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Iontophoretic delivery of apomorphine: from in-vitro modelling to the Parkinson patient

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Abstract

Apomorphine is a mixed dopamine D_1/D_2 receptor agonist which is potentially useful in the treatment of Parkinson's disease. The delivery of apomorphine is however complicated because it is not absorbed orally and other delivery routes with the exception of the intravenous route seem to fail. The most interesting route for controlled delivery of apomorphine is transdermal iontophoresis because this could enable the Parkinson patient to directly control the needed amount of apomorphine by increasing or decreasing the drug input in order to achieve optimal drug therapy ('on-demand') with a minimum of toxic side effects. The typical features of Parkinson's disease could be used to monitor the needed drug input and even more elegantly by means of suitable chip sensors which are able to directly measure bradykinesia, akinesia and/or tremor and to regulate in such a way the drug input. Such a chip-controlled iontophoretic system would be the first closed-loop system monitoring not pharmacokinetic data (blood levels) but more importantly externally measurable pharmacodynamic effects of Parkinson's disease. This scenario is more feasible as skin irritation and toxicity studies have proven that iontophoresis is a safe route of treatment. This review describes the basics of iontophoresis and the development of a transdermal iontophoretic delivery system on the basis of integrated pharmacokinetic/pharmacodynamic (PK/PD) investigations in patients with idiopathic Parkinson's disease. Transdermal iontophoretic transport of apomorphine was studied both in vitro with human stratum corneum using a newly developed iontophoretic continuous flow-through transport cell and in vivo in a first exploratory study in patients with Parkinson's disease. These studies showed that the delivery of apomorphine is feasible and furthermore the rate of delivery can be controlled by variation of the current densities. Additionally the pretreatment of the skin either with a mono-surfactant or a vesicular suspension of elastic liquid-state vesicles may be useful to further increase the apomorphine flux across the skin in combination with iontophoresis.

Keywords: Transdermal iontophoresis; Apomorphine; Parkinson's disease; Iontophoretic in vitro diffusion cell; Pharmacokinetic–pharmacodynamic correlation; Skin irritation after iontophoresis; Increased iontophoretic flux by chemical enhancement

Contents

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1. Introduction to iontophoresis

Iontophoresis is a non-invasive technique which uses a mild electric current to enhance and facilitate the dermal, but especially the transdermal, delivery of a variety of drugs, especially of hydrophilic drugs such as small peptides and hydrophilic compounds such as apomorphine, enalaprilat and others, which are not passively absorbed in the gastrointestinal tract. For this class of drugs, the enhancement of transport across the human skin is about 50–1000-fold in comparison to their passive transdermal flux and intestinal absorption.

The recent attention given to this ~100-year-old technology stems in part from advances made in the following technologies. Firstly, the widespread patient acceptance and financial success of passive transdermal patches such as nicotine, nitroglycerin, estradiol, fentanyl and others have stimulated interest to expand the range of therapeutic agents for this delivery route. Secondly, technological breakthroughs in the microelectronics industry have enabled miniaturization of programmable electronic components at lower cost. Finally, the advances in recombinant DNA technology and rational drug design have yielded several therapeutically potent peptides.

1.1. Benefits

Iontophoretic therapy will allow for the rapid onset/offset of drug action whereby the drug action is programmable and controllable resulting after about 1 h in smooth and continuous plasma levels with a minimum of inter- and intra-subject variability.

From human iontophoretic clinical data on peptides and non-peptides it appears that electrically assisted transdermal delivery of drugs into the systemic circulation is rapid and that plasma levels decline rapidly on termination of the applied current. This 'on–off' feature provides another dosing scenario in that the iontophoretic patch could be employed to deliver 'on-demand' doses of a drug, either in the case of acute pain management through patient activation of a convenient 'bolus' button or by direct measuring of 'disease parameters' via a sensor or an externally worn microchip (such as, for example, fever or heart beating frequency).

1.2. How the patch works

A simplified representation of the components of an iontophoretic patch is given in Fig. 1.

These iontophoretic patches are basically comprised of three distinct components.

