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

Ackaert, O.

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Comparing different salt forms of rotigotine to improve transdermal iontophoretic delivery

Oliver W. Ackaert^a, Jacob Eikelenboom^a, H-Michael Wolff^b, and Joke A. Bouwstra^a

^aDivision of Drug Delivery Technology, Leiden/Amsterdam Center for Drug Research, Leiden, The Netherlands

^bUCB Schwarz Biosciences GmbH, Monheim, Germany

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Abstract

The transdermal delivery of a new salt form of the dopamine agonist rotigotine, rotigotine.H₃PO₄ is presented and compared with rotigotine.HCl. A comparison was made on the level of solubility, passive and iontophoretic delivery. Different aspects of the delivery were investigated: delivery efficiency, maximum flux, donor pH, electroosmotic contribution and transport number. Changing the salt form from rotigotine.HCl to rotigotine.H₃PO₄ increases significantly the solubility and rules out the influence of NaCl on the solubility by the absence of the common ion effect. At low donor concentration no difference in transdermal delivery was observed between the salt forms. Due to an increase in the maximum solubility of rotigotine.H₃PO₄, a 170% increase in maximum flux, compared to rotigotine.HCl, was achieved. A balance between solubility and delivery efficiency can be obtained by choosing the correct donor pH between 5 and 6. A slight increase in electroosmotic contribution and transport number was observed. Using the parameters, determined by modeling the *in vitro* transport, *in vivo* simulations revealed that with iontophoresis therapeutic levels can be achieved with a rapid onset time and be maintained in a controlled manner by adjusting the current density.

Keywords: solubility, transdermal, iontophoresis, rotigotine, salt form, modeling

1 Introduction

Transdermal drug delivery is an attractive alternative for oral drug delivery and hypodermic injections. Different delivery methods have been investigated over the years to increase the drug delivery through the skin. One of the possibilities is iontophoresis. By applying a small current across the skin it is possible to enhance the transdermal delivery of small charged ionic molecules. One of the interesting properties of this technique is the possibility to modulate the transport rate into and through the skin. This is an important advantage for drugs with a narrow therapeutic window, such as dopamine agonists. Rotigotine is a dopamine agonist used for the symptomatic treatment of Parkinson's disease. Studies, investigating the transdermal iontophoretic delivery of rotigotine.HCl revealed that by applying an electrical current across the skin higher steady state fluxes can be achieved with a shorter lag time compared to passive delivery. However in these studies the maximum solubility of rotigotine.HCl in the donor phase appeared to be the limiting factor for its iontophoretic transport through the skin [1-3]. Increasing the solubility of rotigotine can be achieved by changing the donor solution, e.g. by adding surfactants or co-solvents or changing the source of Cl⁻-ions. Another possibility to improve the solubility is altering the salt form of the drug of interest. In transdermal iontophoretic delivery the chloride anion is often added to the donor phase at the anodal side to feed the electrochemical reaction. To increase the solubility of rotigotine, another counterion may be chosen to destabilize the crystal structure and therefore to increase the solubility of rotigotine in the buffer solution. In the current study, H₃PO₄ was chosen as an alternative salt form for rotigotine. H₃PO₄ accounts for 2% of the FDA approved NCE's in the period from 1995 to 2006, is biocompatible, has a low molecular weight and circumvents the common ion effect in the donor phase, making it a very interesting candidate as salt form for iontophoresis [4]. Moreover in literature very few reports, investigating different salt forms for transdermal delivery were described. Fang *et al.* studied the passive and iontophoretic delivery of three different salts of the anion diclofenac, showing that the counter ion can affect the transdermal delivery [5-6].

In the current study the solubility and transdermal delivery of rotigotine.H₃PO₄ were evaluated in comparison to rotigotine.HCl to illustrate the influence of the choice of a salt form on the passive and iontophoretic transport through the skin. In addition the *in vitro* transport of rotigotine.H₃PO₄ was analyzed with compartmental modeling and the parameters were used to evaluate the iontophoretic delivery *in vivo* in a series of simulations.

2 Materials and methods

2.1 Materials

Rotigotine.H₃PO₄ and rotigotine.HCl were kindly supplied by UCB Schwarz Biosciences GmbH (Monheim, Germany). Silver, silver chloride (purity>99.99%), Trypsin (Type III from bovine pancreas), trypsin inhibitor (Type II-S from soybean) and methanesulfonic acid were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Acetaminophen was purchased from Brocacef (Maarsen, The Netherlands) and D-Mannitol was obtained from Scharlau Chemie S.A. (Barcelona, Spain). Spectra/Por[®] RC dialysis membrane sheets (cut off value of 6000-8000 Da) were purchased from Spectrum laboratories, Inc (Rancho Dominguez, Ca, USA). Acetonitrile was purchased from Biosolve (Valkenswaard, the Netherlands). All other chemicals and solvents were of analytical grade. All solutions were prepared in Millipore water with a resistance of more than 18 MΩ.cm.

2.2 Solubility of rotigotine.H₃PO₄

In order to compare the maximum solubility of rotigotine.H₃PO₄ with rotigotine.HCl, the solubility studies of rotigotine.H₃PO₄ were carried out as described by Nugroho *et al.* [3], who determined the solubility of rotigotine.HCl. Briefly, rotigotine.H₃PO₄ was solubilized in 10 mM citric buffer at pH 4.0, 5.0 and 6.0 with and without the presence of NaCl. Subsequent adjustment of pH in each test tube was performed by alternately adding small quantities of 1M NaOH under continuous shaking and subsequent pH measurements, until the pH of each solution had stabilized at the original buffer value. Each solution was shaken for an additional 48h, after which each solution was centrifuged and filtered. The concentration in each solution was determined by HPLC.

