. Data are presented as mean $\pm$ SD ($n=3-4$).

3.3.3 Influence of the pH of the acceptor phase on the 5-OH DPAT iontophoretic flux

All 5-OH-DPAT steady state fluxes (Flux_{ss}) during iontophoresis with different acceptor phase are summarized in Table 2. At a concentration of 1.5 mM 5-OH-DPAT no difference in steady state flux was observed ($p>0.05$), however at increased 5-OH-DPAT concentrations of 3.9 mM and 7.0 mM an acceptor phase at pH 6.2 resulted in a dramatically lower steady state flux ($p<0.001$) compared to the 5-OH-DPAT iontophoretic Flux_{ss} using an acceptor phase at pH 7.4.

As the pH value of the acceptor phase had a strong affect on the 5-OH-DPAT iontophoretic flux, several studies were performed to unravel the underlying mechanism. A reduction in the pH in the acceptor phase may reduce the charge density of the HSC and therefore reduce its permselectivity. The permselectivity with an acceptor phase of either pH 7.4 or pH 6.2 was studied by iontophoretic transport of acetaminophen. A reduction in pH of the acceptor phase from pH 7.4 to pH 6.2

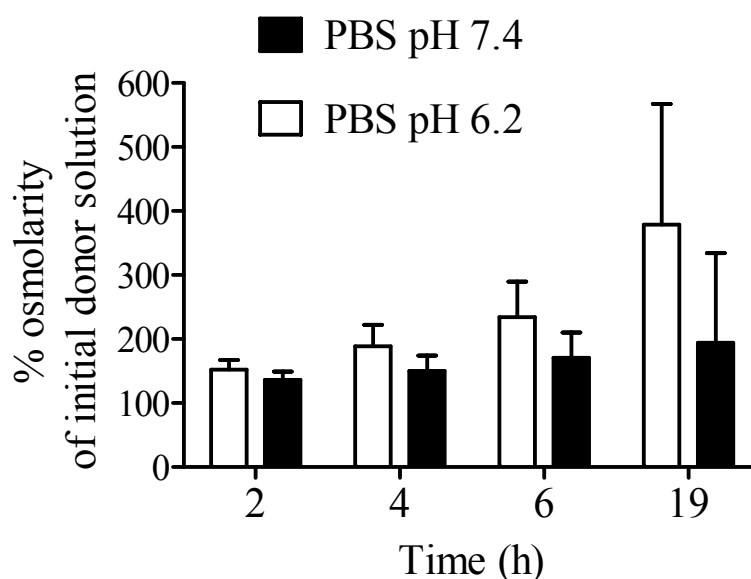


Figure 4: Bar plot of the osmolarity of the donor phase at different time points when using PBS pH 6.2 (white bar) or PBS pH 7.4 (black bar) as acceptor phase. The donor solution, buffered at pH 6, contained 10 mM Na⁺, a salt bridge was used and a current density of 500 $\mu\text{A}.\text{cm}^{-2}$ was applied. Data are presented as mean $\pm$ SD (n=4-5).

resulted in a decrease in the Flux_{ss} of acetaminophen from $86.4 \pm 5.5 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$ to $51.2 \pm 16.1 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$ ($p < 0.05$) (Figure 3), demonstrating that the permselectivity of the stratum corneum is indeed reduced at the acceptor phase of 6.2. The flux pattern remained unaffected.

A change in the permselective properties may influence the flux of ions during iontophoresis across the stratum corneum from donor to acceptor phase and from acceptor to donor phase and therefore affect the osmolarity in the donor phase when changing the pH in the acceptor phase from 7.4 to 6.2. The osmolarity of the donor phase was measured using the same iontophoretic conditions as in the studies described in the previous section. The osmolarity expressed as % osmolarity of the original donor solution, at different time points during current application, is depicted in Figure 4. Simultaneous linear regression of osmolarity vs time shows a good correlation for both conditions ($R^2 = 0.8642$ for pH 7.4 and $R^2 = 0.9835$ for pH 6.2) and shows a significant difference between the slopes of both regression lines (t-test; $p < 0.05$). Decreasing the pH of the acceptor phase from 7.4 to 6.2 causes a higher increase in osmolarity in the donor phase.