1. The drug reservoir is aqueous in nature and is typically a biocompatible gel or adsorbent pad material which conforms readily to the skin surface and electrode component of the patch. The pH of the reservoir is chosen to optimize the iontophoretic delivery and is adjusted to be tolerable to the skin, i.e. in the pH range 4–8. If

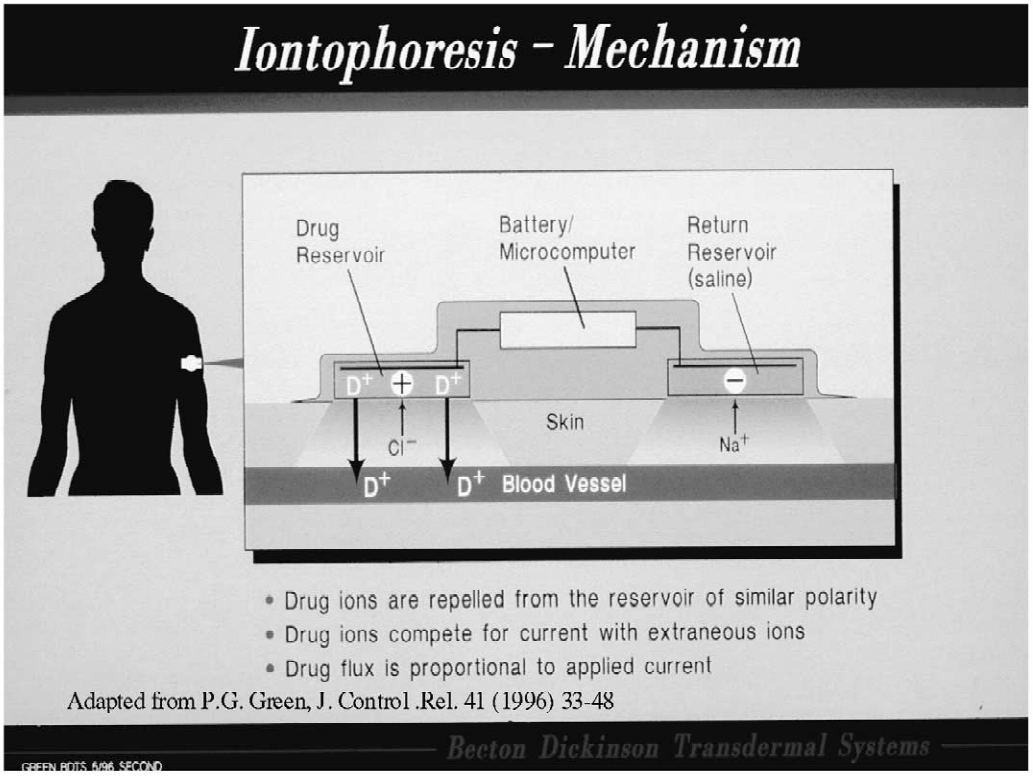

Fig. 1. Simplified representation of the components of a transdermal iontophoretic patch.

the drug has a positive charge under these conditions it is placed at the positive pole (anode) of the patch. Negatively charged peptides are formulated within the negative electrode (cathode).

2. The return reservoir is typically a saline or buffer formulation of a suitable pH value which acts to complete the electronic circuit.
3. The electronic controller contains the battery and programmable microcomputer which provide the driving force and control for the iontophoretic drug delivery. This more expensive component of the patch can be easily removed after each use and be reused or recharged several times whereas the patches are disposable [1].

1.3. Requirements for drug candidates

The desirable features of a drug candidate should be as follows.

The drug should be storable in dry or liquid form in the patch and should be stable. It should have adequate aqueous solubility for delivery. It should be charged and the isoelectric point should be in the range of smaller than 4 or greater than 7.4. The expected potency of the iontophoretic device is to deliver:

20–50 mg drug/day of a molecular weight of 300 Da,
2–5 mg drug/day of a molecular weight of 1000 Da, and
100 μg drug/day of a molecular weight of 5000 Da [1].

1.4. Basic facts about iontophoretic fluxes

The overall iontophoretic flux of a drug is composed of

$$J_{\text{total}} = J_{\text{passive}} + J_{\text{electric}} + J_{\text{convective}}.$$

The passive flux of a hydrophilic drug in transdermal drug delivery can normally be neglected. The electric current-driven skin permeation flux (electrorepulsion) can be derived from Faraday's law, where the mass of drug delivered across the skin is proportional to the applied current and the duration of current application. Furthermore, it is dependent on the molecular weight of the drug, its molecular charge and the current efficiency. In many cases the amount of drug transported across the skin is directly dependent on the applied current density (preferentially 250–500 $\mu A\ cm^{-2}$).

