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International Journal of Pharmaceutics 266 (2003) 61–68
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Iontophoretic R-apomorphine delivery in combination with surfactant pretreatment: in vitro validation studies

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Received 25 November 2002; accepted 11 March 2003

Abstract

To validate the efficacy and controllability of a newly developed transdermal delivery system for R-apomorphine in combination with the surfactant pretreatment, iontophoresis was performed in three-chamber continuous-flow-through diffusion cells in vitro. The transdermal iontophoretic transport of R-apomorphine was examined with both human SC and freshly dermatomed human skin, at room temperature and at 32 °C. Furthermore, the relationship between current density and iontophoretic flux was investigated. By increasing the temperature from 22 to 32 °C, the iontophoretic transport rate of R-apomorphine in human SC was increased 1.9-fold. Also the iontophoretic flux increased linearly with the increase in the current density from 100 to 500 $\mu A/cm^2$. When using dermatomed human skin instead of SC, the iontophoretic flux at a current density of 500 $\mu A/cm^2$ was decreased from 362 ± 45 to 259 ± 30 $nmol/cm^2$ h, and the corresponding lag time was prolonged from 0.8 to 2.8 h. In conclusion, the combination of non-occlusive pretreatment with the surfactant formulation and iontophoresis has shown to substantially increase the transdermal transport rate of R-apomorphine. A linear relationship between current density and R-apomorphine flux indicates that the iontophoretic delivery combined with surfactant pretreatment allows a controlled and individualised administration of R-apomorphine.
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Keywords: Iontophoresis; Surfactant pretreatment; R-apomorphine; Parkinson's disease

1. Introduction

R-apomorphine as a potent D_1 and D_2 dopamine receptor agonist is potentially useful in the treatment of severe motor fluctuations in patients with Parkinson's disease (Colosimo et al., 1994; Muguet et al., 1995). However, its inherent instability, negligible oral bioavailability, short elimination half-life ($t_{1/2}$ = 41 min), narrow therapeutical window and high intra- and inter-individual variation in pharmacokinetics and pharmacodynamics complicate its application in clinical practice. Therefore, several alternative modes and routes of administration have been investigated (Frankel et al., 1990; Kleedorfer et al., 1991; Montastruc et al., 1991; van Laar et al., 1995). So far, the best effect has been achieved with continuous

Abbreviations: SMBS, sodium meta bisulphite; TEACl, tetraethylammonium chloride; $C_{12}EO_3$, laureth-3 oxyethylene ether; $C_{12}EO_7$, laureth-7 oxyethylene ether; SC, stratum corneum; ER, enhancement ratio

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doi:10.1016/S0378-5173(03)00381-8

subcutaneous infusions using a portable minipump (Frankel et al., 1990). However, the complexity of the method and the occurrence of local side-effects at the site of the injection limit the widespread application.

Transdermal delivery systems are capable of maintaining a stable plasma–drug concentration over a prolonged period. More importantly, the rate of drug input into the blood circulation can be substantially enhanced and exactly controlled by iontophoresis (Nair et al., 1999; Guy et al., 2000). The feasibility of transdermal iontophoresis of R-apomorphine has been established previously (van der Geest et al., 1997a,b). The results of these investigations show that the transdermal iontophoretic transport rate of R-apomorphine is linearly proportional to the applied current density, and the observed local skin irritation resulting from the electric field was at a clinically acceptable level. Nevertheless, the transport efficiency of R-apomorphine from such a system was still too low to provide effective plasma concentrations in most patients (van der Geest et al., 1997b).

Recently, two approaches have been simultaneously attempted in our laboratory to maximise the transport rate of R-apomorphine via iontophoresis. The first approach focused on the optimisation of drug donor formulation with respect to drug stability and transport efficiency, taking into consideration the mechanism of transport (Li and Danhof, 2001). The optimal donor formulation was proposed to contain 137.9 mM tetraethylammoniumchloride (TEACl) and 1 g/l ascorbic acid instead of 137.9 mM NaCl and 1 g/l sodium meta bisulphite (SMBS) in the 15 mM R-R-apomorphine hydrochloride donor solution (pH 5.0). In the second approach (Li et al., 2002), a non-occlusive pretreatment with non-ionic surfactant formulations was combined with iontophoresis. Interestingly, a twofold enhancement in the iontophoretic R-apomorphine flux across human stratum corneum (SC) was observed upon the pretreatment with 40 μl/cm^2 of a formulation containing laureth-3 oxyethylene ether ($C_{12}EO_3$)/laureth-7 oxyethylene ether ($C_{12}EO_7$)/cholesterol sulphate in molar ratio of 70:30:5 in PBS. This formulation is fairly stable over a period of at least 6 months.