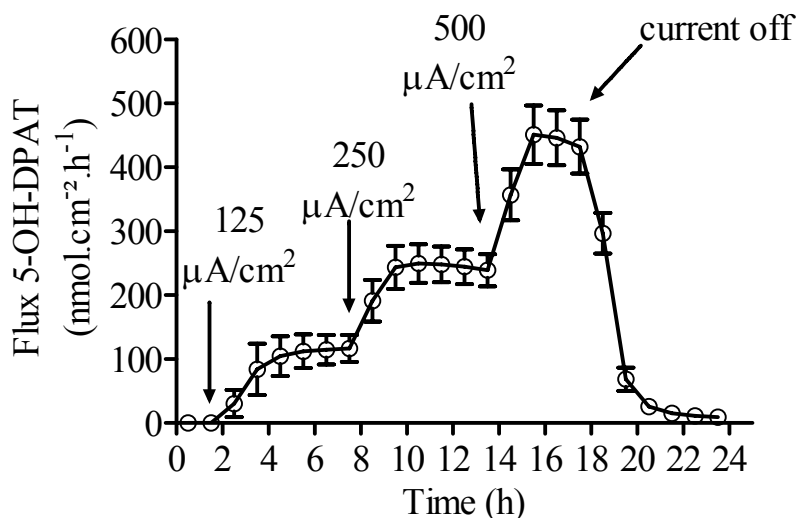


Figure 5: Iontophoretic flux vs time profile of 5-OH-DPAT, using a salt bridge. The following protocol was used: 2h passive+ 6h 125 $\mu\text{A}.\text{cm}^{-2}$ + 6h 250 $\mu\text{A}.\text{cm}^{-2}$ + 4h 500 $\mu\text{A}.\text{cm}^{-2}$ + 6h passive. The anodal compartment consisted of 3.9 mM 5-OH-DPAT in citric buffer at pH 6, containing 10 mM Na^+ and the acceptor compartment consisted of PBS pH 7.4. Data are presented as mean $\pm$ SD (n=6).

3.3.4 Effect of current density on iontophoretic transport of 5-OH-DPAT

The relationship between the Flux_{ss} and the current density was studied when 3.9 mM 5-OH-DPAT and 10 mM Na^+ were present in the donor compartment (pH=6.0). During iontophoretic transport studies the current density was increased using 6h time intervals from 125 $\mu\text{A}.\text{cm}^{-2}$ to 250 $\mu\text{A}.\text{cm}^{-2}$ and finally to a current density of 500 $\mu\text{A}.\text{cm}^{-2}$. As shown in Figure 5, in which the flux vs time profile is plotted, an increase in the current density every 6h resulted in a significant increase in flux, which reaches steady state within the 6h of iontophoresis. A current density of 125, 250 and 500 $\mu\text{A}.\text{cm}^{-2}$ resulted in a Flux_{ss} of $116.9 \pm 20.9 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$, $239.2 \pm 25.2 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$ and $432.3 \pm 42.2 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$, respectively. Linear regression analysis of the steady state flux vs the current density, showed an excellent linear correlation ($R^2 = 0.996$).

4 Discussion

The principal transport mechanisms during iontophoresis of small charged medium hydrophilic compounds, like 5-OH-DPAT, are electroosmosis and electromigration. To calculate the relative contribution of electroosmosis, neutral hydrophilic marker molecules are used. The first aim of this study is to evaluate acetaminophen as marker molecule for the electroosmotic flow. The second aim is to investigate the transport mechanisms of iontophoretic delivery of 5-OH-DPAT and to optimize the iontophoretic delivery of this potent dopamine agonist.