The passage of electrical current through the skin during iontophoresis has been shown to be accompanied by a convective flux of water from anode to cathode. This is known as electroosmosis and is likely to be responsible for the in vitro facilitated transdermal delivery of a number of small hydrophilic neutral molecules such as sugars. In addition, electroosmosis has been reported to be responsible for the extraction of glucose from the skin of human volunteers during 'reverse' iontophoresis. Briefly, electroosmosis arises because at physiological conditions the skin has a net negative charge (above ~pH 4) due to its predominance of acidic carboxylate groups. During the passage of electrical current highly mobile positively charged ions such as sodium are driven from the anode (positive pole) to the cathode (negative pole) reservoir. At the same time, but in the opposite direction, highly mobile negatively charged ions are repelled from the cathode to the anode. The efficiency of delivery across the skin for positively charged ions is greater than for similar size negative ones due to the negative charge of the skin (Donnan exclusion principle) [1].

2. Development of an iontophoretic continuous flow-through transport cell

Transport cells that are used to study transdermal iontophoretic transport in vitro are usually modified Franz flow-through diffusion cells or modified static diffusion cells [2–5]. Flow-through cells have the advantage that the experimental set-up may be automated, allowing for a rapid accumulation of diffusion data. These cells have been extensively validated for the in vitro determination of drug flux through the skin [6–8]. In all of these studies the drug flux is directly derived from the drug concentration of the collected samples.

Under non-steady state conditions, the actual flux through the membrane may deviate significantly from the derived flux from the acceptor volume concentration, depending on experimental set-up and cell design. In this case it may be necessary to derive an equation that describes the flux versus time profile in order to calculate the actual flux. Recently the effects of experimental parameters on the passive flux of permeants through one or more membranes were assessed by several investigators [9,10] and in one case a transfer function, based on diffusion theory, was derived [11]. Experimental variables such as tube volume, acceptor volume, sampling interval and flow-rate were shown to directly affect the absolute value of the apparent flux and may be significantly different from the intrinsic flux through the skin. The deviation from this intrinsic flux depends on the absolute value of each variable.

In order to prevent these problems, cells with a small acceptor volume, a small tube volume and a high flow-rate can be used. The deviations of the apparent flux from the intrinsic flux are minimal in this case. Flow-through cell designs with an ultra-small acceptor volume have been developed for the characterisation of passive diffusion through membranes [12,13]. Due to their geometric configuration these cells are not suitable for iontophoretic protocols.

A 'three chamber in-line' diffusion cell with a large static acceptor chamber was first presented by Bellantone et al. [14]. In our group a new transport cell was developed. This cell design allows for a minimisation of the acceptor volume and therefore an increased flow-rate to volume ratio is obtained. Furthermore, since a separate piece of skin is used on each side of the acceptor chamber openings, lateral transport of the donor formulation is prevented [15].

2.1. Design of the diffusion cell

A schematic drawing of the basic design is given in Fig. 2. The miniaturization of the volume is limited by the requirement of temperature control

