Transdermal iontophoretic delivery of dopamine agonists: in vitro - in vivo correlation based on novel compartmental modeling

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Chapter 5

TRANSDERMAL IONTOPHORESIS OF THE DOPAMINE AGONIST 5-OH-DPAT IN HUMAN SKIN *IN VITRO*

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ABSTRACT

The feasibility of transdermal iontophoretic delivery of a potent dopamine agonist 5-OH-DPAT was studied *in vitro* in side by side diffusion cells across human stratum corneum (HSC) and dermatomed human skin (DHS) according to the following protocol: 6 hours of passive diffusion, 9 hours of iontophoresis and 5 hours of passive diffusion. The influences of the following parameters on the flux were studied: donor solution pH, NaCl concentration, drug donor concentration, current density and skin type. A current density of 0.5 mA cm^{-2} was used, except for one series of experiments to study the current density effect.

Probably due to the influence of the skin perm-selectivity and the competition with H^+, increase in pH from 3 to 5 resulted in a significant increase in flux. Further increase in pH to 6 did not further increase the flux. The iontophoretic transport was found to increase linearly with concentration and current density, providing a convenient way to manage dose titration for Parkinson's disease therapy. Increase in concentration of NaCl dramatically reduced the flux of 5-OH-DPAT as a result of ion competition to the transport. When DHS was used, the iontophoretic transport was less. Also, with DHS the response in flux profile, by switching the current on and off, was shallower than that with HSC. With the optimum condition, a delivery of 104 μg of 5-OH-DPAT per cm^2 patch per hour is feasible, indicating that the therapeutic level could be achieved with a smaller patch size than required in case of rotigotine. Thus, based on this *in vitro* study, transdermal iontophoretic delivery of 5-OH DPAT is very promising.

A. INTRODUCTION

The use of dopamine agonists to manage Parkinson's disease syndrome becomes more and more important since the gold standard therapy with levodopa exhibits several serious side effects (1). However, most dopamine agonists containing a phenolic moiety, are subject to extensive first-pass metabolism (2-4) making the per oral delivery route is not feasible. Therefore, an alternative delivery route is of great interest.

For this reason, transdermal iontophoresis, a method of drug delivery by application of a low current across the skin (5), has become a subject of research for many years. As benefited from the circumvention of the hepatic "first-pass" effect, the possibility to deliver the drug continuously and the possibility to provide a non-invasive self-used therapy device, make this delivery route an attractive and important approach for dopamine agonist therapy. Most importantly, this method also offers the possibility of an individualized dose titration by modulation of the current density (5). This is an important advantage in Parkinson's disease therapy in which individual dose escalation is usually required to obtain an optimum therapy with the minimum side effects (6).

Previously, several investigations on the transdermal iontophoretic delivery of dopamine agonists have been reported. Luzardo-Alvarez *et al*. reported promising results on the delivery of ropinirole by iontophoresis across the full thickness piglet skin (7). Moreover, the potential of the iontophoretic delivery of R-apomorphine has been intensively investigated *in vitro* (8-12) as well as *in vivo* (13,14). Most recently, the feasibility of transdermal iontophoretic delivery of rotigotine, a new and potent dopamine agonist with a high selectivity for the D_2 dopamine receptor (15), was reported (16,17). Despite those promising results, the search for a more ideal dopamine agonist candidate for iontophoretic delivery continues.

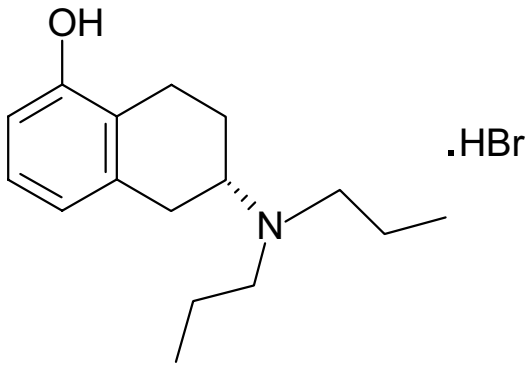

Fig. 1. Structure and physical properties of 5-OH-DPAT.HBr: M.W.= 328.3, *log P* =2.19, *pKa*=8.12

One of these alternatives is the very potent dopamine agonist 5-hydroxy-2-(N,N,-di-n-propylamino)tetralin (5-OH-DPAT) (18) (Fig. 1). 5-OH-DPAT has a similar potency and structure to rotigotine (19,20). Although very potent, its utility in per-oral delivery is limited due to a low bioavailability (approximately 1% (21)) as a consequence of an extensive metabolism in the hepatic circulation. Therefore the research on the feasibility of transdermal iontophoresis as the alternative for the delivery of 5-OH-DPAT is of great interest.