4.1 Acetaminophen as marker molecule for electroosmosis

The hydrophilic neutral permeant, acetaminophen, is a generally accepted marker for quantification of the electroosmotic contribution to the iontophoretic delivery of different compounds [17-18, 23-25]. When a marker molecule is used to quantify the electroosmotic flow 3 assumptions are made: (i) the compound of interest and the marker molecule are transported by the convective flow in the same manner, (ii) the flux of the marker molecule is proportional to its concentration in the donor compartment and (iii) the marker molecule does not influence the flux of the drug of interest [20, 24]. If the molecular weight of the compound of interest is in the same range as the marker molecule ($< 2 \times$ Mw of acetaminophen), it is reasonable to assume that both molecules are transported similarly by the current induced solvent flow.

First with respect to the proportionality of the steady state flux of acetaminophen to the donor concentration our data show that the Flux_{ss} of acetaminophen is linearly proportional to its donor concentration ($R^2=0.995$). According to Equation 1, these results show that the water flow, V_w , quantified by the Flux_{ss} of acetaminophen divided by the donor concentration, remains constant in the selected concentration range.

Secondly with respect to the effect of acetaminophen on the 5-OH-DPAT iontophoretic transport, acetaminophen did not affect the iontophoretic 5-OH-DPAT transport. Our results show that acetaminophen is an excellent marker molecule to determine the electroosmotic flow contribution to the iontophoretic transport of 5 OH-DPAT.

4.2 Electromigrative and electroosmotic contribution

In subsequent studies acetaminophen was used as a marker molecule to determine the contribution of the osmotic flow to the total iontophoretic 5-OH-DPAT transport.

At a pH of 6.0 the relative contributions of the J_{EO} and J_{EM} were estimated between 6.5-13.5% and between 86.5-93.2%, respectively. Reducing the pH of the donor phase from 6.0 to 5.0 gave a slight but not statistically significant decrease in the electroosmotic transport of 5-OH-DPAT. Furthermore, 5-OH-DPAT did not inhibit the electroosmotic flow across the skin during iontophoresis. Even when a salt bridge is used and a high amount of 5-OH-DPAT is transported across the skin, no change in electroosmotic flux was observed, which can be explained by the relative high hydrophilicity of 5-OH-DPAT [6, 26-27].

4.3 Effect of competing ions in the donor phase

Addition of NaCl to the donor solution is required for feeding the electrochemical reaction. However, Na^+ competes with 5-OH-DPAT as a carrier of charge transfer for iontophoretic transport through the skin. The steady-state flux of 5-OH-DPAT reduced dramatically when the NaCl concentration was doubled from 70 to 140 mM [13]. The reduction of Na^+ level in the donor phase from 78 mM to 10 mM resulted in a 2.5 to 3 fold increase in 5-OH-DPAT Flux_{ss} . This increase in transport can be attributed to an increase in the electromigration, since the electroosmosis was unaffected by the amount of Na^+ present in the donor solution.

The steady state flux of 5-OH-DPAT reaches a plateau towards the maximum solubility of 5-OH-DPAT in the donor phase (Figure 2B). When using low concentrations 5-OH-DPAT a dependency of the transport number on the donor concentration can still be observed. The plateau towards the maximum Flux_{ss} , depending on the physicochemical properties of 5-OH-DPAT is reached at lower donor concentrations when competition of co-ions was reduced [28]. Thereby focusing on the flux pattern during iontophoresis, as shown in Figure 2A, a concentration of 1.5 mM 5-OH-DPAT shows a steady state flux when 78 mM Na^+ is present in the donor phase. However when Na^+ concentration is reduced to 10 mM, the steady state 5-OH-DPAT flux is reduced gradually during the 6h iontophoresis period, which can be explained by a depletion of 5-OH-DPAT in the donor phase due to the high 5-OH-DPAT transport after the iontophoretic phase: almost 40.2 % has been transported through HSC.

