Iontophoretic Delivery of Apomorphine II: An *In Vivo* Study in Patients with Parkinson's Disease

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Purpose. Transdermal transport rates of the dopamine agonist R-apomorphine were determined in patients with idiopathic Parkinson's disease (IPD). Apomorphine was applied by iontophoresis at two current densities.

Methods. In ten patients apomorphine was applied passively for one hour. Thereafter, in the first five patients, a current density of 250 $\mu A.cm^{-2}$ was applied for one hour and a current density of 375 $\mu A.cm^{-2}$ in the second group. The individual pharmacokinetic parameters were obtained separately following a 15-minute zero-order intravenous infusion of 30 $\mu g.kg^{-1}$. Skin resistance was measured during current delivery. Current-induced irritation was measured by Laser Doppler Flowmetry (LDF). The pharmacodynamics were quantified by a unilateral tapping score. Qualitative clinical improvements (decreased tremor, rigidity or cramp) were also recorded.

Results. In all patients increasing plasma concentrations of R-apomorphine were found during the interval of current application. The maximum concentrations that were attained were related to the applied current density: 1.3 ± 0.6 $ng.ml^{-1}$ at 250 $\mu A.cm^{-2}$ and 2.5 ± 0.7 $ng.ml^{-1}$ at 375 $\mu A.cm^{-2}$. When the current was switched off all concentrations returned to baseline values in about 90 minutes. By mathematical deconvolution of the profiles it was shown that steady-state fluxes were reached within the one-hour interval of current driven transport. Steady-state fluxes were calculated to be 69 ± 30 $nmol.cm^{-2}.h^{-1}$ at 250 $\mu A.cm^{-2}$ and 114 ± 34 $nmol.cm^{-2}.h^{-1}$ at 375 $\mu A.cm^{-2}$. Individual drug input rates were inversely related to the overall resistance. Significantly elevated LDF values were found after patch removal, indicating mild current induced erythema. Only subtherapeutic plasma concentrations were obtained in all patients except for one.

Conclusions. The results show that current-dependent delivery of apomorphine is possible *in vivo* at acceptable levels of skin irritation. Excellent correlation was found between the calculated *in vivo* transport rates and the rates that were previously obtained *in vitro*.

KEY WORDS: iontophoresis; Parkinson's disease; human; pharmacodynamics; transdermal delivery; apomorphine.

INTRODUCTION

Dopamine agonists were developed in the early seventies for the treatment of Parkinson's disease. Disease progression results in so-called "on-off" phenomena in over 50 percent of the patients over a period of ten years. These phenomena can not be treated effectively with oral dosing of levodopa (1). Apomorphine is currently the most effective dopamine agonist with a potency comparable to levodopa (2,3). Additionally, it has shown improvement in the treatment of on-off effects (4). However, systemic side effects such as nausea, vomiting and dizzyness occur frequently.

Delivery of apomorphine is not without difficulty and different problems occur when the drug is dosed via different routes. These problems are mainly due to instability and—possibly as a result of this—local toxicity. Cotzias *et al.* showed that oral absorption is minimal and that the first-pass effect is high (5). At very high doses beneficial effects occur but long-term administration via this route is not possible due to drug-induced nephrotoxicity. The development of alternate routes of administration has resulted in a significant improvement of therapy, but the serious problems associated with long-term dosing have prevented its widespread acceptance.

Subcutaneous administration is currently the method of first choice but invariably results in the appearance of subcutaneous nodules. Responses comparable to subcutaneous administration were observed for sublingual, rectal and nasal administration. However, for sublingual administration, systemic side effects occurred more frequently and inconsistency of dissolution and unpleasant taste were noted (6). Furthermore, in a follow-up study 50 percent of the patients developed stomatitis (7). No local toxicity was observed following rectal administration (8). However, the limited acceptability of this route will probably limit its chronic use. A reduction of systemic side effects was observed for nasal administration (9). Prolonged use resulted in moderate to severe nasal irritation in all patients (10).

As described earlier, accurate control of the input rate of apomorphine will result in maximal benefit to the patient and minimal systemic side effects (11). In studies on the iontophoretic delivery of fentanyl in humans it has been shown that current-dependent delivery can be achieved with this technique (12). However, as for the other alternate routes, local toxicity may limit its success. Therefore, the applicability of this technique for the controlled delivery of apomorphine in patients with Parkinson's disease will equally depend on the control of dosing and on local drug-induced toxicity. Therefore these two aspects should be studied concurrently.