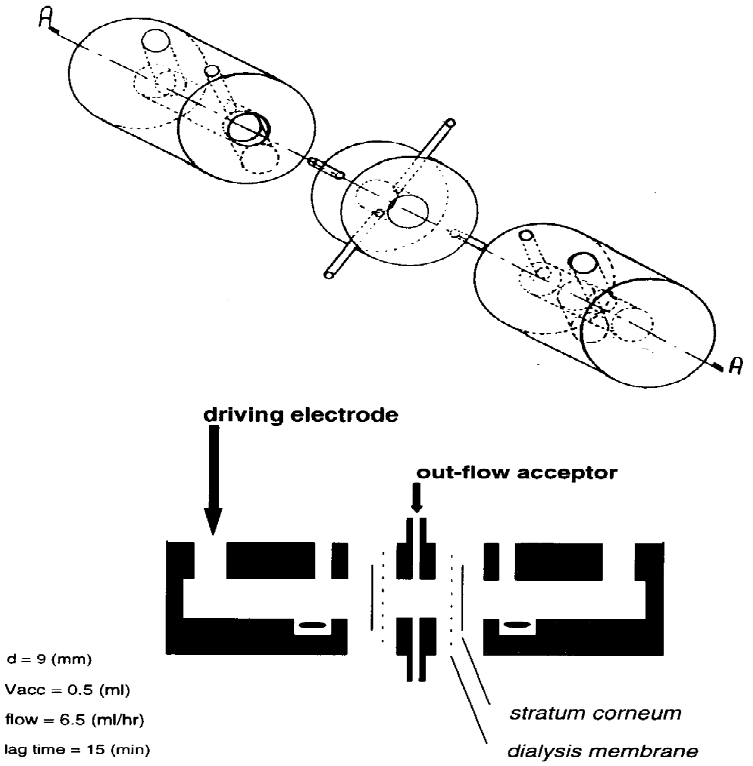

Fig. 2. Schematic (lower) and spacial (upper) drawing of the three-chamber continuous flow-through transport cell. The two outer chambers have a volume of 2 ml and contain the electrodes. They are continuously stirred. The acceptor volume (V_{acc}) is 0.5 ml. The exposed surface area for transport is 0.64 cm^2 ($d = 9$ mm). With a flow-rate of 6.5 ml h^{-1}, the lag time due to the system geometry was calculated to be 15 min.

and the in- and out-flow tubes of this chamber. The outflow is connected to the highest tube to prevent accumulation of air in the acceptor chamber. The resulting acceptor volume is 0.54 ml.

The circular diffusion areas with a diameter of 9 mm (0.64 cm^2) are positioned perpendicular to the in- and out-flow openings. The two outer chambers contain vertical openings on the top side for the electrodes and a downward protruding volume close to the diffusion surface for stirring. The volume of both the outer chambers is 2 ml. Cells and membranes (supporting membranes and human stratum corneum sheets) are held together by a perspex clamp. The cells were custom made by the Glass Technical Department of the Gorlaeus Laboratories of Leiden University [16].

2.2. Diffusion cell procedures

The stratum corneum (ϕ 14 mm) was hydrated for 2 h by floating the dermal side on PBS. A dialysis membrane was used as a support membrane (boiled for 30 min in doubly distilled water prior to use). Dermatomed skin (ϕ 16 mm) was used and supported by a piece of Whatman paper of the same diameter. One membrane was then clamped between each outer chamber and the middle chamber. The two skin membranes separate the outer electrode chambers from the middle acceptor chamber, facing the electrodes with their anatomical surfaces. To mimic physiological circumstances the acceptor chamber, but not the two outer chambers, could be thermostated at 37 °C by a surrounding socket. The

flux experiments were conducted at room temperature. After assembling the system, the acceptor chamber was flushed for 30 min. PBS was used as acceptor fluid (flow-rate: 6.5 ml h^{-1}) and in the cathodal chamber. To maintain viability 1 g l^{-1} glucose was added to the PBS in the dermatomed skin experiments. In all experiments the donor chamber contained a citrate-buffered apomorphine hydrochloride solution (NaCl 8.18 g l^{-1}, 5 mM citrate buffer: citric acid/Na_3-citrate = 0.62/0.63, 0.37/0.96, 0.12/1.30 g l^{-1} for pH 4, 5, 6, respectively).

2.3. Iontophoretic protocols

A silver plate electrode was used in the anodal compartment and a silver/silver chloride electrode was used in the cathodal compartment. The electrodes were connected to a 9-channel computer-controlled current source that was able to deliver both constant-pulsed current of variable frequency and duty cycle. The system was equipped with two differential input channels per current source channel, enabling on-line resistance measurements of the individual membranes of each transport cell. The maximum voltage for each channel was 40 V. The current source was custom made at the electronics department of the Gorlaeus Laboratories.

Typically, current was applied for 5 or 6 h. Resistance was measured during current application at 30- or 60-s intervals. In some cases, samples were collected after termination of the current to analyze passive transport.

Protocol I: passive flux of apomorphine through human stratum corneum at a 15 mM donor concentration, pH 5 at 20 °C.

Protocol II: 5 h of iontophoretic delivery at 500 μA cm^{-2} followed by 3 h of passive delivery.

Protocol III: relationship between the applied current density and the steady state flux of apomorphine through human stratum corneum at 15 mM donor concentration, pH 4 at 20 °C.