In the present investigation, the effect of combining the new R-apomorphine formulation and pretreatment with the surfactant formulation on the transdermal iontophoretic transport was studied. Specifically, the objectives of the investigation were to determine: (1) whether there is an extra effect resulting from the combination of the optimal donor formulation with the pretreatment; (2) whether the enhancement is also observed under physiological conditions, such as a temperature of 32 °C and with freshly dermatomed skin instead of human SC; (3) whether the enhanced iontophoretic flux combined with pretreatment is linearly proportional to the externally applied current density.

2. Materials and methods

2.1. Materials

R-apomorphine hydrochloride was obtained from OPG (Utrecht, The Netherlands). Purity was tested by high performance chromatography on a chiral column and found to be >99%. Silver and silver chloride were obtained from Aldrich (Bornem, Belgium) and were more than 99.99% pure. Ascorbic acid, SMBS and TEACl were purchased from Sigma chemicals (Hilversum, The Netherlands). Dialysis membrane disks (cut-off value: 5000 Da) were obtained from Diachema (München, Germany). Non-ionic surfactant $C_{12}EO_3$ and $C_{12}EO_7$ were gifts from Servo (Delden, The Netherlands). Sodium sulphosuccinate was a gift from Cytec Industries (Rotterdam, The Netherlands). HPLC grade acetonitrile (Rathburn, Walkerburn, UK) was used as a solvent in the HPLC analysis. All other chemicals and solvents were of analytical grade. All solutions were prepared in Millipore water (resistivity $\geq$ 18 MΩ).

2.2. Non-occlusive pretreatment with surfactant formulation

The surfactant formulation consisted of $C_{12}EO_3$, $C_{12}EO_7$ and sodium sulphosuccinate in a molar ratio of 70:30:5 and was prepared by a modification of the sonication method described by Baillie et al. (1985). Cholesterol sulphate was replaced by sodium sulphosuccinate since excess cholesterol sulphate is known to disturb the skin desquamation process (Sato et al., 1998) and consequently may cause skin irritation in vivo. Briefly, the three surfactants were dissolved in

ethanol. The solvent was evaporated overnight in a vacuum centrifuge, and the remaining surfactant film was hydrated with PBS. The formulations were sonicated for 15 s at room temperature. The sonicator used was a Branson Sonifier 250 (Branson Ultrasonics Corp., Danbury, CT, USA) with a 1/8 inch microtip at a 60 W energy output. The surfactant formulations were pre-examined for size distribution and stability, and found to be rather stable for over half a year at room temperature. The formulations have characteristics that are very similar to the previous formulation containing cholesterol sulphate (Li et al., 2002).

Human abdomen skin was obtained after cosmetic surgery and processed on the same day. Both dermatomed skin (thickness 200–250 μm) and isolated human SC were used in the study. Their preparation was as described previously (Li and Danhof, 2001). Both SC and dermatomed skin were punched into circular samples of 18 mm diameter. Prior to use, the SC was hydrated for 1 h by floating them with the dermal side on a PBS solution at pH 7.4. The circular pieces of SC or dermatomed skin were placed in Franze-type diffusion cells with the SC side facing the donor compartment. The receiver compartment was filled with PBS pH 7.4 and heated to a temperature of 32 °C. Following the mounting of the skin, 40 μl/cm^2 of the fresh surfactant formulation was applied to the skin. The pretreatment took 3 h under a non-occlusive condition. For comparison, the SC/dermatomed skin treated with 40 μl/cm^2 PBS buffer solution served as the control.

2.3. Iontophoretic apparatus

A nine-channel computer-controlled power supply was used to provide a constant current (Central Electronics Department, Gorlaeus Laboratories, Leiden University, The Netherlands). A silver plate electrode was used as an anode a silver/silver chloride electrode as a cathode. All diffusion experiments were carried out in three-chamber continuous-flow-through diffusion cells. At least, three different skin specimens were used for each experimental condition studied.