2.3 Capillary electrophoresis

The electrophoretic mobility of rotigotine.HCl and rotigotine.H₃PO₄ was investigated with capillary electrophoresis (CE). These experiments were performed according to a method, described elsewhere [7]. Briefly, the electrophoretic mobility of the samples (0.5 mM) was determined in triplicate using a phosphate buffer pH 7.4 (Na₂HPO₄: 5.82 g.l⁻¹; NaH₂PO₄: 5.1 g.l⁻¹) as electrolyte solution (mobile phase).

2.4 Preparation of human stratum corneum

The preparation of human stratum corneum (HSC) was performed according to the method described previously [8]. 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} , Na_2HPO_4 : 2.86 g.L^{-1} , KH_2PO_4 : 0.2 g.L^{-1} , KCl : 0.19 g.L^{-1}) overnight at 4 $^{\circ}\text{C}$ and subsequently for 1h at 37 $^{\circ}\text{C}$ 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 N_2 environment.

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 experiments were carried out, using a three chamber continuous flow through cell as described elsewhere [8]. The donor formulation, buffered with a 10 mM citric buffer, was applied at the anodal side. The cathodal chamber was filled with PBS pH 7.4. The acceptor phase, maintained at 32 $^{\circ}\text{C}$, was continuously perfused with PBS pH 6.2 (NaCl : 8 g.L^{-1} , KCl : 0.19 g.L^{-1} , $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$: 0.43 g.L^{-1} , KH_2PO_4 : 0.97 g.L^{-1}) at a flow rate of 7.0 ml.h^{-1} . Unless described elsewhere, the following protocol for the iontophoresis experiments was used: 6h of passive diffusion, followed by 9h of iontophoresis with a current density of 500 $\mu\text{A.cm}^{-2}$ and 5h of passive diffusion. Samples were collected every hour with an automatic fraction collector (ISCO Retriever IV, Beun De Ronde BV, Abcoude, The Netherlands). The specific conditions of the individual transport studies are described below.

2.5.1 Flux of rotigotine. H_3PO_4 vs rotigotine.HCl

The donor concentration of rotigotine. H_3PO_4 and rotigotine.HCl was kept constant (3.7 mM). The donor phase was buffered at pH 5.0 and contained 68 mM NaCl.

2.5.2 Flux_{ss} vs donor concentration rotigotine. H_3PO_4 and influence of pH

In these transport studies 4 different concentrations rotigotine. H_3PO_4 (4.4 mM, 9.5 mM, 22.2 mM and 47.5 mM), buffered at pH 5.0, were used. Thereby the transport of rotigotine. H_3PO_4 (4.4 and 13.0 mM), buffered at pH 6.0 was investigated as well.

All transport experiments were performed in the presence of 68 mM NaCl in the donor phase.

2.5.3 Electroosmotic flow

The donor concentration of rotigotine.H₃PO₄ was 3.9 mM and 11.6 mM. Acetaminophen (15 mM) was added to the donor phase as a marker for the electroosmotic flow. The donor phase was buffered at pH 5.0 and contained 68 mM NaCl.

2.5.4 Current density flux-relationship

The relationship between the current density was studied with a rotigotine.H₃PO₄ concentration of 31.3 mM, buffered with a citric buffer at pH 5.5, containing 68 mM NaCl. The following protocol was used: 6h passive+ 6h 166 $\mu\text{A.cm}^{-2}$ + 6h 333 $\mu\text{A.cm}^{-2}$ + 6h 500 $\mu\text{A.cm}^{-2}$ + 6h passive.

2.6 Analytical method

Prior to and at the end of a transport study the pH of donor and acceptor compartment was 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). Rotigotine was detected using a scanning fluorescence detector (Waters[™] 474, Millipore, Milford, MA, USA) at excitation and emission wavelengths of 276 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 60% H₂O (v/v), 40% ACN (v/v) and 0.05% methanesulfonic acid (v/v). The injection volume was 50 μL and the flow rate was set to 1.0 mL.min⁻¹.

The concentration of rotigotine was quantified according to 3 standards with a concentration of 0.005, 2 and 5 $\mu\text{g.mL}^{-1}$. The intra-assay variation of the retention time and of the area was less than 2.0%. For acetaminophen, calibration curves showed a linear response when using concentrations of compounds between 0.1 and 40 $\mu\text{g.mL}^{-1}$ ($R^2 > 0.9999$). The limit of detection (LOD) and limit of quantification (LOQ) of acetaminophen were experimentally determined at 5.8 and 9.6 ng.mL⁻¹ respectively. According to literature the limit of detection of rotigotine (base) was 11 ng.mL⁻¹ [2].

2.7 Determination of the passive, electroosmotic and electromigrative flux

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 neutral permeant acetaminophen was added to the donor solution as a marker molecule for quantification of the electroosmotic flow during iontophoretic transport. The electromigrative flux is calculated by subtracting the passive and electroosmotic flux from the total flux.

2.8 Data analysis

To calculate the steady state flux during passive and iontophoretic transport, the cumulative flux of the transport was plotted as a function of time. The steady state flux was estimated from the linear part of the slope of this plot according to the permeation lag-time method [8]. All data are presented as mean $\pm$ standard deviation (SD). When a statistical analysis was performed comparing only 2 groups, a Student's t-test was used. When 3 or more groups were compared, a 1-way ANOVA statistical analysis was executed. If the overall p-value was less than 0.05, a bonferonni post-test was applied to compare different groups. For all statistical analysis a significance level of $p < 0.05$ was used.