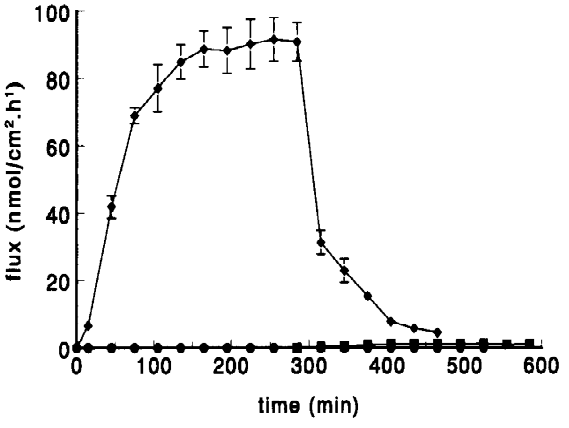

Fig. 3. Iontophoretic and passive flux versus time profile of apomorphine through human stratum corneum at a 15 mM donor concentration, pH 5 at 20 °C. (1) Five hours of iontophoretic delivery at 500 μA cm^{-2} followed by 3 h of passive delivery (♦), (2) passive flux after 5 h of current pretreatment (500 μA cm^{-2}) without apomorphine (■), (3) passive flux through non-pretreated skin (●).

2.4. Flux experiments

Fig. 3 shows that no appreciable amount of apomorphine was delivered by passive diffusion when protocol I was applied. The application of an iontophoretic current according to protocol II greatly enhanced the delivery of apomorphine across human stratum corneum to a steady-state flux of 90±6 nmol cm^{-2} h^{-1} (Fig. 3). A steady-state flux was attained in ~3 h, corresponding with a lag time of 40 min. Reversibility of the enhanced membrane permeability to the 'normal' state was tested by measuring the passive drug flux after switching off the current following the 5-h period of iontophoretic transport at the highest current density applied (500 μA cm^{-2}). In a period of only 3 h, the flux had decreased exponentially to a value ~1/20th the steady-state iontophoretic flux. This value was still elevated significantly compared to the passive flux of apomorphine. In a control experiment, stratum corneum was exposed to the same anodal current density for 5 h without the co-application of apomorphine. Thereafter, passive diffusion of apomorphine was studied for several hours. A significantly enhanced passive diffusion was observed due to current treatment alone.

The relationship between current density and steady-state flux was determined at a 15 mM donor concentration at pH 4. As shown in protocol III the current density range 0 to 500 μA cm^{-2} was studied. A linear relationship ($r^2 = 0.98$) between current

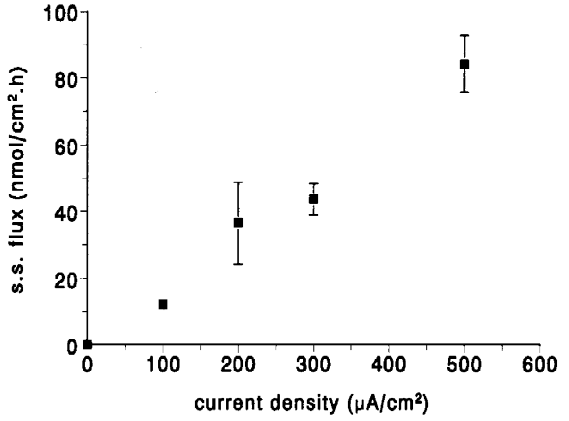

Fig. 4. Relationship between the applied current density and the steady state flux of apomorphine through human stratum corneum at a 15 mM donor concentration, pH 4 at 20 °C (current density range is 100–500 μA cm^{-2}).

density and steady state flux was found (Fig. 4). For all current densities tested, the time to reach a steady-state flux was less than 2 h. A detailed review of the physicochemical parameters on the in vitro iontophoretic delivery of apomorphine has recently been published by Li et al. [17]. From these in vitro results some calculations can be done in order to estimate the patch size needed for a successful in vivo iontophoretic treatment of Parkinson patients.

The therapeutic infusion rate for a population EC_{50} and EC_{90} is 1.4 and 2.4 mg h^{-1}, respectively. The maximum iontophoretic flux so far obtained is about 100 nmol h^{-1} cm^{-2}. The total patch area needed for successful therapeutic dosing would then be 50 cm^2 and 88 cm^2, respectively, which may be too big for rational therapeutic use. As a result of this, further improvement of the iontophoretic flux across human stratum corneum has to be achieved in order to obtain therapeutic apomorphine levels based on a reasonable size of the patches.

3. Chemical enhancers for increased iontophoretic flux

In order to increase the iontophoretic transdermal flux of apomorphine, two approaches of chemical flux enhancement have been investigated.

1. Chemical flux enhancement by use of single penetration enhancers of suitable physicochemical properties and devoid of incompatibilities with the physical iontophoretic flux increase.
2. Chemical flux enhancement by use of elastic liquid-state vesicles.