The absence of the thienyl group in the side chain of 5-OH-DPAT makes this compound less lipophilic than rotigotine. The reduced lipophilicity of 5-OH-DPAT provides at least 3 benefits for the delivery of this compound by iontophoresis: 1) the increase of drug solubility, which offers a possibility to increase the concentration of the drug in the donor phase, 2) the less retardation of the drug in the skin during iontophoresis, which reduces the risk of a skin depot effect, and 3) the increase of the chance of the drug ions to carry the current across the skin, which results in a more efficient transport due to electro-repulsion.

Thus our aim in this study was to determine the feasibility of the delivery of 5-OH-DPAT by transdermal iontophoresis across human stratum corneum (HSC) and dermatomed human skin (DHS) *in vitro*. Specifically, the effects of 5-OH-DPAT donor concentration, pH and NaCl concentration on the iontophoretic transport of 5-OH-DPAT were studied. We also examined the relationship between the iontophoretic transport of 5-OH-DPAT and the current density in dermatomed human skin (DHS). The concomitant skin electric resistance during transdermal iontophoretic transport of 5-OH-DPAT was also measured.

B. MATERIALS AND METHODS

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 and silver chloride (purity > 99.99%) were obtained from Aldrich (Borneum, Belgium). Ascorbic acid, sodium meta bisulphite, trypsin (Type III, from a bovine pancreas) and trypsin inhibitor (Type II-S from soybean) were purchased from Sigma Chemicals (Zwijndrecht, The Netherlands). Dialysis membrane disks (cut off value of 5000 Da) were purchased from Diachema (München, Germany). HPLC grade acetonitrile was obtained from Rathburn (Walkerburn, UK). All other chemicals and solvents were of analytical grade. All solutions were prepared in Millipore water with resistivity of more than 18 MΩ.

2. Distribution coefficient of 5-OH-DPAT and the determination of *log P* and *pKa*

The 1-octanol/water distribution coefficient (*log D*) was measured at pH 7.4 and 6.2. Briefly, after 24 hours of equilibration between the 1-octanol and water phases (0.15 M of PBS pH 7.4 and 6.2), 5.0 ml of 0.02 mg ml^{-1} of 5-OH-DPAT solution in buffer was mixed with 0.5 ml of 1-octanol. Then the mixtures were shaken at room temperature for 72 hours (GFL 1086, Salm en Kip BV, Breukelen, The Netherlands). After equilibration, the mixtures were centrifuged for 15 minutes at a speed of 1800 rpm. The water and 1-octanol phases were then separated manually. The concentration of 5-OH-DPAT was analyzed in both phases using HPLC. The 1-octanol/water distribution coefficient was calculated according to equation 1.

$$log\, D = log \frac{C_{octanol}}{C_{water}} \quad (1)$$

in which $C_{octanol}$ and C_{water} refer to 5-OH-DPAT concentration in the 1-octanol and water phases respectively. As the distribution coefficient at two different pH values (i.e. 7.4 and 6.2) has been measured, *log P* (1-octanol/water partition coefficient) as well as *pKa* (the equilibrium dissociation constant) of 5-OH-DPAT could be estimated using the equation below:

$$log\, D = log\, P - log\, (1+10^{pKa-pH}) \quad (2)$$

in which *pH* is the pH of the water phase.

3. Preparation of Dermatomed Human Skin (DHS) and Human Stratum Corneum (HSC)

Within 24 hours after surgical removal of the human skin (abdominal or breast skin from female donor), residual subcutaneous fat was removed. To avoid interference with contaminating subcutaneous fat, the skin surface was carefully wiped with a tissue paper soaked in successively 70% ethanol and Millipore water. DHS was obtained by dermatoming the skin to a thickness of about 300 μm using a Padgett Electro Dermatome Model B (Kansas City, USA). In order to obtain HSC, DHS was incubated with the dermal side on Whatman paper soaked in a solution of 0.1% trypsin in 0.15 M phosphate buffered saline (PBS) pH 7.4 (NaCl: 8 mg ml^{-1}, Na_2HPO_4: 2.86 mg ml^{-1}, KH_2PO_4: 0.2 mg ml^{-1}, KCl: 0.19 mg ml^{-1}) overnight at 4 ^{0}C and subsequently for 1 hour at 37 ^{0}C after which HSC was peeled off from the underlying epidermis and dermis. Remaining trypsin activity was blocked by bathing in a 0.1% trypsin inhibitor solution in PBS pH 7.4. HSC was subsequently washed several times in water and stored in a silica gel containing desiccator in a N_2 environment to inhibit oxidation of HSC lipids.

4. *In vitro* Iontophoretic 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 the iontophoresis. The system has two differential input channels per current source channel enabling on-line measurement of the skin electric resistance in each diffusion cell. The maximum voltage in each channel was 40 Volts (22). One pair of the driver electrodes, made from a silver plate (anode) and a silver wire coated with silver chloride (cathode). The skin electric resistance was measured using another pair of electrodes, i.e. the measuring electrodes, which were situated only 2 mm from the anatomical site of HSC or DHS. The measurement electrodes were made from silver/silver chloride.