The good correlation between the Flux_{ss} and the current density (Figure 5) and between the Flux_{ss} and donor concentration in presence and absence of NaCl in the donor phase are very important for the therapeutic treatment of Parkinson's disease. By modulation of these two parameters it will be possible to titrate the administered dose of this dopamine agonist, adjusted to the demand of the patient and to account for the inter- and intra-individual variability.

In the assumption that the same flux can be achieved *in vivo* and *in vitro*, it is possible to deliver approximately $1.00 \mu\text{mol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$. Previous studies showed that 5-OH-DPAT is more potent compared to rotigotine [12, 29]. Therefore it is reasonable to assume that the required dose of 5-OH-DPAT equal or lower than that of Rotigotine. The rotigotine patch Neupro[®] (UCB, Schwarz Pharma, Monheim, Germany) delivers 8 mg rotigotine in 24h, which corresponds with approximately 25 μmol rotigotine base ($M_w=315.45 \text{ g/mol}$) [7, 30-31]. This means that for 5-OH-DPAT (35 mM in the patch) in the presence of 68 mM NaCl and applying a current density of $500 \mu\text{A} \cdot \text{cm}^{-2}$ a patch size of approximately 2.0 cm^2 (anode and cathode patch) would be sufficient to provide the required amount of 5-OH-DPAT in combination with iontophoresis. When the patch size is increased, the donor concentration and the current density can be reduced. In addition, the use of a salt bridge results in even higher fluxes and therefore it will be possible to administer the same dose with a reduced amount of 5-OH-DPAT or a reduced current density.

4.4 Influence of the composition of the acceptor compartment

Conducting iontophoresis experiments with a different acceptor phase can provide more detailed insight in the transport mechanism during iontophoresis, important for selecting those conditions that result in the most efficient iontophoretic drug transport. Therefore a series of transport studies were conducted to compare the iontophoretic delivery of 5-OH-DPAT with a pH of 6.2 or 7.4 in the acceptor phase. In the present study the acceptor phase pH was varied in order to provide more insight in the mechanisms involved in the transport of 5-OH-DPAT. Our studies show that at a pH of 7.4 in the acceptor phase, 5-OH-DPAT is transported more efficiently than at a pH of 6.2. Similar results were found by Lai *et al.*, who observed that a decrease in acceptor pH from 7.4 to 4.5 resulted in a decrease in the iontophoretic transport of a series of local anaesthetics [32]. Whether this is due to an increase in the negative charge density in the stratum corneum and a subsequent reduction in Cl^- transport, can be studied by measuring the acetaminophen transport and osmolarity in the donor compartment. As demonstrated in Figure 3, the pH of the acceptor phase influences the permselective properties of the stratum corneum, as the flux of acetaminophen, when using an acceptor pH of 6.2 is significantly lower compared to an acceptor pH of 7.4. Whether this also increases the ion transport from acceptor to donor compartment is indirectly monitored by measuring the osmolarity in the donor compartment (Figure 4). During the iontophoretic period a higher increase in osmolarity is observed with the acceptor phase at pH 6.2 compared to that at pH 7.4, most probably due to a higher ion transport (Cl^- -ion) from acceptor to donor compartment. This results in an increased competition for charge transfer

and thus in a reduction in 5-OH-DPAT transport at pH of 6.2 compared to the transport at pH of 7.4 in the acceptor phase. As clearly shown the transport of 5-OH-DPAT is strongly affected by the composition of the acceptor phase, which is in agreement with the high sensitivity of 5-OH-DPAT iontophoretic transport for competitive ions such as Na^+ in the donor phase.

In conclusion, the research described in this paper demonstrates the high transport efficiency of 5-OH-DPAT and elucidates different mechanisms involved in its iontophoretic transport. Optimization of the composition of the donor phase resulted in high fluxes, making the drug an excellent candidate for treatment of Parkinson's disease using transdermal iontophoresis. Acetaminophen, identified as a good marker molecule for the quantification of the electroosmotic flux can be used in future transport studies to characterize the iontophoretic delivery of other molecules.

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