Compounds that enhance the percutaneous penetration of a drug by modification of the barrier (e.g. the stratum corneum) have been applied widely in passive transdermal studies [18]. Neutral, anionic and cationic enhancers have been used for flux enhancement. The applicability of these compounds in humans is mainly limited by the level of local skin irritation that they evoke. Skin irritation generally depends on the charge of the enhancer, but depending on their specific structure, wide variability in skin irritating properties has been observed [19]. Many investigators have studied the relationship between the structure of the enhancer and the enhancement properties. A number of these studies indicate that the enhancement depends on the carbon chain structure of the enhancer [19,20]. Maximal enhancement is generally attained for enhancers with a carbon chain length range of 10–14. This optimal range was found for anionic, cationic and neutral enhancers [21].

3.1. Chemical flux enhancement by single penetration enhancers

Little is known about the mechanism of interaction of chemical and iontophoretic treatment of the skin. Apparently contradicting reports are known on the enhancing potential of this combinatorial delivery strategy. In some studies additional enhancement of the iontophoretic flux is observed [22,23], but in other cases no further enhancement was obtained [24,25]. Depending on their chemical structure, enhancers can have a significant effect on post-iontophoretic skin impedance [26].

Therefore, it was interesting to investigate the effect of potential enhancers on the transdermal transport of apomorphine and to determine the enhancement mechanism of this enhancer in combination with iontophoresis. Three compounds with equal length carbon chains but different charges on

the headgroup were selected: dodecyltrimethylammonium bromide (DTAB), lauric acid (LA) and trioxyethylenedodecyl ether ($C_{12}EO_3$) in comparison to propylene glycol (PG) which was used as solvent for the three surfactants. Careful consideration was given to the effect on the barrier properties of the skin as a result of this combined enhancement strategy. Pretreatment with $C_{12}EO_3$ resulted in the highest iontophoretic steady state flux of apomorphine (244 ± 34 nmol cm^{-2} h^{-1}) which is shown in Fig. 5. An enhancement factor (EF) of 61 compared to the pre-iontophoretic passive flux was found. For PG pretreatment, an absolute steady state iontophoretic flux of 106 ± 13 nmol cm^{-2} h^{-1} and an enhancement factor of 28 compared to pre-iontophoretic passive delivery was obtained. Comparable flux versus time profiles were obtained for all four pretreatments of human stratum corneum: (I) steady state fluxes were achieved and maintained within all three delivery intervals (pre-iontophoretic, iontophoretic and post-iontophoretic) in which the flux was measured. (II) Application of current to human stratum corneum resulted in a significant increase of the transdermal transport compared to pre-iontophoretic passive delivery. (III) All fluxes rapidly declined during the post-iontophoretic passive delivery interval. The post-iontophoretic steady state flux was comparable to the pre-iontophoretic steady state flux of non-iontophoresed stratum corneum.

The steady state fluxes for all pretreatments and delivery intervals are given in Fig. 6. All calculated values are the average of the data obtained from 6 to 8 transport cells. Pretreatment with LA resulted in a slight increase of the iontophoretic steady state flux (119 ± 6 nmol cm^{-2} h^{-1}) compared to PG-pretreated stratum corneum: EF = 1.13. DTAB pretreatment resulted in a slight inhibition (steady state flux = 83 ± 14 nmol cm^{-2} h^{-1}) of iontophoretic transport: EF = 0.78. These steady state fluxes are significantly different from each other ($P<0.05$). An enhancement factor of 2.3 was found for $C_{12}EO_3$ pretreatment.

The rank order of the lag times during iontophoretic delivery is inversely related to the steady state fluxes that were achieved. No lag time was observed for the highest iontophoretic flux that was measured, after $C_{12}EO_3$ pretreatment. Similar lag times for iontophoretic delivery were observed after PG and LA pretreatment (±5 min) and the longest lag time (±55 min) was found for the lowest iontophoretic flux: after DTAB pretreatment.