The aim of this study was to explore the feasibility of *in vivo* iontophoretic apomorphine application for the treatment of patients with Parkinson's disease. Constant apomorphine concentrations were applied at two current densities to investigate if transport rate can be controlled and manipulated by the externally applied current. Furthermore, the occurrence of possible side effects on the skin as a result of this application was determined.

MATERIALS AND METHODS

Patients

Ten patients with idiopathic Parkinson's disease (IPD) were included (6 men and 4 women) with a mean age of 55 ± 5 (range 46–64) years. The mean weight was 73 ± 11 (range 52–91) kg and all patients were internally and neurologically

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stable. All patients gave informed consent. The protocol of the study was approved by the Medical Ethical Committee of the University Hospital of Leiden.

Materials

R-apomorphine was obtained from OPG B.V. (Utrecht, Holland), N-propylnorapomorphine was obtained from Research Biochemicals International (RBI), (Natick, USA). Tetraoctylammoniumbromide (TOABr) and diphenylborinic acid ethanolamine ester (DPBEA) were obtained from Aldrich (Bornem, Belgium). Sterile aqueous apomorphine formulations were prepared at the Department of Clinical Pharmacy and Toxicology of the University Hospital of Leiden. The formulations contained 15 mM apomorphine, 140 mM NaCl, 0.1 % sodium meta bisulfite and 5 mM citrate buffer pH 5. This formulation was derived from previously performed *in vitro* transport experiments, in which human stratum corneum and Ag/AgCl electrodes were used (11). In the patient study, open chamber TransQ-E® patches, containing Ag/AgCl electrodes, with a skin exposed surface area of 20 cm^2, were kindly provided by Iomed Inc. (Utah, USA). The anodal compartment was filled with 3 ml of the formulation. The cathodal compartment was filled with 0.9% sterile saline (NPBI, Emmer-Compascuum, The Netherlands). A (pulsed-) constant current carry-on power supply (V_{max} = 27 Volts, I = 0–10 mA) was manufactured by the electronics department of the Gorlaeus Laboratories (Leiden, The Netherlands).

Iontophoretic Application and Pharmacokinetics

Each patient received R-apomorphine intravenously (30 $\mu g.kg^{-1}$) and by transdermal iontophoresis according to a randomized cross-over design. The wash-out period between each treatment was at least one week. All dopaminergic medication was stopped at 24:00 hours, the night before the study day. Thirty mg t.i.d. domperidone, a peripheral dopamine antagonist, was administered starting two days before each session and was stopped at the end of each treatment.

For the iontophoretic treatment, application at two current densities was tested. For safety reasons the first five patients were treated with the lowest current density. The anodal compartment was filled with 3 ml of the apomorphine solution. The two patches (anode and cathode) were applied on the volar side of the forearm. An i.v. catheter was inserted in the opposite arm for blood sampling and the infusion of saline. Blood samples were obtained at 0, 20, 40, 60, 70, 80, 90, 100, 110, 120, 125, 130, 135, 140, 150, 165, 185, 210, 240, 275, and 315 minutes. Patches were applied at t = 0. Electrical current was applied during one hour starting at t = 60 min. Resistances were measured at t = 65, 90 and 115 minutes. Resistance data were corrected for the voltage drop across the electrode-electrolyte interface. This voltage drop was 1.2 Volts. Local skin erythema was assessed visually and quantified by laser Doppler flowmetry (LDF) (Diodopp, Appl.Laser Technology, The Netherlands), before and after current application (t = 0, 245 and 317 minutes).

The pharmacokinetic parameters of apomorphine were determined upon intravenous infusion. The patient was given a zero-order intravenous infusion of 30 $\mu g.kg^{-1}$ for 15 minutes through a permanent catheter in the forearm vein of one arm. A second i.v. catheter was inserted in the opposite arm. The other i.v. catheter was again used for blood sampling and infusion of saline. 5 ml blood samples were obtained at 0, 3, 7, 11, 15, 19, 23, 27, 31, 35, 40, 45, 50, 60, 70, 85, 105, 130, 160, 200, 225 and 260 minutes.

All blood samples were collected in tubes containing 5 mg of sodium metabisulphite and 15 mg of EDTA and were placed in ice immediately. Plasma was obtained from the blood samples by centrifugation. At the end of the session all plasma samples were stored at −70dgC.