Following pretreatment, the SC/dermatomed skin was transferred to the iontophoretic set up. The anodal chamber was filled with R-apomorphine hydrochloride solution and the cathodal chamber with PBS at pH 7.4. The intermediate chamber was continuously perfused with PBS buffer at pH 7.4 at a rate of 7 ml/h. Samples were collected hourly with a fraction collector. During the experiments, both anodal and cathodal chambers were magnetically stirred at 375 rpm. Precautions were taken to prevent oxidation of R-apomorphine by using N_2 gas, keeping the system in dark and adding 200 μl anti-oxidant solution (0.05% EDTA, 0.5% SMBS dissolved in 25% H_3PO_4) in the collecting tubes.

2.4. Experiments

2.4.1. Finalisation of donor formulation and pretreatment

The iontophoretic experiments were conducted at room temperature following the pretreatment. According to the previous report (Li and Danhof, 2001), the optimal donor solution containing 15 mM R-apomorphine, 137.9 mM TEACl and 1 g/l ascorbic acid in a 5 mM citrate buffer at pH 5.0 was used. The total course of iontophoresis included three stages: 6 h of passive diffusion, 9 h of iontophoresis and 5 h of post-iontophoresis passive diffusion. In addition, for comparison, a similar iontophoretic experiment across the PBS-treated SC was repeated using the original control donor formulation (15 mM R-apomorphine, 137.9 mM NaCl and 1 g/l SMBS in a 5 mM citrate buffer at pH 5.0).

2.4.2. Effect of temperature

The donor formulation was selected on the basis of the outcome of the experiment described above and consisted of 15 mM R-apomorphine, 137.9 mM NaCl and 1 g/l ascorbic acid in a 5 mM citrate buffer at pH 5.0. The iontophoretic experiments were performed using SC at room temperature and at 32 °C. The total course of experiment included half-an-hour passive diffusion and 9 h iontophoresis. This protocol was chosen, since it mimics the protocol for in vivo studies that will be carried out in patients in future.

2.4.3. Effect of current density

The iontophoretic experiments was performed across human SC at 32 °C and at four current densities: 100, 250, 375 and 500 μA/cm^2. The total course of experiment included half-an-hour passive diffusion

and 9 h iontophoresis, which was chosen for the same reasons as described above.

2.4.4. Dermatomed skin vs. stratum corneum

The iontophoretic experiments were conducted across freshly dermatomed human skin (200–250 μm) at 32 °C. The duration of total diffusion experiment included half-an-hour passive diffusion, 11 h iontophoresis at a current density of 500 μA/cm^2 and 5 h post-iontophoresis passive diffusion.

2.5. Analytical methods

The concentration of R-apomorphine in the diffusion samples was analyzed by HPLC. Briefly, the samples were injected directly into an HPLC system consisting of an SP8810 LC pump (Spectra-Physics Inc., CA, USA), a Gilson 234 autoinjector (Gilson Medical Electronics Inc., WI, USA) and a fluorescence detector (Jasco 821-FP, H.I. Ambacht, The Netherlands). A nucleosil 100, 5 μm C-18 column was used (200 mm × 4.6 mm i.d.). The mobile phase consisted of acetonitrile/0.1 M phosphate buffer (25/75 v/v). The phosphate buffer at pH 3 contained 0.1 M NaH_2PO_4, 20 mg/ml 1-octanesulphonic acid and 10 mg/ml EDTA. Detection was at an excitation wavelength of 280 nm and an emission wavelength of 460 nm (Gain 10 and attenuation 1). The injection volume was 20 μl. Freshly made standard solutions were used to get calibration curves for each experiment. The calibration curves were linear ($r > 0.999$) in the concentration range of 125–2500 ng/ml. The intra- and inter-assay variations were <5% for all concentrations tested. The detection limit under these conditions was 10 ng/ml.

2.6. Data analysis

The cumulative amount of R-apomorphine permeated per unit skin area was plotted against time. The slope of the linear portion of this plot was estimated as the steady-state flux, whereas the lag time was calculated from the intercept in *x*-axis. The enhancement ratio (ER) was calculated by iontophoretic steady-state flux across surfactant-treated skin divided by iontophoretic steady-state flux across PBS-treated skin. Results were presented as mean values ± standard deviations. Student's *t*-test, at a level of significance $P < 0.05$, was used to compare data sets.