2.9 *in vitro* modeling and *in vivo* simulation

Next to the permeation lag-time method the data of the *in vitro* transport of rotigotine.H₃PO₄ at the selected condition (donor pH 5.0, donor conc 47 mM) was analyzed using non-linear mixed effects modeling with the compartmental models described elsewhere [9]. Briefly, in these models the starting point was a zero order mass transport from the donor solution into the skin during and after iontophoresis. The equations, to describe *in vitro* iontophoretic transport during current application:

$$J(t) = \frac{I_0}{S} (1 - e^{-K_R \cdot (t-t_L)}) \quad (1)$$

$$J_{ss} = \frac{I_0}{S} \quad (2)$$

$$J(t) = \frac{P_{PI}}{S} (1 - e^{-K_R \cdot (t-t_L)}) + \frac{I_0}{S} (1 - e^{-K_R \cdot (T-t_L)}) \cdot e^{-K_R \cdot (t-T)} \quad (3)$$

Where $J(t)$ is the flux at time t and S is the diffusion area, K_R is a first order skin release rate constant, I_0 the zero-order iontophoretic mass transfer from the donor compartment into the skin compartment during current application, t_L is the kinetic lag time parameter, introduced to address the time required for drug molecules to enter the skin compartment, T is time of current application and P_{PI} is the zero order drug input due to the passive driving force in the post iontophoretic period. Fitting the data was performed using the subroutines ADVANCE6 TRANS1 TOL=5 from PREDPP in NONMEM (NONMEM version VI). Interindividual variability was modeled using an exponential error model and the residual error was characterized by an exponential and additive error model. The estimates of the population parameters were performed using a conventional first order method. The model was evaluated graphically by plotting the population predicted vs the observed flux and the individual predicted vs the observed flux. Finally, from the final parameter estimates a number of 100 samples were simulated. Post processing of NONMEM simulations creating plots of visual predictive check (VPC) was done using xpose 4, implemented in the software R (R version 2.7.0, R-foundation) [10].

The pharmacokinetic model used to simulate the iontophoretic delivery of rotigotine was based on a model, described elsewhere [9]. Assuming a 1-compartment model, different protocols were investigated for simulating the pharmacokinetic profile of iontophoretic delivery of rotigotine.H₃PO₄ during 24h using a patch size of 10 cm²:

Protocol 1: 24h 350 $\mu\text{A}.\text{cm}^{-2}$

Protocol 2: 5h 350 $\mu\text{A}.\text{cm}^{-2}$ + 19 h 150 $\mu\text{A}.\text{cm}^{-2}$

At time=24h the patch was removed. In this case the simulations were performed with the \$SIMULATION function provided in NONMEM. A population of 5 subjects was simulated and the simulated population prediction was displayed graphically.

3 Results

3.1 Solubility and electrophoretic mobility of rotigotine.H₃PO₄

The results of the solubility assay of rotigotine.H₃PO₄ are presented in Table 1. At the selected pH values the addition of NaCl did not affect significantly the solubility of rotigotine.H₃PO₄ (2-way ANOVA; $p>0.05$). This is in contrast with the results obtained with rotigotine.HCl by Nugroho *et al.* [3]. For rotigotine.HCl the solubility

Table 1: The solubility of Rotigotine.H₃PO₄ and Rotigotine.HCl in different medium at pH 4.0, 5.0 and 6.0 in the presence and absence of 68 mM NaCl (n=2-3).

pH	Rotigotine.H ₃ PO ₄		Rotigotine.HCl*	
	No NaCl	68 mM NaCl	No NaCl	68 mM NaCl
	mM	mM	mM	mM
	(mean)	(mean)	(mean ± SD)	(mean ± SD)
4.00	83.48 ^a	80.08 ^c	24.39 ± 4.66 ^{a,b}	6.75 ± 0.31 ^{b,c}
5.00	41.89 ^a	42.95 ^c	22.45 ± 0.63 ^{a,b}	6.35 ± 0.34 ^{b,c}
6.00	15.67	14.44 ^d	15.87 ± 1.25 ^b	6.52 ± 0.17 ^{b,d}

* values obtained from literature: [3]

a-c: p<0.001; d: p<0.01

reduced tremendously after adding 68 mM NaCl. Furthermore decreasing the pH of the donor phase from 6.0 to 5.0 and again to pH 4.0 resulted in a significant increase in the solubility of rotigotine.H₃PO₄ (2-way ANOVA; p<0.05). The electrophoretic mobility of rotigotine.H₃PO₄ ($1.53 \pm 0.02 \times 10^{-4} \text{ cm}^2 \cdot \text{s}^{-1} \cdot \text{V}^{-1}$) showed no significant difference with the electrophoretic mobility of rotigotine.HCl ($1.49 \pm 0.04 \times 10^{-4} \text{ cm}^2 \cdot \text{s}^{-1} \cdot \text{V}^{-1}$) (t-test; p=0.134).