Pharmacodynamics

A unilateral tapscore was performed by pressing, as quickly as possible and with one hand, two buttons that are 30 cm apart. The number of taps during 30 seconds, using the arm that was most affected by the disease, was used as effect parameter (13). The tapscore was assessed at t = 0, 5, 9, 17, 25, 33, 42, 52, 72, 90, 132, 180, 227 and 262 minutes for the i.v. infusion and at t = 0, 22, 42, 72, 82, 92, 112, 132, 152, 187, 232, 277 and 317 minutes for the iontophoretic application. An increase of at least 25% compared to the tapscore at t = 0 was defined as a positive clinical response. Clinical improvement (decreased tremor, rigidity or cramp) was recorded using standard rating scales (14). The blood pressure was monitored and adverse effects (nausea, dizziness) were also recorded.

Drug Analysis

An enantio-selective assay according to Van der Geest *et al.* (15) was used for the quantification of R-apomorphine in plasma. Briefly, the method was as follows: 30 µl of internal standard N-propylnorapomorphine (2 mg/ml) was added to 1 ml of plasma sample. The samples were extracted with 0.5 ml DPBEA buffer and 1.5 ml TOABr. The pH of the buffer was 8.45 for plasma analysis and 9 for urine analysis. After 2 min of shaking and 15 min of centrifugation at 5°C, the organic phase was taken off and 3 ml of octanol and 0.5 ml of aqueous phase (0.05 M H3PO4, 0.1 % sodium-metabisulphite and 0.01 % of EDTA) were added. After 2 min of shaking and 15 min of centrifugation at 5°C, 50 µl sample was injected into the HPLC system.

The HPLC system consisted of a Spectroflow 400 solvent delivery system (Applied Biosystems, Ramsey, NJ, USA), a WISP™-710 B autosampler (Millipore-Waters, Milford, MA, USA) and an Antec Electrochemical Detector (Antec, Leiden, The Netherlands). The chromatograms were recorded by a Chromatopack C-R3A reporting integrator (Shimadzu, Kyoto, Japan). For the separation of R-apomorphine a 10-µm Chiralcel OD-R chiral column (200 mm × 4.6 mm I.D.) (Diacel Chemical Industries, LTD. Tokyo, Japan) was used. Acetonitrile/buffer (35/65) was used as a mobile phase at a flow rate of 0.9 $ml.min^{-1}$. The buffer consisted of 0.1 M NaH_2PO_4, 0.1 M $NaClO_4.H_2O$, 10 mg/l EDTA, pH 4. The voltage of the detector was 0.7 V. The calibration curves for both enantiomers were linear ($r > 0.995$) and the intra- and inter-assay variations were <5% for all concentrations tested (2.5, 12.6 & 25.1 $ng.ml^{-1}$), (n=5). The detection limit was 0.2 $ng.ml^{-1}$ for R-apomorphine and 0.6 $ng.ml^{-1}$ for S-apomorphine at a signal to noise ratio of 3.

Data Analysis

The pharmacokinetics of apomorphine were quantified for all patients. The data obtained upon intravenous administration were fitted to a poly-exponential equation, describing the plasma concentration time profile for intravenous infusion (16). The non-linear least squares-regression program Siphar was used for this (Simed SA, Creteil, France):

$$C(t) = \Sigma_{i=1}^{n} \frac{C_i}{\lambda_i \cdot T} \cdot (1 - e^{-\lambda_i \cdot t}) \qquad t \leq T \qquad (1)$$

$$C(t) = \Sigma_{i=1}^{n} \frac{C_i}{\lambda_i \cdot T} \cdot (e^{-\lambda_i \cdot (t-T)} - e^{-\lambda_i \cdot t}) \qquad t > T \qquad (2)$$

where C(t) is the plasma concentration of apomorphine at time t, T is the infusion duration and A_i is the coefficient associated with the i-th exponent λ_i. Different exponential models were investigated and the most suitable model, based on best fit, was chosen for each patient. In the non-linear regression analysis a weight factor of w=1/y was used. Basic pharmacokinetic parameters as elimination half-life ($t_1/_2$), volume of distribution (V_d) and total clearance (Cl) were calculated from the coefficients and exponents of the fitted function. Area under the curve (AUC) was calculated using the trapezoidal rule.