3. Results

3.1. Selection of the iontophoretic conditions

In Fig. 1, the permeation profiles of R-apomorphine across human SC are plotted. During iontophoresis, a donor formulation with either SMBS and NaCl or ascorbic acid and TEACl was used. For all conditions during 6 h pre-iontophoresis passive diffusion, the amount of R-apomorphine in the receptor chamber was below the detection limit of the HPLC method. After switching on the current, the R-apomorphine flux was dramatically increased and reached at a steady state. Following switching off the current, the flux decreased to a final post-iontophoretic plateau that was slightly higher than the pre-iontophoresis passive level. Table 1 summarises the calculated fluxes and ERs.

In the absence of surfactant pretreatment, replacing SMBS and NaCl with ascorbic acid and TEACl, respectively, in the donor solution, resulted in a 1.4 enhancement ($P < 0.01$) in iontophoretic flux of

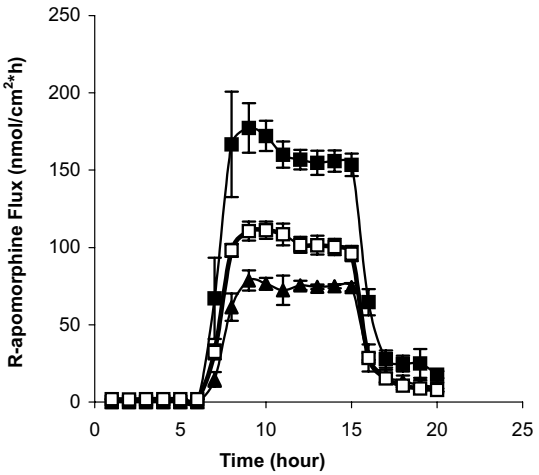

Fig. 1. Permeation profiles of R-apomorphine across human SC at room temperature during 6 h passive diffusion, 9 h iontophoresis at a current density of 500 μA/cm^2 and 5 h post-iontophoresis passive diffusion. Each data represents the mean of 5 to 6 determinations. (▲) with PBS pretreatment; donor solution contained 15 mM R-apomorphine, 137.9 mM NaCl and 1 g/l SMBS in a 5 mM citrate buffer of pH 5.0; (□) with PBS pretreatment and (■) with surfactant formulation pretreatment: donor solution contained 15 mM R-apomorphine, 137.9 mM TEACl and 1 g/l ascorbic acid in a 5 mM citrate buffer of pH 5.0.

Table 1
The effect of the surfactant pretreatment on the iontophoretic delivery of R-apomorphine across human SC at room temperature

Condition	Iontophoretic steady-state flux (nmol/cm^2 h)	Enhancement ratio	Post-iontophoresis passive flux[a] (nmol/cm^2 h)
Donor formulation containing ascorbic acid and TEACl[(1)]			
PBS pretreatment ($n = 5$)	104.5 ± 5.5	1.4[b]	7.8 ± 0.9
Surfactant formulation pretreatment ($n = 6$)	162.0 ± 9.7	1.6[c]/2.2[d]	17.7 ± 2.3
Donor formulation containing SMBS and NaCl[(2)]			
PBS pretreatment ($n = 6$)	74.3 ± 2.8	–	9.7 ± 3.0

[a] Passive fluxes at the 5th hour of post-iontophoresis.
[b] Enhancement ratio = [iontophoretic flux with PBS pretreatment from donor solution (1)]/[iontophoretic flux with PBS pretreatment from donor solution (2)].
[c] Enhancement ratio = [iontophoretic flux with surfactant pretreatment from donor solution (1)]/[iontophoretic flux with PBS pretreatment from donor solution (1)].
[d] Enhancement ratio = [iontophoretic flux with surfactant pretreatment from donor solution (1)]/[iontophoretic flux with PBS pretreatment from donor solution (2)].