3.2 Passive diffusion of rotigotine.H₃PO₄

A series of iontophoretic transport studies under various conditions were performed. During 6h prior to iontophoresis no current was applied and passive transport reached steady state conditions within this period. From the slope of the linear part of the cumulative flux vs time profile the passive steady state flux (Flux_{pss}) was calculated. Firstly, rotigotine.H₃PO₄ and rotigotine.HCl both at a concentration of 3.7 mM, buffered at pH 5.0, showed no significant difference in Flux_{pss} (t-test; p>0.05) (Table 2). Secondly, the results of transport studies of rotigotine.H₃PO₄ at various donor concentrations, comparing a donor pH of 5.0 and 6.0 are depicted in Figure 1. A non-linear hyperbolic fit showed a correlation between the Flux_{pss} and the donor concentration at pH 5.0 ($R^2=0.889$). Increasing the pH of the donor phase from 5.0 to

Table 2: Comparison of the steady state passive flux (Flux_{pss}) and the steady state iontophoretic flux (Flux_{ss}) of Rotigotine. H_3PO_4 with Rotigotine.HCl at pH 5.0 in the presence of 68 mM NaCl. Results are presented as mean $\pm$ SD (n=3-4).

		Flux_{pss}	Flux_{ss}
	mM	nmol. $\text{cm}^{-2}.\text{h}^{-1}$ (mean $\pm$ SD)	nmol. $\text{cm}^{-2}.\text{h}^{-1}$ (mean $\pm$ SD)
Rotigotine.HCl	3.77	1.7 ± 0.8	69.4 ± 6.4
Rotigotine. H_3PO_4	3.74	1.6 ± 0.4	73.8 ± 12.6

6.0 increased the passive flux of rotigotine. H_3PO_4 quite drastically: Close to saturation of rotigotine. H_3PO_4 in the donor phase the maximum flux that could be achieved was $10.8 \pm 1.9 \text{ nmol.cm}^{-2}.\text{h}^{-1}$ at pH 5 and $24.9 \pm 2.5 \text{ nmol.cm}^{-2}.\text{h}^{-1}$ at pH 6.0.

3.3 Iontophoresis of rotigotine. H_3PO_4

A series of iontophoretic transport studies was conducted to investigate the iontophoretic transport of rotigotine. H_3PO_4 specially focusing on: (i) comparison with the iontophoretic flux of rotigotine.HCl, (ii) the relationship between the flux and donor concentration, (iii) the influence of the pH, (iv) the contribution of the electroosmotic flow and (v) the determination of the transport number.

3.3.1 Flux of rotigotine. H_3PO_4 vs rotigotine.HCl

During the transport studies after 6h of passive diffusion a current density was applied of $500 \mu\text{A.cm}^{-2}$ during 9h. The steady state flux (Flux_{ss}) of rotigotine. H_3PO_4 during iontophoresis showed no significant difference to the Flux_{ss} of rotigotine.HCl ($p > 0.05$) (Table 2).

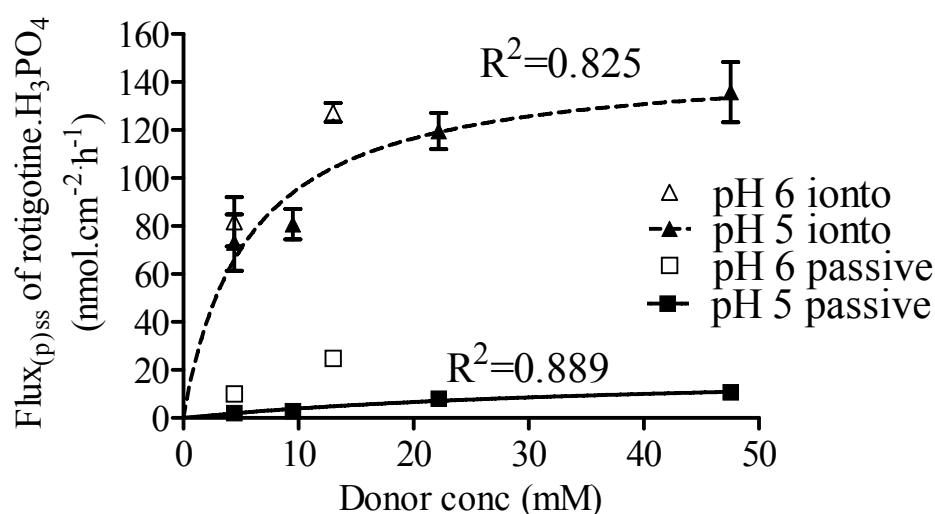


Figure 1: The $\text{Flux}_{(p)ss}$ of rotigotine. H_3PO_4 during the passive phase (square) (no current) and the iontophoretic phase (triangle) (current density= $500 \mu\text{A}.\text{cm}^{-2}$) at various donor concentrations at donor pH 5.0 (closed) and pH 6.0 (open). The correlation of the $\text{Flux}_{(p)ss}$ vs donor concentration was determined at pH 5.0. The line of correlation of passive Flux_{pss} vs donor concentration is full and the line of correlation of the iontophoretic Flux_{ss} vs donor concentration is intermittent. Data are presented as mean $\pm$ SD ($n=3$)

3.3.2 Flux_{ss} as function of donor concentration rotigotine. H_3PO_4 and influence of pH in donor formulation

As shown in Figure 2, current application resulted in an immediate increase in the flux of rotigotine. H_3PO_4 , which reached steady state within 4h. The results of a series of iontophoretic transport studies of rotigotine. H_3PO_4 at pH 5.0 and pH 6.0 are depicted in Figure 1. A non-linear relationship could be described between the Flux_{ss} and the donor concentration at pH 5.0 ($R^2=0.825$). Thereby the Flux_{ss} at equal rotigotine. H_3PO_4 concentration increased with increasing pH of the donor solution. However, close to saturation in the donor phase, the Flux_{ss} at pH 5.0 ($135.8 \pm 12.5 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$) was not significantly different from the Flux at pH 6.0 ($127.3 \pm 4.0 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$) (t-test; $p>0.05$).