Iontophoretic fluxes (J) of apomorphine were also determined. Equation (3) was used to calculate the average iontophoretic apomorphine flux. Equation (4) was used to calculate the steady-state iontophoretic apomorphine flux,

$$J_{av} = (Cl^*AUC_{td})/(\tau^*A) \qquad (3)$$

$$J_{ss} = (C_{ss}^*Cl)/A \qquad (4)$$

in which J_{av} is the average apomorphine flux, J_{ss} is the steady state apomorphine flux, Cl is the clearance determined by intravenous infusion, AUC_{td} is the area under the plasma concentration time curve for transdermal delivery, τ is the delivery interval, and A is the current exposed area of the skin. C_{ss} is the extrapolated steady-state plasma concentration for iontophoretic delivery of apomorphine by fitting up-slope of the curve to equation (1) that describes the plasma concentration time profile for a zero-order intravenous infusion. Mathematical deconvolution (Loo-Riegelman method) was applied to derive the *in vivo* iontophoretic input profile of apomorphine, using the program Siphar (Simed SA, Creteil, France). Differences were tested for significance using a students' *t*-test. All calculated values are expressed as the average ± SD.

RESULTS

Pharmacokinetics

Figure 1 shows the plasma concentration time profiles resulting from the iontophoretic current delivery protocol at two current densities. In none of the patients elevated levels of apomorphine were found during the first hour of patch application (when no current was applied). In the two patients that had been treated by chronic subcutaneous apomorphine infusion, relatively constant background levels of apomorphine were found. The plasma concentration - time profiles resulting from the iontophoretic protocol of these two patients were appropriately corrected. In all patients elevated levels of apomorphine were found between t = 60 and 120 minutes, during which period apomorphine was applied by iontophoresis. The concentration of apomorphine steadily increased at both current densities. Due to a continuous uncomfortable level of current sensation in one patient, the current density was lowered from 250 to 200 $\mu A.cm^{-2}$. The results from this patient were not included in the average values. At t = 120 min. the plasma concentration resulting from a one-hour current passage at 250 $\mu A.cm^{-2}$ was 1.3 ± 0.6 $ng.ml^{-1}$ and at 375 $\mu A.cm^{-2}$ the concentration was 2.5 ± 0.7 $ng.ml^{-1}$. This indicates that the rate of delivery of apomorphine is current dependent. For both current densities plasma concentrations returned to baseline after approximately 90 minutes.

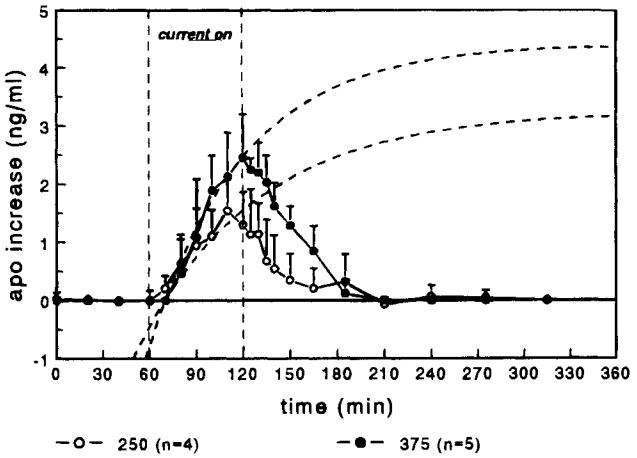

Fig. 1. Average plasma concentration time profiles of apomorphine upon transdermal iontophoretic delivery at current densities of 250 $\mu A.cm^{-2}$ (-o-) (n = 4) and 375 $\mu A.cm^{-2}$ (-●-) (n = 5) for one hour, starting at t = 60 min. Steady state plasma concentration were predicted by fitting the plasma concentration time profiles between 60 and 120 min. to equation (1).