All transport experiments were carried out by using three-chamber continuous flow through diffusion cells as previously described (22). HSC was used in all transport studies except in two series of experiments to study the effect of skin membrane type and the effect of current density to the iontophoretic transports. In these studies DHS was used. HSC and DHS (∅=18 mm, the diffusion area is 0.64 cm^2) were hydrated for two hours in PBS pH 7.4 *prior* to mounting in the cells. Two pieces of HSC or DHS were placed between the anodal and the acceptor side, and between the acceptor and the cathodal side, with the dermal sides of HSC or DHS facing the acceptor compartment. At least three skin specimens were used for each experimental condition examined. Dialysis membrane (cut-off: 5000 Da) was used as supporting membrane. The solution of 5-OH-DPAT was applied at the anodal side. The donor formulation of 5-OH-DPAT was buffered with 5 mM citric acid. The cathodal side was filled with PBS pH 7.4. The acceptor chamber was continuously perfused using a peristaltic pump with PBS pH 7.4 at a flow rate of 6.5 ml h^{-1}. To maintain osmolarity an appropriate amount of D-mannitol was added to the donor solution. During the experiment, the acceptor phase was maintained at 32 ^{0}C, which is the temperature of the skin *in vivo*. The current was switched on for 9 hours after 6 hours of passive diffusion. The iontophoresis was followed by 5 hours of passive diffusion. Unless specially mentioned, the current density used was 0.5 mA cm^{-2}. Samples were collected every hour with an automatic fraction collector (ISCO Retriever IV, Beun De Ronde BV, Abcoude, The Netherlands). During the experiments both the anodal and the cathodal compartment were magnetically stirred at 375 rpm. The composition of donor solutions for individual studies is outlined below.

a. The influence of donor pH

The pH of the donor solution was either 3, 4, 5, or 6. In all experiments the concentration of 5-OH-DPAT was 1.3 mg ml^{-1} in the presence of 0.07 M NaCl.

b. The influence of 5-OH-DPAT concentration
The concentration of 5-OH-DPAT in the donor solution was either 0.25, 0.5, 1.3, or 2.3 mg ml^{-1}. For the donor phase a pH of 5 was chosen. All studies were performed in the presence of 0.07 M NaCl.

c. The influence of NaCl concentration at the donor phase
The concentration of NaCl in the donor compartment was either 0.07 or 0.14 M at a pH of 5. The concentration of 5-OH-DPAT was 1.3 mg ml^{-1}.

d. The influence of using DHS compared to HSC
The iontophoretic 5-OH-DPAT transport was examined across DHS. The donor solution contained 2.3 mg ml^{-1} of 5-OH-DPAT at pH 5 and 0.07 M NaCl.

e. The influence of current density
The iontophoretic transport of 5-OH-DPAT across DHS at different current density was examined. The current density applied was either 0.25 mA cm^{-2} or 0.5 mA cm^{-2}. The donor solution contained 2.3 mg ml^{-1} of 5-OH-DPAT at pH 5 and 0.07 M NaCl.

5. Analytical Method

Collected samples during iontophoresis were injected directly into the HPLC system and analyzed by using a scanning fluorescence detector (Waters™ 474, Millipore Corporation, Milford, MA, USA) at excitation and emission wavelengths of 275 and 303 nm, respectively. The attenuation and gain were set at 1 and 10 respectively. A Superspher® 60, RP-select B, 75 mm-4 mm column (Merck KGaA, Darmstadt, Germany) was used. The mobile phase consisted of acetonitrile/0.1 M acetate buffer at pH 3.6 (40/60) v/v with a flow rate of 0.7 ml $minute^{-1}$. The calibration curves were linear ($r>0.999$) in the concentration range of 0.01 to 10 μg ml^{-1}. The intra and inter-assay variation were less than 5% for all concentrations tested. The detection limit under these conditions was 3 ng ml^{-1}.

6. Data Analysis

The flux and the cumulative amount of the iontophoretic transport of 5-OH-DPAT were plotted as a function of time. From the latter plot, the *estimated* steady-state flux ($Flux_{ss}$) was calculated based on the permeation lag time method (23,24). The term *estimated* is given, as for most conditions, the real steady-state situation was not clearly observed during 9 hours of iontophoresis. For this reason the $Flux_{ss}$ was calculated from the slope of linear portion of the plot between 5 and 9 hours of iontophoresis ($r>0.999$). Moreover, the concomitant values of the skin electric resistance were measured during iontophoresis. The steady state skin electric resistance during iontophoresis (R_{ss}) were determined based on the average values of the resistance during the last 5

hours of iontophoresis. All results were expressed as mean values ± standard deviations. Statistical analysis was performed by using one-way ANOVA followed by Newman-Keuls multiple comparison tests. When a statistical analysis was performed for only 2 groups, a Student's t-test was used. For all statistical analysis, the probability value of less than 0.05 was considered significant.