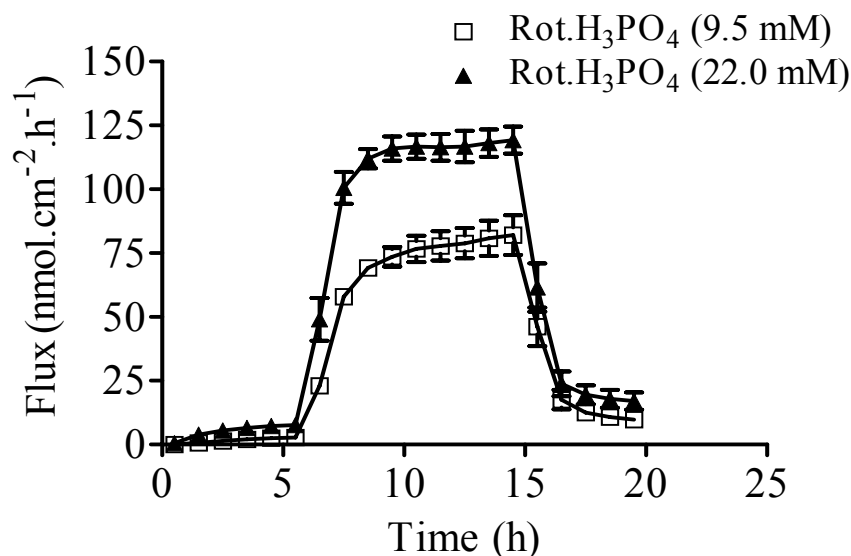


Figure 2: The iontophoretic flux vs time profile of rotigotine.H₃PO₄ dissolved in citric buffer pH 5 at 2 different concentrations, 9.5 mM (open square) and 22.0 mM (closed triangle). Data are presented as mean $\pm$ SD (n=3)

3.3.3 Electroosmotic contribution

To quantify the electroosmotic contribution acetaminophen was co-transported with rotigotine. The electroosmotic flux was investigated at two different concentrations rotigotine.H₃PO₄ (3.9 and 11.6 mM) at pH 5.0 in the donor phase. The relative contributions of J_{pass} , J_{EO} and J_{EM} are depicted in Figure 3. With increasing donor concentrations the relative contribution of the passive flux significantly increased from $13.1 \pm 0.1\%$ for 3.9 mM to $17.6 \pm 0.1\%$ for 11.6 mM (t-test; $p < 0.05$). An increase in rotigotine.H₃PO₄ concentration from 3.9 mM to 11.6 mM resulted in a significant decrease in the relative contribution of the electroosmotic flow from $5.1 \pm 0.1\%$ to $2.8 \pm 0.3\%$ (t-test; $p < 0.05$). Electromigration was the main driving force and accounted for 81.8 ± 0.03 and $79.5 \pm 0.1\%$ of the total flux of 3.9 mM and 11.6 mM rotigotine.H₃PO₄, respectively.

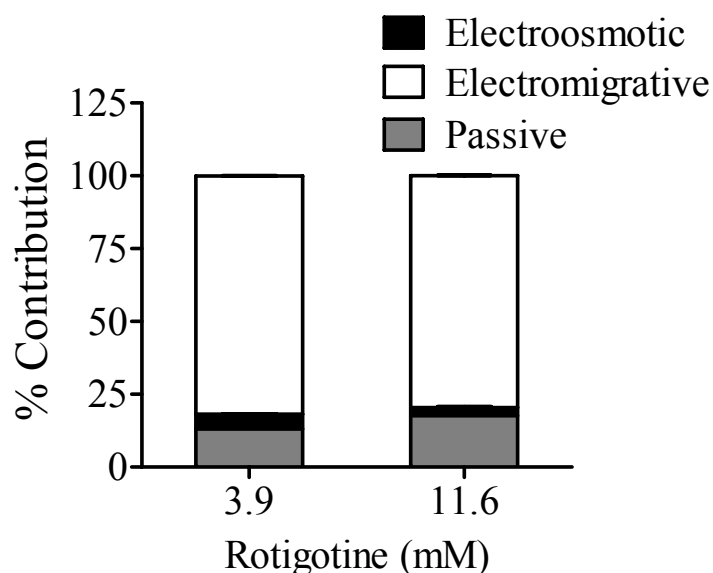


Figure 3: The relative contribution of the passive (grey), electroosmotic (black) and electromigrative (white) flux to the total (100%) flux of rotigotine.H₃PO₄ at 2 different concentrations (3.9 and 11.6 mM). The donor phase was buffered at pH 5 and the applied current density was 500 $\mu\text{A}.\text{cm}^{-2}$. Data are presented as mean $\pm$ SD (n=3)

3.3.4 Determination of transport number

With increasing pH from 5.0 to 6.0 a decrease in solubility, but an increase in delivery efficiency was observed. At pH 5.5 a balance is expected between maximum solubility and delivery efficiency. At this selected pH under near to saturated conditions the transport number was determined. Therefore in a single experiment the relationship between the Flux_{ss} and the current density was studied with a donor phase containing 31.3 mM rotigotine.H₃PO₄, which was 90% of the maximum solubility of rotigotine.H₃PO₄.

An increase in the current density resulted in a significant increase in flux, which reached steady state within 6h of current application. A current density of 0 $\mu\text{A}.\text{cm}^{-2}$ (passive phase), 166 $\mu\text{A}.\text{cm}^{-2}$, 333 $\mu\text{A}.\text{cm}^{-2}$ and 500 $\mu\text{A}.\text{cm}^{-2}$ resulted in a Flux_{ss} of $24.4 \pm 1.9 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$, $65.8 \pm 9.3 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$, $109.7 \pm 15.7 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$ and $154.5 \pm 27.0 \text{ nmol}.\text{cm}^{-2}.\text{h}^{-1}$, respectively. An excellent linear correlation could be observed between the Flux_{ss} and the current density ($R^2=0.999$). The transport number was calculated from the slope of the correlation at 0.7%.