The pharmacokinetic parameters for each individual patient are given in Table I. All the plasma concentration-time profiles were fitted to a bi-exponential equation. The iontophoretic transport rates were calculated using these parameters. Application of equation (3), using the individual clearances and

Table I. Pharmacokinetic Parameter Estimates of R-Apomorphine After Intravenous Administration of 30 $ug.kg^{-1}$ in 15 Minutes in Patients with Idiopathic Parkinson's Disease

Pnt	Gender	Dose (mg)	Clearance ($ml.min^{-1}.kg^{-1}$)	V_{dist} ($l.kg^{-1}$)	$t^1/_2$ (min)
p1	m	2.73	39.6	0.79	34.1
p2	f	1.60	21.2	1.53	85.4
p3	m	1.95	24.6	2.00	82.4
p4	m	2.25	38.7	0.63	19.6
p5	f	2.28	31.6	1.16	59.2
p6	m	2.40	48.8	2.06	43.5
p7	m	2.19	37.0	1.41	43.3
p8	f	1.83	41.6	1.02	29.5
p9	f	2.34	19.2	0.73	41.4
p10	m	2.40	47.5	2.35	48.2
Mean±std.		2.20±0.32	35.0±10.5	1.37±0.61	48.7±21.4

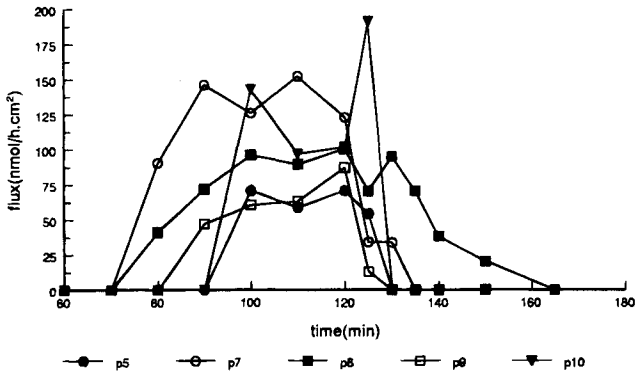

Fig. 2. Calculated individual transdermal transport rates of apomorphine at an applied current density of 375 $\mu A.cm^{-2}$ for one hour. Mathematical deconvolution (Loo-Riegelman method) was applied on the plasma concentration-time profiles, using the individual pharmacokinetic parameters.

AUC's, resulted in an average apomorphine flux of 44.0 ± 3.1 $nmol.cm^{-2}.h^{-1}$ at 250 $\mu A.cm^{-2}$ and 91 ± 28 $nmol.cm^{-2}.h^{-1}$ at 375 $\mu A.cm^{-2}$. Equation (4) was used to calculate the steady-state flux. C_{ss} for patients treated with the same current density was estimated from the average concentrations at t=120 min, using the average pharmacokinetic parameters of the patients treated with the same current density. A lag time of 10 minutes was used. As can be observed in Figure 1, a satisfying fit of the up-slope of the curve was obtained. The calculated flux values for 250 $\mu A.cm^{-2}$ and 375 $\mu A.cm^{-2}$ were 69 ± 30 $nmol.cm^{-2}.h^{-1}$ and 114 ± 34 $nmol.cm^{-2}.h^{-1}$, respectively. Deconvolution of the obtained plasma concentration-time profiles at 375 $\mu A.cm^{-2}$ showed that steady-state transport rates were obtained 10 to 20 minutes after the drug appeared in the blood (Fig. 2). The value of the steady-state flux was 101 ± 43 $nmol.cm^{-2}.h^{-1}$. This is in fair agreement with 114 ± 34 $nmol.cm^{-2}.h^{-1}$, the value that was obtained when equation (4) was applied.

A good linear correlation was found between AUC_{td} and resistances, both at t = 65 min and at 115 min. (Figure 3). No improvement in the correlation was found when the AUC values were corrected for the individual clearances.

Pharmacodynamics

Two patients at the low current density and three patients at the high current density experienced clinical improvement during or after current application. Decreased rigidity of the extremities, tremor and yawning were the observed effects. No adverse dopaminergic effects were observed. In only one patient treated with the high current density, the qualitative clinical improvement could be confirmed by a > 25 % increase in tapping score. It should be noted, however, that the study was not blinded, so placebo effects can not be excluded.

Clinical observations and/or tapping score improvements following intravenous infusion served as a positive control. Tapscore increase as a result of intravenously administered apomorphine was observed in seven out of ten patients at concentrations above approximately 3 $ng.ml^{-1}$. In this case five out of ten patients experienced side effects associated with higher plasma concentrations of apomorphine (> 20 $ng.ml^{-1}$). The reported side effects were nausea and dizziness.