R-apomorphine, which is similar to previously published data (Li and Danhof, 2001). When prior to iontophoresis the surfactant formulation treatment was performed, the iontophoretic transport rate was further increased from 104.5 ± 5.5 to 162.0 ± 9.7 nmol/cm^2 h ($P < 0.01$). Also the post-iontophoresis passive transport was promoted. However, when combined with surfactant pretreatment, substitution of NaCl by TEACl did not result in a further increase in the iontophoretic flux ($P > 0.05$) compared to the previously reported 181.5 ± 22.6 nmol/cm^2 h, using the drug formulation containing NaCl and SMBS (Li et al., 2002). Therefore, all the additional diffusion studies were carried out with NaCl in the donor formulation.

3.2. Effect of temperature

Upon increasing the temperature from 22 to 32 °C, an increase in the R-apomorphine iontophoretic flux from 92 ± 14 to 185 ± 33 nmol/cm^2 h ($P < 0.01$) was observed for PBS-treated SC, and from 182 ± 23 to 362 ± 46 nmol/cm^2 h ($P < 0.01$) for surfactant formulation-treated SC (Fig. 2). The ERs were identical at both temperatures.

3.3. Effect of current density

The relationship between the current density and the iontophoretic transport rate of R-apomorphine was examined at 32 °C across human SC pretreated with the surfactant formulation. A linear relationship (correlation coefficient, $r^2 = 0.95$) was observed between the applied current density and the transdermal transport rate of R-apomorphine (Fig. 3).

3.4. Dermatomed skin vs. stratum corneum

The transport through freshly dermatomed human skin was also studied at 32 °C. The iontophoresis interval was prolonged from 9 to 11 h, since in previous

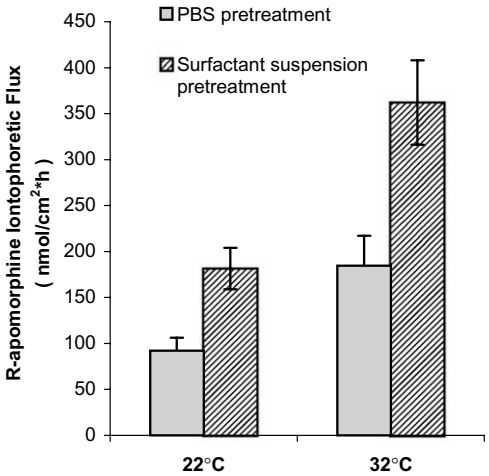

Fig. 2. Effect of temperature on the iontophoretic transport of R-apomorphine across human SC. Donor solution contained 15 mM R-apomorphine, 137.9 mM NaCl and 1 g/l ascorbic acid in a 5 mM citrate buffer of pH 5.0. Each data represents the mean of 5 to 6 determinations.

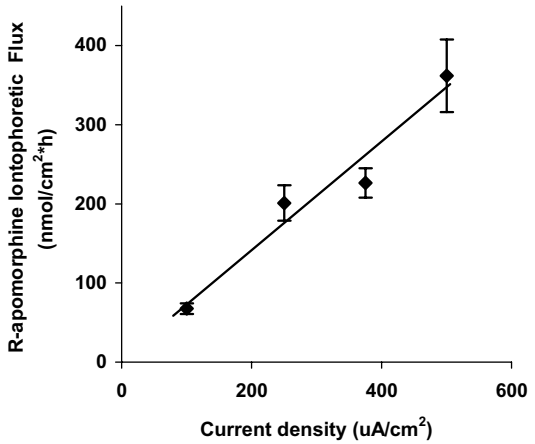

Fig. 3. Iontophoretic flux of R-apomorphine across the surfactant formulation-pretreated human SC as a function of current density applied at 32 °C. Donor solution contained 15 mM R-apomorphine, 137.9 mM NaCl and 1 g/l ascorbic acid in a 5 mM citrate buffer (pH 5.0). Each data point represents the mean (±S.D.) of 5 to 8 determinations.