Table 3: Best fit-results of the estimated parameters, steady state flux (Flux_{ss}) release constant (K_R), the lag time (t_L) and the passive flux post iontophoresis (pass) and comparison to the value obtained with the permeation lag time method. The data are presented as mean and standard error of the mean (SEM). Furthermore values of the elimination constant (k_e), clearance (Cl) and apparent volume of distribution, considering the bioavailability (V_d/F) of rotigotine as found in literature, are depicted.

	Unit	Model prediction		Permeation- lag time method		Literature values [*]
		Best fit value	SEM	Best fit value	SEM	
Flux_{ss}	$\text{nmol.cm}^{-2}.\text{h}^{-1}$	135	3.56	135.8	4.18	
K_R	h^{-1}	1.08	0.04	n.d.	n.d.	
t_L	h^{-1}	negligible		n.d.	n.d.	
pass	h	12.3	0.4	n.d.	n.d.	
k_e	h^{-1}					0.125
Cl	L.h^{-1}					450
V_d/F	L					3600

*values obtained from ref. [11-17]

n.d. not determined

3.4 Modeling *in vitro* iontophoretic transport of rotigotine. H_3PO_4

The *in vitro* iontophoretic transport across HSC was analyzed using compartment modeling. The kinetic model to describe the flux was based on zero-order mass transport from donor to skin and a first order release from skin to acceptor. The best-fit results and the standard error of the mean (SEM) of Flux_{ss} , the first order release constant (K_R), the lag time (t_L) and the passive flux post iontophoresis (pass) can be found in Table 3. No significant difference could be observed between the best fit of Flux_{ss} , estimated by the compartmental model and by the permeation lag-time method (t-test; $p > 0.05$). The quality of fitting and model parameter estimation was evaluated graphically. The diagnostic plots of the model can be found in Figure 4. The population predicted (PRED) vs the observed flux (Figure 4A) and the

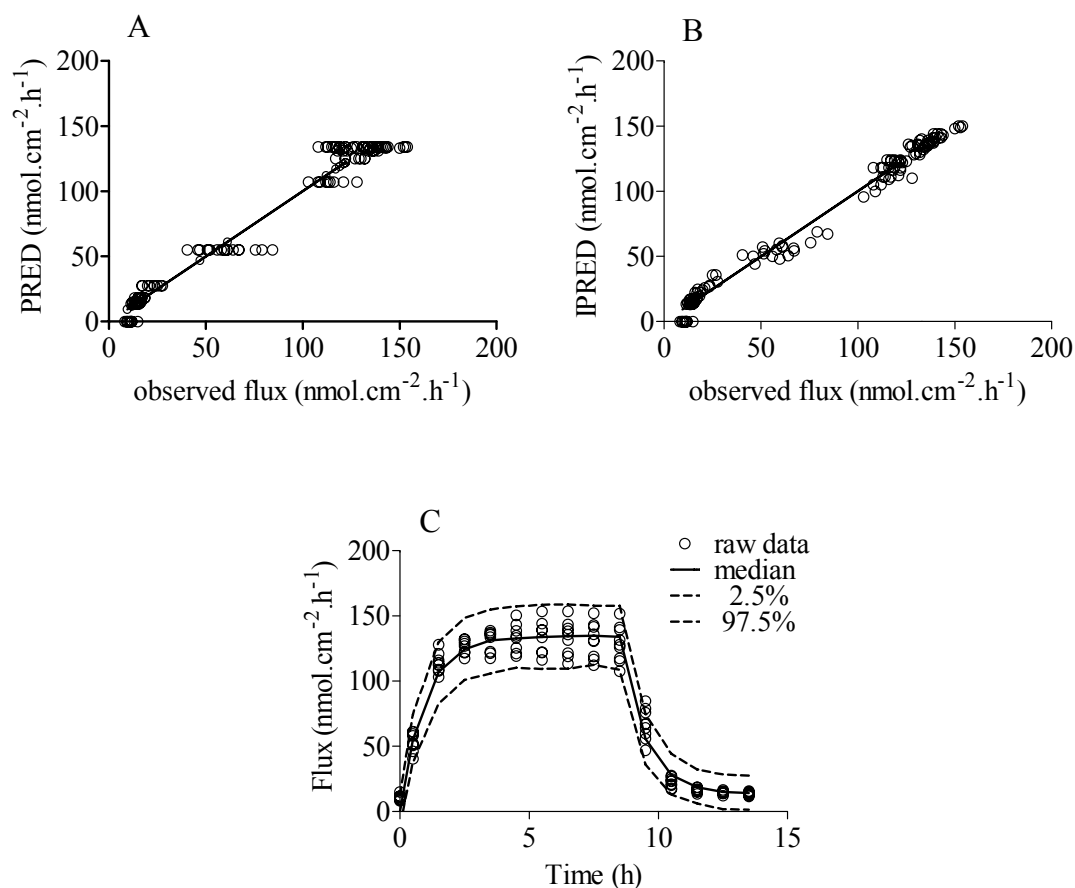


Figure 4: Diagnostic plots of the compartmental modeling of the *in vitro* iontophoretic transport of rotigotine (47 mM), buffered at pH 5. **A:** plot of the population predicted flux (PRED) vs the observed flux. **B:** plot of the individual predicted flux (IPRED) vs the observed flux. **C:** Visual predictive check of the *in vitro* model: raw data superimposed on median, 2.5th and 97.5th percentiles of data simulated from model. Median (full line); 2.5th and 97.5th percentile (dashed line; raw data (open circle))

individual predicted (IPRED) vs the observed flux (Figure 4B) show that the model can describe adequately the *in vitro* iontophoretic transport. Moreover the visual predictive check, displayed in Figure 4C demonstrates that the observed data are well distributed in the 95% confidence interval of the simulated data. This suggests that the variability, predicted by the model, represents the variability of the actual observed data.