C. RESULTS

1. Distribution coefficient and the determination of *log P* and *pKa* of 5-OH-DPAT

The values of the 1-octanol/water distribution coefficient of 5-OH-DPAT (*log D*) at a pH of 7.4 and 6.2 were 1.39 ± 0.02 and 0.26 ± 0.02, respectively. The values for *log P* and *pKa* of 5-OH-DPAT were estimated as 2.19 and 8.12 respectively.

2. Flux *versus* Time Profile of 5-OH-DPAT Iontophoretic Transport

Typical examples of the flux *versus* time profile of 5-OH-DPAT across HSC *prior* to, during and after iontophoresis are shown in Fig. 2 panel A. The passive pre-iontophoresis fluxes in all conditions were negligible. By switching on the current at 6 hours, the fluxes dramatically increased and when the current was switched off at 15 hours, a dramatic decrease in flux was observed. However in most experiments, a real steady-state flux was not achieved during 9 hours of iontophoresis. Interestingly, the passive flux at 5 hours post iontophoresis was still higher than the passive flux values observed *prior* to iontophoresis.

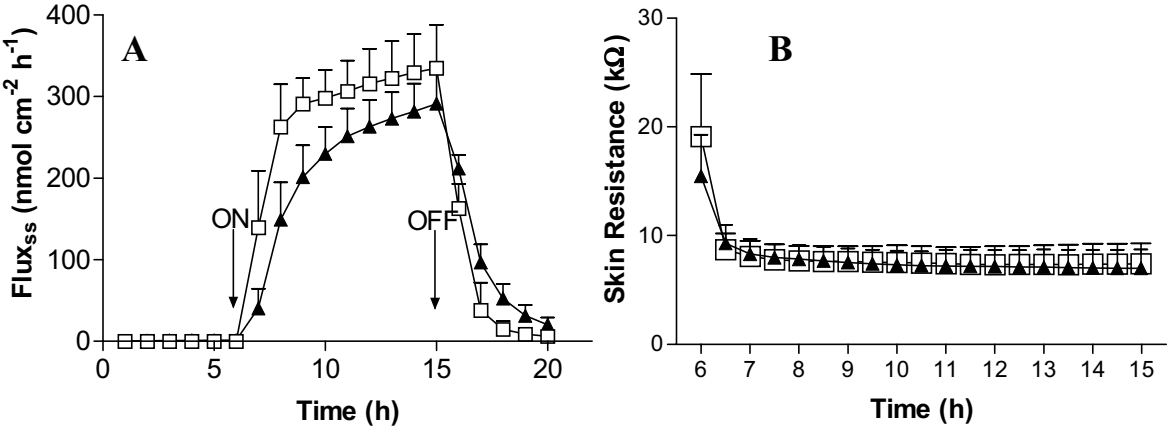

Fig. 2. The profiles of flux *versus* time (panel A) and skin electric resistance *versus* time (panel B) of the iontophoretic transport of 2.3 mg ml^{-1} 5-OH-DPAT across HSC (open square) and across DHS (close triangle). Current was switched on at 6 hours and off at 15 hours. Data are presented as mean + SD (n=6).

The typical example of the profile of the skin electric resistance *versus* time during the iontophoresis of 5-OH-DPAT across HSC is presented in Fig. 2 panel B. As demonstrated in the figure, the resistances dropped immediately after switching on the current and reached steady-state values after approximately 1 hour of iontophoresis.

3. Effect of Donor Solution pH

The effect of donor solution pH on 5-OH-DPAT $Flux_{ss}$ is presented in Fig. 3. Increase in pH from 3 to 4 resulted in a dramatic increase in $Flux_{ss}$ from 25.8 ± 2.8 to 115.4 ± 41.6 nmol cm^{-2} h^{-1} ($p<0.001$). Increase in pH from 4 to 5 resulted in a slight increase in $Flux_{ss}$ to 187.6 ± 36.2 nmol cm^{-2} h^{-1} ($p<0.05$) and when the pH was further increased to 6, no further increase in $Flux_{ss}$ (172.1 ± 28.1 nmol cm^{-2} h^{-1}) was observed ($p>0.05$).