Effect of Current on the Skin

Tingling sensation was felt in all patients during the first 2–4 minutes of current passage. One patient (#4) that was treated with the low current density indicated continued uncomfortable levels of tingling sensation of the skin area below the anodal chamber. For this reason the current density was lowered to 200 $\mu A.cm^{-2}$ at t = 65 min. This patient, who had red hair and a very light skin, reported that his arms had been exposed to elevated levels of UV light the day before the study. The concentration time profile of this patient was therefore not included in the overall results. The apomorphine concentrations in this patient were comparable to the levels found in the other patients that were treated with a current density of 250 $\mu A.cm^{-2}$. Figure 4 shows the individual erythematous responses measured with LDF. Relatively high responses are observed in patient #4 (as expected from the severe tingling sensation) and in patient #8. Patient #8 had previously experienced thrombosis in the upper arm resulting in a continuously increased redness of this arm. Therefore the LDF values from these two subjects were excluded from the overall results (Figure 5). As shown in Figure 5 significantly increased LDF values are observed as a result

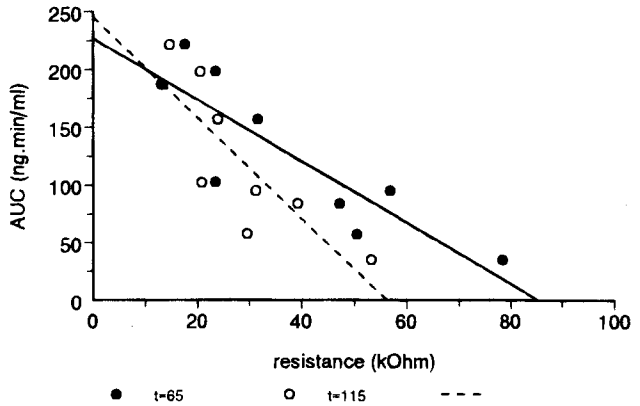

Fig. 3. Relationship between the resistance of the skin (at t = 65 and 115 min.) and the area under the plasma concentration time curve (AUC) obtained after iontophoretic application of apomorphine at 250 and 375 $\mu A.cm^{-2}$ (r = 0.7).

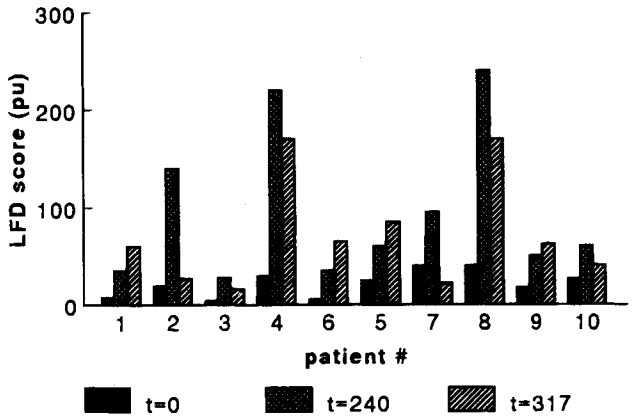

Fig. 4. Individual LDF values of the skin, post-current exposure, measured at t = 0, 245 and 317 minutes. Patients 1,2,3,4 and 6 were treated with apomorphine at 250 $\mu A.cm^{-2}$. Patients 5,7,8,9 and 10 were treated with apomorphine at 375 $\mu A.cm^{-2}$.

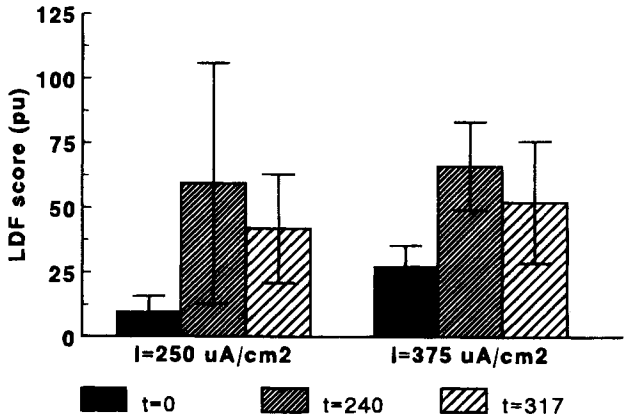

Fig. 5. Average LDF values at t = 0, 245 and 317 minutes of the skin, that had been exposed to iontophoretic apomorphine treatment at either 250 or 375 $\mu A.cm^{-2}$.

of current treatment. The increase in LDF value was not dependent on the current density and tended to decrease after one hour.