4 Discussion

4.1 Solubility

In a previous study one of the major limitations in the iontophoretic transport of rotigotine.HCl was its low solubility. The maximum solubility of rotigotine.HCl was only 7.1 ± 0.4 mM at pH 5.0. In that study the iontophoretic transport at varying rotigotine.HCl concentrations between 1.4 and 3.9 mM showed a linear relationship between the Flux_{ss} and the donor concentration [2]. This demonstrated that the maximum iontophoretic flux of rotigotine was not yet achieved, but the low solubility of rotigotine.HCl was the limiting factor for further increasing the iontophoretic flux. Higher donor concentrations could be achieved by replacing HCl by another salt [20-21]. As shown in Table 1, in the presence of 68 mM NaCl, the solubility of rotigotine increased substantially when HCl was replaced by H_3PO_4 . Compared to rotigotine.HCl the solubility of rotigotine. H_3PO_4 is 2, 6, 10 fold higher at pH 6.0, 5.0 and 4.0, respectively. Furthermore in contrast to the solubility of the HCl-salt the presence of NaCl did not affect the solubility of rotigotine. H_3PO_4 . This can be explained by an absence of the common ion effect. The next step was to investigate the transdermal delivery of rotigotine as H_3PO_4 salt.

4.2 *in vitro* transdermal delivery

The transdermal passive delivery of rotigotine. H_3PO_4 (3.77 mM) was investigated in comparison with rotigotine.HCl at the same donor concentration (3.74 mM). At pH 5.0 no difference in passive delivery could be observed. The solubility of rotigotine.HCl at pH 5.0 was lower compared to the solubility of rotigotine. H_3PO_4 . The higher thermodynamic activity of rotigotine.HCl under these conditions was expected to result in a higher passive flux for rotigotine.HCl [22]. It seems that replacement of HCl by H_3PO_4 in the rotigotine salt not only increases the solubility of rotigotine, but also increases the efficiency in passive transport of rotigotine across HSC. In analogy to the passive flux, no difference in total iontophoretic flux could be observed between rotigotine. H_3PO_4 (3.77 mM) and rotigotine.HCl (3.74

mM). This observation is strengthened by the observed equal electrophoretic mobility of both salt forms, determined with capillary electrophoresis. This indicates that under these conditions an equal part of the current is carried by rotigotine, regardless its salt form, resulting in the same total iontophoretic flux.

The use of rotigotine.H₃PO₄ compared to that of rotigotine.HCl has two major advantages. (i) Increasing the donor pH from 5.0 to 6.0 significantly increases the passive flux of rotigotine.H₃PO₄. The maximum solubility of rotigotine.H₃PO₄ at pH 6.0 is much lower than at pH 5.0. Consecutively the thermodynamic activity at equal concentration of this compound is higher at pH 6.0, which results in an increased passive diffusion [23]. Due to this increase in passive flux, an increase in total flux during the iontophoresis period was also observed with increasing pH. In contrast, the total iontophoretic flux of rotigotine.HCl did not change when the pH was increased from 5.0 to 6.0 [3]. (ii) The second advantage is the increase in maximum solubility of rotigotine.H₃PO₄ compared to that of rotigotine.HCl. Due to a higher maximum solubility of the phosphate salt, higher donor concentrations of rotigotine can be obtained. Iontophoretic delivery of higher donor concentrations results in an increase in the maximum iontophoretic transport. At pH 5.0 the maximum iontophoretic flux of rotigotine.HCl was $80.2 \pm 14.4 \text{ nmol.cm}^{-2}.\text{h}^{-1}$, while with rotigotine.H₃PO₄ a maximum flux of $135.8 \pm 12.5 \text{ nmol.cm}^{-2}.\text{h}^{-1}$ was achieved [2]. This means that the maximum flux can be increased with 170% by replacing the HCl salt with a H₃PO₄ salt. Besides a higher flux another practical advantage can be established when using a high donor concentration at pH 5.0. Calculations revealed that after 24h, maintaining a maximum flux of $135.8 \text{ nmol.cm}^{-2}.\text{h}^{-1}$, the amount rotigotine.H₃PO₄ in the donor phase decreased with 35%. This decrease in donor concentration would result only in a decrease of 10% in steady state flux. This shows that with a high donor concentration a high flux can be maintained for a long time. Taking these results together, one must seek a balance between transport efficiency and donor concentration by choosing the pH of the donor solution. On one hand by increasing the pH it is possible to increase the transport efficiency, however the limited solubility of the compound at pH 6.0 prevents the use of a high concentration. On the other hand at pH 5.0 the transport efficiency is lower, nonetheless a high flux can be established for a long time due to the higher solubility of rotigotine.H₃PO₄.

The total flux is driven by the passive, the electromigrative and the electroosmotic flux. The electroosmotic contribution of rotigotine.H₃PO₄, estimated at 5%, is higher than the electroosmotic contribution of rotigotine.HCl, calculated at 2.3% [2]. This is mainly due to the higher concentration of rotigotine.H₃PO₄ in the donor formulation.