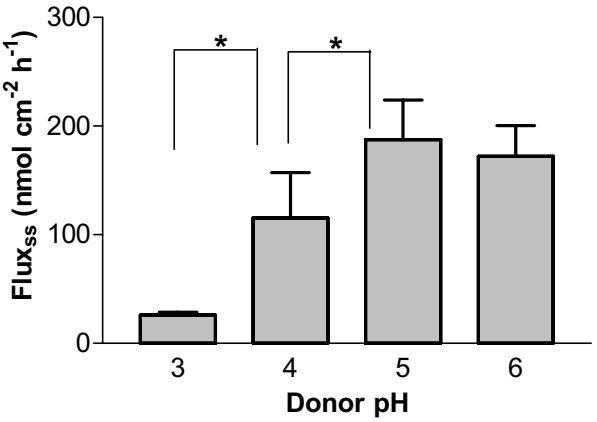

Fig. 3. The correlation of $Flux_{ss}$ *versus* drug donor pH during the iontophoresis of 1.3 mg ml^{-1} of 5-OH-DPAT in the presence of 0.07 M of NaCl. Data are presented as mean + SD (n=5-7). * indicates a significant difference ($p<0.05$).

The values of the steady state skin electrical resistance during iontophoresis (R_{ss}) of 5-OH-DPAT at the various pH values in the donor compartment are presented in Table I. The values of R_{ss} at pH 3 and 4 were 14.5 ± 3.4 and 16.6 ± 3.8 kΩ and were statistically identical ($p>0.05$). Similarly, the value of R_{ss} at pH 5 and pH 6 were not significantly different (8.1 ± 1.7 and 7.8 ± 3.4 kΩ respectively, $p>0.05$). The values of R_{ss} at pH 5 and 6 were approximately a half of those at pH 3 and 4 ($p<0.05$).

4. Effect of NaCl concentration

As demonstrated in Fig. 4, an increase in NaCl concentration in the donor compartment from 0.07 to 0.14 M resulted in a dramatic reduction in 5-OH-DPAT $Flux_{ss}$ from 187.6 ± 36.2 to 17.4 ± 1.6 nmol cm^{-2} h^{-1} ($p<0.0001$). At both concentrations of NaCl, the R_{ss} values were not significantly different ($p>0.05$) (Table I).

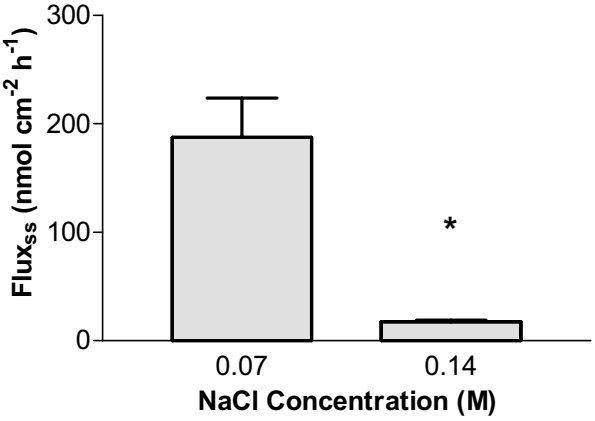

Fig. 4. The correlation of $Flux_{ss}$ *versus* NaCl concentration in the donor phase during the iontophoresis of 1.3 mg ml^{-1} of 5-OH-DPAT at pH 5. Data are presented as mean + SD (n=4-7). * indicates a significant difference ($p<0.05$).

5. Effect of 5-OH-DPAT concentration

As depicted in Fig. 5, increasing the concentration of 5-OH-DPAT from 0.25 to 0.5, 1.3 and 2.3 mg ml^{-1} resulted in a linear increase in $Flux_{ss}$ (R^2=0.997) from 39.1 ± 3.1 to 78.4 ± 6.8, 187.6 ± 36.2 and 318.2 ± 43.2 nmol cm^{-2} h^{-1}, respectively. Similar to the effect of variation in NaCl concentration, a change in 5-OH-DPAT concentration did not result in a significant change in R_{ss} ($p>0.05$) (Table I).

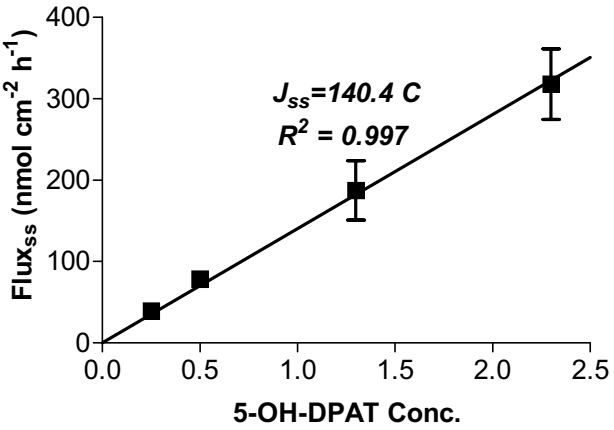

Fig. 5. The linear correlation of $Flux_{ss}$ *versus* drug donor concentration during 5-OH-DPAT iontophoresis at pH 5 in the presence of 0.07 M of NaCl. Data are presented as mean ± SD (n=5-7).