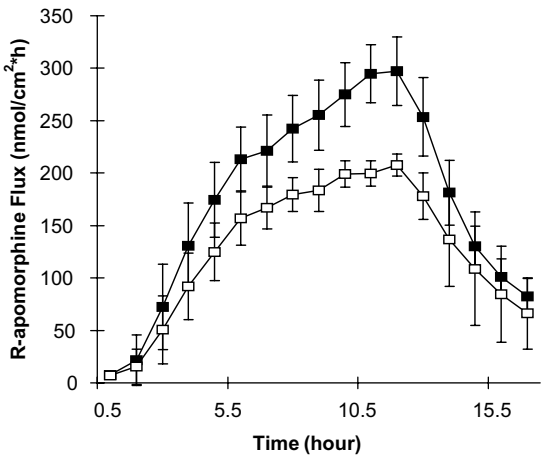

Fig. 4. Iontophoretic flux of R-apomorphine across dermatomed human skin as a function of time during half an hour passive diffusion, 11 h iontophoresis at a current density of 500 μA/cm^2, and 5 h post-iontophoresis passive diffusion at 32 °C. (□) with PBS pretreatment ($n = 10$); (■) with surfactant formulation pretreatment ($n = 11$).

studies, it was reported that the lag time was strongly increased when using dermatomed human skin. The diffusion profiles are plotted in Fig. 4. The fluxes and lag times are summarised in Table 2.

The surfactant formulation effectively enhanced the iontophoretic transport of R-apomorphine compared to the PBS control in dermatomed human skin. However, the ER was only 1.4, which is substantially lower than that observed using SC. The post-iontophoresis passive fluxes were not affected significantly ($P > 0.05$). In addition, the lag time was largely prolonged to 2.8 h.

4. Discussion

Our previous study on the transdermal iontophoretic delivery of R-apomorphine in vitro has suggested that using ascorbic acid as an anti-oxidant and TEACl as a source of Cl^-, the drug formulation instead of SMBS and NaCl affects the transdermal iontophoretic transport rate favourably (Li and Danhof, 2001). Furthermore, a non-occlusive pretreatment with the non-ionic surfactant formulation in PBS also results in an increase in the iontophoretic transport of R-apomorphine from the control formulation

Table 2
Iontophoretic delivery of R-apomorphine across human SC/dermatomed skin in combination with pretreatment of the surfactant formulation at 32 °C

Skin type	Iontophoretic steady-sate flux (nmol/cm^2 h)	Iontophoresis lag time (hour)	Enhancement ratio[a]	Post-iontophoresis passive flux[b] (nmol/cm^2 h)
Human SC				
PBS pretreated ($n = 5$)	185 ± 33	0.51	1.96	–
Surfactant formulation pretreated ($n = 5$)	362 ± 46	0.80		
Dermatomed human skin				
PBS pretreated ($n = 10$)	185 ± 15	2.78	1.4	69 ± 41
Surfactant formulation pretreated ($n = 11$)	259 ± 30	2.84		84 ± 20

[a] Enhancement ratio = [iontophoretic flux with surfactant-pretreated skin]/[iontophoretic flux with PBS-pretreated skin].
[b] Passive fluxes at the 5th hour of post-iontophoresis.

(Li et al., 2002). Therefore, it was considered of interest to investigate whether a further enhancement could be achieved on the basis of a combination of these two approaches.

The present observation has shown that although a 1.4-fold enhancement was obtained by the replacement of NaCl and SMBS by TEACl and ascorbic acid in the donor formulation, eventually a steady-state iontophoretic flux of $162.0 \pm 9.7\,\text{nmol/cm}^2\,\text{h}$ was observed upon pretreament with surfactant formulation. This value of transport rate is not significantly different from the previously reported $181.5 \pm 22.6\,\text{nmol/cm}^2\,\text{h}$ ($P > 0.05$), when using the drug formulation containing NaCl and SMBS (Li et al., 2002). It can therefore be concluded that the effect of the ascorbic acid and TEACl was masked by the surfactant pretreatment. A possible explanation may be related to the changes in skin structure as consequences of the surfactant formulation pretreatment. Recent studies in our laboratory (unpublished results) suggest that the combination of surfactant pretreatment and electrical enhancement increases the water content in the SC. More likely, this alteration might especially facilitate the iontophoretic transport of large molecules (such as R-apomorphine and TEACl) and hence the original size-selectivity of skin to drug diffusion is reduced. Other researchers (Kalia and Guy, 1997; Bhatia and Singh, 1998; Mitragotri, 2000) have also proposed this hypothesis.

To validate the efficacy and controllability of this iontophoretic delivery system in combination with the pretreatment, further investigations have been taken under conditions similar to the in vivo situations.