Nonetheless the main driving force of the iontophoretic delivery of both salt forms remains electromigration, and in case of rotigotine.H₃PO₄ accounts for approximately 80% of the total flux at two different concentrations (Figure 3). The transport number of rotigotine.H₃PO₄ at pH 5.5 was estimated from the slope of relationship between the Flux_{ss} and the current density at 0.7%. This is higher than the transport number of rotigotine.HCl (0.4%) at pH 5.0, which can be explained by a higher donor concentration of rotigotine.H₃PO₄ [2].

4.3 From *in vitro* modeling to *in vivo* simulation

After characterizing and optimizing the transdermal delivery of this promising compound *in vitro*, the potential of the iontophoretic delivery of rotigotine *in vivo* was evaluated. A series of simulations was performed, using pharmacokinetic modeling. The first step was to determine the parameters driving the iontophoretic delivery *in vitro* across human stratum corneum of rotigotine.H₃PO₄ (47 mM), buffered at pH 5.0. The value of the Flux_{ss} corresponds well with the value estimated by the permeation lag time method (Table 3). In addition, diagnostic plots of the data modeling (Figure 4) confirm that this model successfully describes the *in vitro*

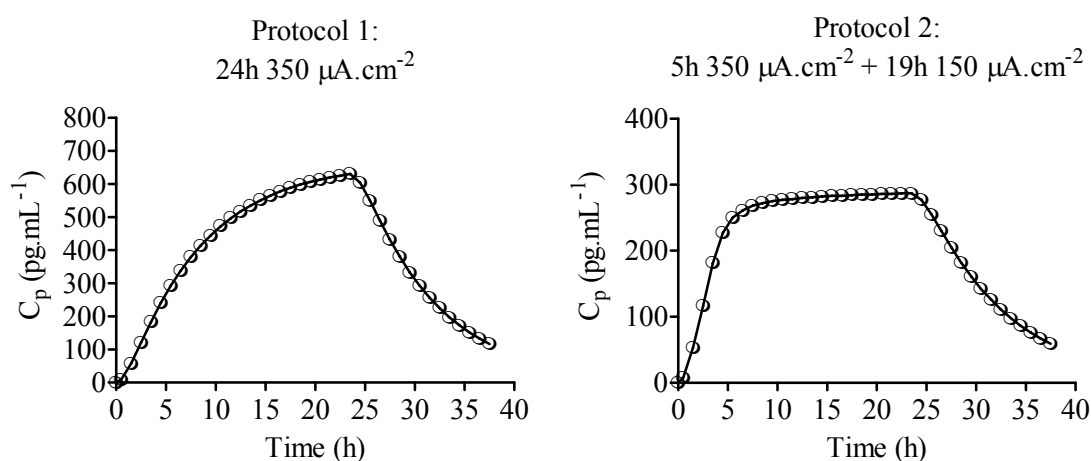


Figure 5: Population prediction of the simulations of iontophoretic delivery of rotigotine.H₃PO₄ (47 mM) using different protocols: Protocol 1: 24h 350 $\mu\text{A}.\text{cm}^{-2}$; Protocol 2: 5h 350 $\mu\text{A}.\text{cm}^{-2}$ + 19h 150 $\mu\text{A}.\text{cm}^{-2}$. The open circles are the population prediction of the simulated plasma concentration (C_p).

iontophoretic transport of rotigotine.H₃PO₄. In the next step the apparent pharmacokinetic parameters of rotigotine, reported in literature, are combined with the best-fit values of Flux_{ss}, K_R and t_L to predict the plasma levels *in vivo* [11-17]. A comparison was made with the passive delivery of rotigotine. Neupro[®], a passive transdermal delivery system of rotigotine, is approved by the EMEA for symptomatic treatment of Parkinson's disease [24]. The patch delivers 1 to 8 mg rotigotine in 24h, depending on the patch size. As reported in literature, passive delivery of rotigotine with a patch size of 10 cm², estimated to deliver 2 mg in 24h, resulted in a maximum plasma concentration (C_{max}) of 215 pg.ml⁻¹ at 16 h [16]. Two different protocols were used to evaluate the iontophoretic delivery of rotigotine (47 mM, pH 5.0) during 24h. As shown in Figure 5 applying a current density of 350 µA.cm⁻² during 24h (protocol 1) is expected to result in C_{max} of 630 pg.ml⁻¹. Not only can a higher flux be established with iontophoresis, but more interestingly already at time=5h a plasma concentration of 240 pg.ml⁻¹ can be reached. Therefore the *in vivo* iontophoretic delivery of rotigotine was simulated using protocol 2, applying initially a current density of 350 µA.cm⁻² for 5h, after which the current density was decreased to 150 µA.cm⁻². These simulations demonstrate two very important advantages of iontophoretic delivery of rotigotine in combination with iontophoresis over transdermal passive diffusion. Firstly, because of active transdermal delivery the onset time to achieve the desired level can be significantly decreased. Secondly, by adjusting the current density a titration of the plasma concentration is possible, making it feasible to individually modulate the delivery according to the desired dosing regimen. Both advantages can be of great benefit for symptomatic treatment of Parkinson's disease.

In conclusion despite the lipophilicity of rotigotine, high levels can be achieved with transdermal iontophoretic delivery with a rapid onset time, making it a very promising compound for symptomatic treatment of Parkinson's disease. Thereby changing the salt form of a drug can improve the physicochemical properties and consequently the maximal transdermal transport of a therapeutic drug, without changing its major transport mechanisms.

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