6. Effect of using DHS in comparison to HSC

As demonstrated in Fig. 2 panel A, the replacement of HSC by DHS changed the flux *versus* time profile. The gradual increase in flux after switching on the current as well as the gradual decrease in flux after switching off the current were less steep in DHS in contrast to HSC. Furthermore, the

$Flux_{ss}$ was significantly reduced from 318.2 ± 43.2 in HSC to 266.2 ± 32.6 nmol cm^{-2} h^{-1} in DHS ($p<0.05$). Interestingly, although the change of the skin membrane from HSC to DHS resulted in an obvious different in the shapes of the profiles of the flux *versus* time, the profiles of the skin electric resistance *versus* time in both systems are identical, as presented in Fig. 2B. Accordingly, the values of R_{ss} were not significantly different ($p>0.05$) in both types of skin membrane (Table I).

Table I. The values of R_{ss} during iontophoresis of 5-OH-DPAT by the difference in the pH of the donor phase, concentration of drug in the donor phase, concentration of NaCl, type of skin membrane and current density.

Conditions					R_{ss}		
5-OH-DPAT conc. (mg ml^{-1})	Current density (mA cm^{-2})	NaCl conc.	pH	Skin membrane	Mean	SD	n
1.3	0.500	0.07	3	HSC	14.5	± 3.4	7
1.3	0.500	0.07	4	HSC	16.6	± 3.8	5
1.3	0.500	0.07	5	HSC	8.1	± 1.7	7
1.3	0.500	0.07	6	HSC	7.8	± 3.4	6
1.3	0.500	0.14	5	HSC	6.6	± 0.8	4
0.25	0.500	0.07	5	HSC	8.0	± 2.2	5
0.5	0.500	0.07	5	HSC	7.1	± 2.5	6
2.3	0.500	0.07	5	HSC	7.4	± 1.7	6
2.3	0.500	0.07	5	DHS	7.1	± 1.5	6
2.3	0.250	0.07	5	DHS	13.5	± 1.1	3

7. Effect of Current Density

When the current density was reduced from 0.5 to 0.25 mA cm^{-2}, the $Flux_{ss}$ was significantly reduced from 266.2 ± 32.6 to 108.5 ± 23.5 nmol cm^{-2} h^{-1} ($p<0.0001$). Moreover, a linear correlation between current density and $Flux_{ss}$ was observed with a correlation coefficient $R^2=0.987$ (see Fig. 6). As expected based on the Ohm's Law, reducing the current density from 0.5 to 0.25 mA cm^{-2} resulted in approximately a two-fold increase in the values of R_{ss} ($p<0.001$) (Table I).

D. DISCUSSION

The flux *versus* time profile of the 5-OH-DPAT iontophoretic transport across HSC in Fig. 2A demonstrated an instantaneous effect on the transport by switching the current on and off. This is somehow different from the transport profiles obtained with rotigotine, where the response of the flux on the current application and termination was somewhat slower (16,17). Most probably this is caused by the difference in lipophilicity between the two compounds. During its

transport across HSC, rotigotine (*log P*=4.03 (16)), which is more lipophilic than 5-OH-DPAT (*log P*=2.19), resulted in a slowing down of the transport rate relative to 5-OH DPAT. As a result, the change in partitioning into the acceptor phase was faster for 5-OH-DPAT after the application or termination of current than for rotigotine. The faster response of the 5-OH-DPAT transport was also obvious from the level of the flux during the post iontophoretic period. The flux at 5 hours of post-iontophoresis for 5-OH-DPAT was approximately 2% while for rotigotine it was approximately 35%. Nevertheless, similar to rotigotine, the real steady-state was not achieved during 9 hours of iontophoresis. This might be caused by the relatively lipophilic nature of 5-OH-DPAT, although it is less lipophilic than rotigotine.

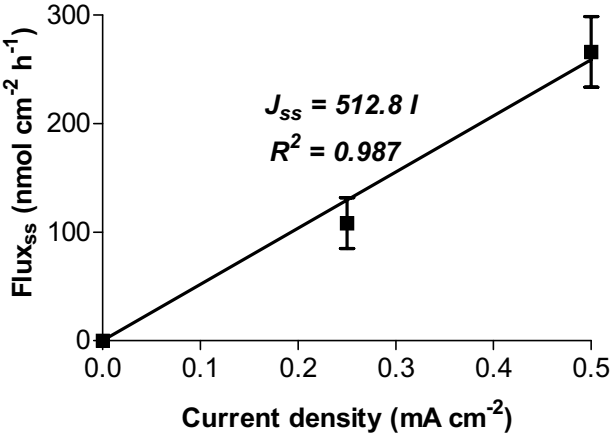

Fig. 6. The linear correlation between $Flux_{ss}$ *versus* current density on iontophoresis of 2.3 mg ml^{-1} 5-OH-DPAT across DHS at pH 5 by the presence of 0.07 M of NaCl. Data are presented as mean ± SD (n= 4 - 6)

Similar to the transport of rotigotine (16) and R-apomorphine (10), the difference in the donor pH resulted in a difference in iontophoretic transport of 5-OH-DPAT across HSC. Increase in pH from 3 to 4 and 5 resulted in an increase in the $Flux_{ss}$ while a further increase in pH to 6 did not further increase the transport. In this respect, at least 2 factors can be considered to play a role.