DISCUSSION

This study shows that subtherapeutic plasma concentrations of apomorphine can be attained in patients with Parkinson's disease, when currents of moderate intensity are applied for one hour. Plasma concentrations continuously increased during this hour. The profile of the up-slope of the plasma concentration-time curve is comparable to the profile that is obtained from a zero-order infusion of apomorphine. The plasma concentration dropped exponentially to baseline levels when the current was switched off, as is to be expected for this rapidly cleared drug (15). At the same time, this indicates that no subcutaneous depot of apomorphine was formed from which the drug was released slowly.

The delivery was closely regulated by the applied current density. Mathematical deconvolution of the data for the intrinsic pharmacokinetic parameters showed that the flux reaches a constant level in 10 to 20 minutes. When the current is switched off, the flux rapidly drops back to zero. The interval needed to reach a steady-state flux, reflects the time it takes to achieve a steady-state flux of apomorphine from the skin surface to the micro-vascular system. The derived transport rate is directly proportional to the applied current density. These results show that precise control of delivery of apomorphine is possible. This is of great importance since it was shown previously that the pharmacodynamic effect of the drug is directly related to the plasma concentration. Moreover, the therapeutic window was shown to be relatively narrow (13).

Proportionality of current and flux was also found for the delivery of apomorphine across human skin *in vitro* (11). Moreover, almost identical absolute values of the steady-state flux were found when the derived rate *in vivo* was compared to the transport rate across dermatomed human skin *in vitro* as is summarized in Table II. Steady-state fluxes were normalized fluxes for a current density of 250 $\mu A.cm^{-2}$, assuming a linear relationship between flux and current. Note that the variability was increased in the *in vivo* situation. Surprisingly, correction of the input profile for the individual differences in PK did not narrow down the amount of variability in apomorphine delivery.

Table II. *In Vitro/In Vivo* Relationship of Transport Kinetics of Apomorphine Through Human Skin Normalised for a Current Density of 250 $\mu A.cm^{-2}$

Membrane	J_{ss} ($nmol.cm^{-2}.h^{-1}$)	tts[a] (min)
in vivo	69 ± 30	10–20
stratum corneum, *in vitro*[b]	101 ± 13	15–30
dermatomed skin, *in vitro*	65 ± 11	300

Note: A donor concentration of 15 mM at a pH of 5 was applied in both cases.

[a] tts = time to reach steady state. For the determination of the *in vivo* transport profiles mathematical deconvolution was used.

[b] *in vitro* experiments were performed with a thermostrated acceptor chamber of 37°C and Ag/AgCl electrodes.

Intra-patient variability in the pharmacokinetics, and increased variability in the measurement of low apomorphine plasma level may contribute to this. The *in vitro* steady state flux across human stratum corneum is also given in table II. This value slightly exceeds the *in vivo* flux. Moreover, no differences in flux kinetics were observed *in vitro* between skin samples obtained from different donors.

A summary of *in vitro/in vivo* correlations for several low molecular ions is given by Phipps *et al.* (18). In contrast to the results presented here, the measured flux *in vivo* was generally higher than the flux *in vitro*. These differences were ascribed to the intrinsic ion concentration of the skin. For apomorphine it was shown that temperature and skin type determine the flux to a large extent (11). The *in vitro/in vivo* correlation is therefore highly dependent on the experimental conditions. Several mechanisms may contribute to the overall transport and may effect *in vitro* and *in vivo* transport rates to a different degree: [a] Skin metabolism: Differences may occur in skin residence time of the substrate and enzyme activity. However, no significant metabolism was observed during iontophoresis *in vitro* under physiological conditions (11) and is therefore not likely to contribute to the overall transport rate. [b] A contribution of the dermis to the barrier function of the skin: This is confirmed by the significantly higher steady-state flux that is observed through stratum corneum alone. Furthermore, the relationship between skin resistance (R) and AUC_{td} was exclusively observed *in vivo* and may originate from inter-individual differences in the dermal region. Differences in skin resistance are not likely to be due to differences in contact between patch and skin, since open chambers in combination with an aqueous vehicle were used. The correlation between the resistance and AUC_{td} should be verified in a larger group of patients, but especially the correlation at early time points opens up the attractive possibility of individual titration of the patient. [c] Differences in dermal clearance: The deconvoluted *in vivo* flux data suggest a rapid uptake of apomorphine in the systemic circulation, as opposed to the long time to steady state (tts) through dermatomed human skin (Table II). This difference presumably reflects the contribution of the dermal blood supply in removing topically applied compounds *in vivo*. The transient erythema that is observed reflects local vasodilatation and may induce a rapid clearance from the dermal region (19). The effect of dermal clearance on the iontophoretic delivery of drugs was studied in detail by Singh *et al.* (20,21) and was shown to contribute to the overall transport rate.