Firstly, upon increasing the temperature to the skin surface physiological temperature of 32 °C, a significant increase in the iontophoretic steady-state flux across human SC was observed. This is observed with and without surfactant formulation pretreatment. Interestingly, the ER relative to the PBS control, however, remained the same (1.96-fold) as that at 20 °C. This illustrates that the intensity of enhancing action of the surfactant formulation is independent of the temperature. At 32 °C, the reduced diffusional barrier of skin due to the higher temperature is further diminished by the surfactant pretreatment.

Secondly, the iontophoretic flux of R-apomorphine across human SC pretreated with the surfactant formulation was shown to be linearly proportional to the applied current density. This was also observed in a previous study with iontophoresis alone, and has been reported for other compounds such as thyrotropin releasing hormone (Burnette and Marrero, 1986), amphotericin (Singh et al., 1998) and gonadotropin-releasing hormone (Miller et al., 1990). However, in all these studies, no pretreatment with chemical enhancers was involved. Based on the proportional relationship between current density and R-apomorphine flux, we can speculate that the input rate of R-apomorphine into blood circulation in vivo can be externally controlled by adjusting the current density. This is of extreme importance, especially for a drug such as R-apomorphine that shows a very narrow therapeutic window and high patient-to-patient variation.

When SC was replaced with fresh dermatomed human skin, an increase in lag time and a reduction in the iontophoretic flux after surfactant pretreatment were observed. These findings are in accordance with results reported previously in the sense of differences between dermatomed skin and SC (van der Geest et al., 1997a). We account these differences on either one of the following issues keeping in mind that skin consists of an epidermis and small layer of dermis: (1) the drug may bind to the tissue/protein in viable epidermis and dermis; (2) the higher pH value in the viable skin may partly neutralise the drug, which makes iontophoresis less effective; (3) in the dermis layer no clearance will be observed in vitro in contrast to the in vivo situation.

An important question is whether with the currently observed transdermal delivery rates, therapeutic plasma concentration might be achieved. Assuming a one-to-one ratio between the transdermal iontophoretic transport rate in vivo and in vitro, the steady-state plasma concentration (Css) in vivo can be predicted by applying the equation $J \times A = \text{Cl} \times \text{Css}$. J is the steady-state flux, A is the current-exposed area of skin and Cl is the intrinsic clearance. It has been found that the clearance was $40.4 \pm 14.9\,\text{ml}\,\text{min}^{-1}\,\text{kg}^{-1}$ (van der Geest et al., 1998) and therapeutical plasma concentration ranged between 3 and 6 ng/l (van Laar et al., 1998). In this study, we could achieve iontophoretic steady-state flux of $259 \pm 30\,\text{nmol}\,\text{cm}^{-2}\,\text{h}$ through the surfactant formulation-treated dermatomed skin at a current density of $500\,\mu\text{A/cm}^2$ at 32 °C. Therefore, assuming the iontophoretic delivery from a patch of $20\,\text{cm}^2$ will result in a plasma concentration of 10.8 ng/ml (Given

60 kg per person). This implies that the required therapeutic levels will be achieved.

In summary, the transdermal transport rate of R-apomorphine from this newly developed iontophoresis system can be substantially enhanced by the application of the surfactant formulation pretreatment. Under the optimal condition observed in this study, the therapeutically relevant transport rate is predicted. More importantly, the linear relationship between current density and R-apomorphine flux indicates that the input rate of the drug can be simply adjusted by varying the external current intensity. Therefore, continuously delivering R-apomorphine from this developed iontophoresis system into blood circulation in a well-controlled and individualised manner will be expected in vivo. Consequently, the treatment of Parkinson's disease with R-apomorphine will also be greatly improved.

References

Baillie, A.J., Florence, A.T., Hume, L.R., Muirhead, G.T., Rogerson, A., 1985. The preparation and properties of niosomes-nonionic surfactant vesicles. J. Pharm. Pharmacol. 37, 863–868.

Bhatia, K.S., Singh, J., 1998. Synergistic effect of iontophoresis and a series of fatty acids on LHRH permeability through porcine skin. J. Pharm. Sci. 87, 462–469.

Burnette, R.R., Marrero, D., 1986. Comparision between the iontophoretic and passive transport of thyrotopin releasing hormone across excised nude mouse skin. J. Pharm. Sci. 75, 738–743.