The first factor is the electro-osmosis and skin perm-selectivity. Due to *pI* of the HSC of approximately 4.8 (25), the reduction of the iontophoretic transport due to the electro-osmosis in the counter direction of the flow of current is maximum at pH 3, which is in agreement with the fact of the lowest value of $Flux_{ss}$. Moreover, the maximum facilitation of electro-osmosis should be obtained at pH 6, which is in contradiction with the fact that the $Flux_{ss}$ at that pH is similar to the value at pH 5. Thus, other factors than electro-osmosis may play more important role in this case.

The second factor is the electro-repulsion particularly the influence of the ion competition between the hydrogen ion to the drug ion to carry the current. The concentration of hydrogen ions ($[H^+]$) was reduced a 10-fold from one to

the one higher unit of pH. The decrease in $[H^+]$ was dramatic from pH 3 to 4, was slight from pH 4 to 5 and was negligible from pH 5 to 6. Interestingly, a dramatic increase in $Flux_{ss}$ was observed at pH 3 to 4, a slight increase in $Flux_{ss}$ was observed from pH 4 to 5 and identical $Flux_{ss}$ values were obtained at pH 5 and 6. Thus, there is a good agreement between decrease in $[H^+]$ with increase in $Flux_{ss}$ in all pHs indicating that ion competition greatly affects the transport due to the difference in pH. This underscores the electro-repulsion as the most important mechanism in the iontophoretic delivery of 5-OH-DPAT.

The influence of ion competition is also demonstrated by the dramatic decrease in $Flux_{ss}$ by increase in NaCl concentration from 0.07 to 0.14 M, which was much stronger than for rotigotine (16) and for R-apomorphine (10). As the concentration of Na^+ increases, the chance of the drug ions to carry the current is reduced. Interestingly, the $Flux_{ss}$ at NaCl concentration of 0.14 M was even lower than the $Flux_{ss}$ at pH 3, in which H^+ was present at the highest concentration. This can be explained by the fact that although Na^+ is less mobile than H^+, its higher concentration makes Na^+ a stronger competitor for charge transfer.

Moreover the decrease in $Flux_{ss}$ can also be understood based on the equation proposed by Phipps and Gyory (26) as follows:

$$J_d = \frac{t_d . I}{F . Z_d} \tag{3}$$

in which J_d, t_d and Z_d are the flux, the transport number and the valence of the 5-OH-DPAT cation, I is the current density and F is the Faraday constant. In this case, the higher the level of ion competition the smaller the chance that the drug carries the charge. As a result the drug transport number (t_d) is reduced and accordingly the flux of the drug is reduced.

When the concentration of the drug increased, the drug transport number also increased and according to equation 3, if electro-repulsion plays important role during iontophoresis then the flux should also increase. This is, however, not always observed especially for some compounds in which electro-osmosis significantly contributes to the iontophoretic transport such as nafarelin (27), leuprolide (28), and propranolol (29). Such compounds may have a certain concentration threshold above which an increase of concentration does not result in a proportional increase of the flux anymore, or in some cases even reduces the transport. However, as in the case of rotigotine (17), for 5-OH-DPAT there is a linear correlation between the flux *versus* the drug concentration. Even at the highest concentration of the drug, the flux still linearly increases with concentration. This is again a confirmation that the electro-repulsion might be the most important factor contributed during the iontophoretic transport of 5-OH-DPAT.

When DHS was used as the skin membrane, the increase and decrease in flux by switching on and off the current was less obvious than that across HSC.

A similar trend was also observed with the rotigotine (17) and R-apomorphine (30). However, in case of rotigotine, probably due to its lipophilic nature, the difference in the flux profile was more extreme. This difference could be related to several factors. First, there is a difference in the membrane thickness from approximately 40 μm in fully hydrated HSC (31) to approximately 300 μm in DHS. This difference may explain the longer time period required for the drug to transport across the skin membrane and the observed delay in the 'response' in the acceptor phase. Secondly, the presence of a stagnant body fluid with the pH of around 7.4 in the epidermal and dermal part of DHS that might entrap unionized 5-OH-DPAT (approximately 16%) in its way to the acceptor phase. This situation might result in a depot effect in DHS that also delays the drug appearance in the acceptor phase. Thirdly, the absence of vesicular clearance in DHS might also render to a depot effect of the drug in the skin.