Local adverse effects such as prolonged inflammation and green colouring as a result of oxidation of apomorphine are known to be major problems in subcutaneous apomorphine delivery (4). None of these effects were observed in this study. Several factors could contribute to the absence of apomorphine associated skin toxicity. Firstly, puncturing the skin could initiate the release of mediators that contribute to the overall inflammatory response of the skin. Scondly, the concentrations per surface area are reduced in the iontophoretic application since the uptake in the micro vasculature is spread over a larger surface area of the skin. Thirdly, skin toxicity occurs only at higher dermal concentrations. If the toxic response is indeed concentration-dependent, skin toxicity can still occur at therapeutic levels. Lees *et al.* proposed that the utilisation of diluted solutions for subcutaneous injection may reduce skin toxicity (22). These considerations underline the necessity to evaluate the relationship between apomorphine concentration in the skin and the local adverse effects.

A transient erythematous response was observed following iontophoretic application of apomorphine. Little is known about the underlying mechanism. Only a handful reports has been published so far investigating these effects (23,24,25). No effects on transepidermal water loss and only transient erythema have been observed for short-term application of moderate current densities. The side effects observed in this study did not differ significantly from the side effects observed in previous studies, in which only vehicle (so no drug) was applied on the skin for 30 minutes. Thus, the observed minor skin irritation appears to be due to the application of transdermal iontophoresis per se, rather than apomorphine. No correlation was observed between LDF values and current density, which also indicates that the immediate erythematous response of the skin is not associated with the amounts of apomorphine that are transported through it. Despite the fact that these initial reports show few side effects, little is known about the effects of long-term application of transcutaneous currents. These considerations are of concern for the treatment of chronic diseases (*e.g.* Parkinson's disease) where drugs must be applied for longer time intervals. In this study low pigmentation of the skin in combination with recent exposure to UV light led to an enhanced sensitivity of one patient. For safety precautions, this combination should be an exclusion criterion for future *in vivo* studies.

Overall pharmacokinetic values were similar to the values obtained in an earlier study (17). Also pharmacodynamic effects were measured in this study. PK/PD relationships for drugs with fast PK and fast equilibration to the effect compartment such as apomorphine are difficult to investigate when plasma concentrations are changing rapidly. PK/PD relationships were investigated in a previous study (13). In this study it was shown that the PK/PD relationship varies significantly from patient to patient and that beneficial effects can be expected at plasma concentrations that are higher than 3–6 $ng.ml^{-1}$. In the present study plasma levels stay just below this level. Therefore, beneficial effects are not likely to occur. Only one patient showed unequivocal improvement due to iontophoretic delivery of apomorphine. Longer current application times at the currently used conditions are expected to result in effective plasma levels. However, further improvement of delivery by optimizing the donor formulation and/or modification of the barrier is desired.

Side effects (nausea, dizziness) occurred in 50% of the patients at higher concentrations (20–50 $ng.ml^{-1}$) following zero-order intravenous infusion of 30 $\mu g.kg^{-1}$ in 15 minutes. This stresses once again that for apomorphine to be a successful therapeutic agent the input rate needs to be closely regulated. Iontophoresis seems to be the ideal tool for this if transport rates can be achieved that are sufficiently high to result in therapeutically relevant significant plasma levels.

CONCLUSION

In this study it was shown that controlled iontophoretic apomorphine delivery in patients with Parkinson's disease may have specific advantages over conventional subcutaneous delivery. The fact that the delivery is directly proportional to the applied current density opens up the attractive possibility of individual titration of the administered dose. However, strategies should be developed to improve the transport efficiency, since little room for individual titration, or additional on-demand bolus dosing, is left under the currently used experimental conditions.

Careful consideration of possible side effects on the skin is needed, especially at longer application times. By further improving the efficiency of apomorphine delivery, current levels can be lowered. This is expected to lower the level of local erythema.

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