Colosimo, C., Merello, M., Albanese, A., 1994. Clinical usefulness of apomorphine in movement disorders. Clin. Neuropharmacol. 17, 243–259.

Frankel, J.P., Lees, A.J., Kempster, P.A., Stern, G.M., 1990. Subcutaneous apomorphine in the treatment of Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 53, 96–101.

Guy, R.H., Kalia, Y.N., Delgado-Charro, M.B., Merino, V., Lopez, A., Marro, D., 2000. Iontophoresis: electroreplusion and electroosmosis. J. Control. Rel. 64, 129–132.

Kalia, Y.N., Guy, R.H., 1997. Interaction between penetration enhancers and iontophoresis: effect on human skin impedance in vivo. J. Control. Rel. 44, 33–42.

Kleedorfer, B., Turjanski, N., Ryan, R., Lees, A.J., Milroy, C., Stern, G.M., 1991. Intranasal apomorphine in Parkinson's disease. Neurology 41, 761–762.

Li, G.L., Danhof, M., 2001. Iontophoretic delivery of apomorphine in vitro: physicochemical considerations. Pharm. Res. 18, 1509–1513.

Li G.L., Danhof M., Frederik P.M., Bouwstra J.A., 2002. Pretreatment with a water-based surfactant formulation affects transdermal iontophoretic delivery of R-apomorphine in vitro. Pharm. Res. (submitted).

Miller, L.L., Kolaskie, C.J., Smith, G.A., Rivier, J., 1990. Transdermal iontophoresis of gonadotropin releasing hormone (LHRH) and two analogues. J. Pharm. Sci. 79, 490–493.

Mitragotri, S., 2000. Synergistic effect of enhancers for transdermal drug delivery. Pharm. Res. 17, 1354–1359.

Montastruc, J.L., Rascol, O., Senard, J.M., Gualano, V., Bagheri, H., Houin, G., Lees, A., Rascol, A., 1991. Sublingual apomorphine in Parkinson's disease: a clinical and pharmacokinetic study. Clin. Neuropharmacol. 14, 432–437.

Muguet, D., Broussolle, E., Chazot, G., 1995. Apomorphine in patients with Parkinson's disease. Biomed Pharmacother. 49, 197–209.

Nair, V., Pillai, O., Poduri, R., Panchagnula, R., 1999. Transdermal iontophoresis. Part I. Basic principles and considerations. Methods Find. Exp. Clin. Pharmacol. 21, 139–151.

Sato, J., Denda, M., Nakanishi, J., Nomura, J., Koyama, J., 1998. Cholesterol sulfate inhibits proteases that are involved in desquamation of stratum corneum. J. Invest. Dermatol. 111, 189–193.

Singh, S., Bi, M., Jayaswal, S.B., Singh, J., 1998. Effect of current density on the iontophoretic permeability of benzyl alcohol and surface characteristics of human epidermis. Int. J. Pharm. 166, 157–166.

van der Geest, R., Danhof, M., Boddé, H.E., 1997a. Iontophoretic delivery of apomorphine I: in vitro optimisation and validation. Pharm. Res. 14, 1797–1802.

van der Geest, R., van Laar, T., Gubbens-Stibbe, J., Boddé, H.E., Danhof, M., 1997b. Iontophoretic delivery of apomorphine II: an in vivo study in patients with Parkinson's disease. Pharm. Res. 14, 1804–1810.

van der Geest, R., van Laar, T., Kruger, P.P., Gubbens-Stibbe, J.M., Boddé, h.e., Roos, R.A.C., Danhof, M., 1998. Pharmacokinetics, enantiomer interconversion and metabolism of R-apomorphine in patients with idiopathic Parkinson's disease. Clin. Neuropharmacol. 21, 159–168.

van Laar, T., Jansen, E.N.H., Neef, C., Danhof, M., Roos, R.A.C., 1995. Pharmacokinetics and clinical efficacy of rectal apomorphine in patients with Parkinson's disease: a study of five different suppositories. Mov. Disord. 10, 433–439.

van Laar, T., van der Geest, R., Danhof, M., Boddé, H.E., Goossens, H., Roos, R.A.C., 1998. Stepwise intravenous infusion of apomorphine to determine the therapeutic window in patients with Parkinson's disease. Clin. Neuropharmacol. 21, 152–158.