Moreover, the similarity in the profiles of resistance *versus* time in HSC and DHS is of interest. As the skin electrical resistance is considered to be one of the indicators for the skin barrier integrity (32), this indicates that the electrical current results in a similar effect on the permeability of both skin membranes. As all experimental conditions in both cases (i.e. the intensity of current, the donor concentration of 5-OH-DPAT, the donor pH, and the concentration of co-ions) are equal, the difference in the level of the iontophoretic transport of 5-OH-DPAT in HSC and DHS might underline the role of three possible sources as discussed above.

The iontophoretic transport of 5-OH-DPAT was linearly correlated to the applied current density. This is another indication that electro-repulsion is very important. Based on the slope of this correlation, the drug transport number of 5-OH-DPAT can be estimated to be approximately 1.4%. Based on the linear correlation of the flux *versus* the concentration of 5-OH-DPAT in the donor phase, the value of t_d at a concentration of 1.3 mg ml^{-1} can be estimated to be approximately 0.8%. This value is a two-fold higher than the value of t_d of an equimolar concentration of rotigotine (1.4mg ml^{-1}), which was reported to be approximately 0.4% (17). This confirms that the iontophoretic transport of 5-OH-DPAT is more efficient than rotigotine, which most probably also due to the less lipophilic nature of the compound.

An interesting fact from the HSC resistance values during iontophoresis is the lower resistance at pH 5 and 6 in contrast to the resistance at pH 3 and 4. When HSC is positively charged at pH 3 and 4, it repels the cations flow carrying the current from the anode across HSC to the cathode resulting in a less mobile cations transport. In contrast, when HSC is negatively charged at pH 5 and pH 6, it attracts the cations-current flow and finally reduces the skin electric resistance during iontophoresis. Interestingly, the difference in the charge intensity both with positive or negative charge did not result in a difference in the resistance value. Therefore the values of skin electric resistance at pH 3 and

pH 4 as well as pH 5 and pH 6 were identical. Further investigation is required to explain this phenomenon.

The linear relationships between the drug concentrations and the current density *versus* the transdermal iontophoretic transport of 5-OH-DPAT are very important. As in the therapy of Parkinson's disease by dopamine agonists, the dose adjustment is essential to account for inter and intra individual differences and the disease progression, the delivery method which provides such feature to easily manage the dose titration will be of great important. By iontophoresis, the dose can be managed based on the concentration or the amount of the drug presence in the patch or by providing several sizes of the patch based on the patient demand. However, the most attractive benefit is the possibility to manage the dose titration by adjusting the current. By this feature, a fixed-size of the patch can be used and the current can be easily adjusted at a certain level which is required for the patient.

In addition, the maximum $Flux_{ss}$ achieved in this study was approximately 318 nmol cm^{-2} h^{-1}, which was achieved at a donor concentration of 2.3 mg ml^{-1} at pH 5. If we assume that the value of the $Flux_{ss}$ *in vitro* can also be achieved *in vivo*, at this optimal condition, it is possible to deliver approximately 104 μg of 5-OH-DPAT per cm^2 patch per hour. Considering the similarity in the structures and potencies of 5-OH-DPAT and rotigotine (19,20), we might assume that the dose required to maintain the therapeutic level of 5-OH-DPAT is similar to that of rotigotine as reported by Calabrese *et al*. (33). Based on this assumption, therapeutically effective plasma concentrations could be achieved with the patch size of less than 10 cm^2, which indicates the feasibility of transdermal iontophoretic delivery of 5-OH-DPAT.

Finally, similar to rotigotine, we did not observe any indication of the degradation products of the drug after the iontophoresis, suggesting the stability of the drug under iontophoretic condition *in vitro*.

In summary, iontophoretic transport was influenced by pH of the donor solution in which the optimum transport was achieved at pH 5. The iontophoretic transport of 5-OH-DPAT linearly increased with drug donor concentration and current density, providing a convenient way to manage dose titration for Parkinson's disease therapy. In agreement with analysis that electro-repulsion is the main mechanism of the iontophoretic transport of 5-OH-DPAT, increase in NaCl concentration in the donor phase exhibited a dramatic decrease in flux as indication of a dramatic role of ion competition to the transport. The change of skin membrane from HSC to DHS significantly reduces the flux and changes the shapes of the flux *versus* time profiles particularly at the periods immediately after the current application and immediately after the current removal. Finally, the delivery of 5-OH-DPAT is quite feasible as is indicated from the possibility to deliver approximately 104 μg of 5-OH DPAT per cm^2 patch per hour, which might indicate that the therapeutic level could be achieved with a smaller patch size than required in case of rotigotine.

E. ABBREVIATIONS:

HSC : Human stratum corneum
DHS : Dermatomed human skin
$Flux_{ss}$: The estimated steady-state flux
R_{ss} : The steady-state skin electric resistance

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