3.2. Chemical flux enhancement by elastic liquid-state vesicles [27]

In the second approach, the elastic vesicle suspension was prepared by a modification of the sonication method described by Baillie et al. [28]. Briefly, the PEG-8-L, sucrose-ester L595, and cholesterol sulfate

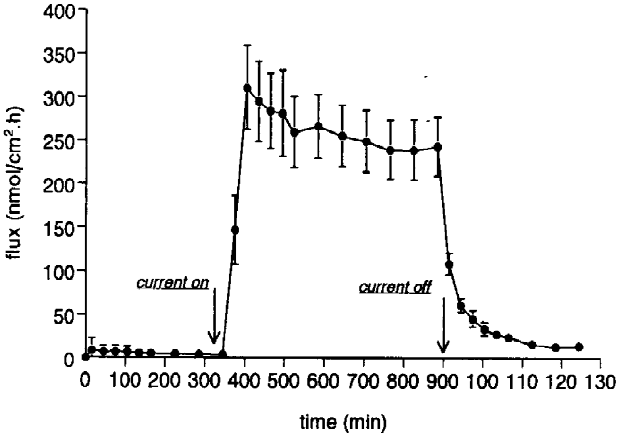

Fig. 5. Flux versus time profile of pre-iontophoretic passive, iontophoretic, and post-iontophoretic passive delivery of apomorphine. Stratum corneum was pretreated with 0.15 M $C_{12}EO_3$ in PG for 24 h. Thereafter 15 mM apomorphine was applied. A 0.5 mA cm^{-2} anodal current was applied for 9 h, starting at $t=6$ h.

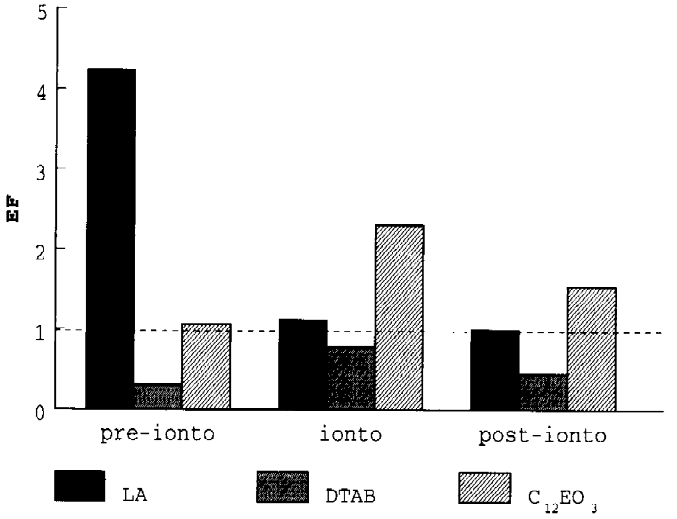

Fig. 6. Calculated enhancement factors for the delivery of apomorphine through human stratum corneum. The level of enhancement following pretreatment with LA, DTAB and $C_{12}EO_3$ was compared to the PG control treatment per time interval (pre-iontophoretic, iontophoretic and post-iontophoretic).

were solubilized in chloroform/methanol (3:1 v/v). The solvent was evaporated overnight in a vacuum centrifuge, and the remaining surfactant film was hydrated with phosphate buffered saline (PBS). The final formulation contained 5% (w/w) of total lipids.

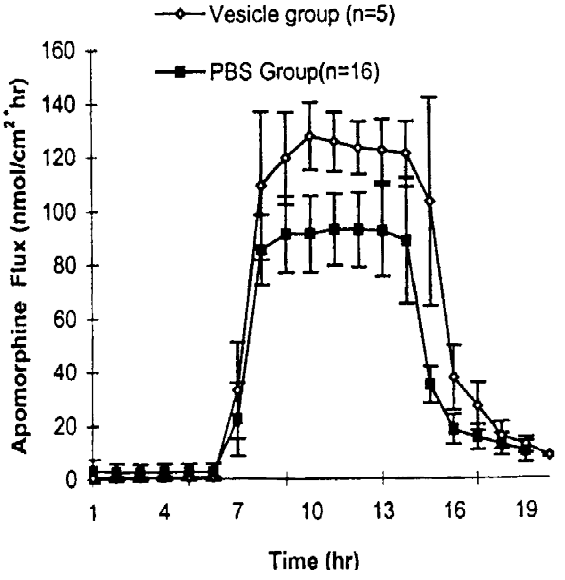

Fig. 7. Permeation profile of apomorphine across human stratum corneum during 6 h passive diffusion, 9 h iontophoresis at current density of 500 μA cm^{-2}, and 5 h post-iontophoresis passive diffusion. (■) Control; (◇) pretreatment with elastic vesicle.

Fig. 7 shows the permeation profiles of apomorphine across human stratum corneum treated with either elastic vesicle suspension of PBS during 6 h passive diffusion, 9 h iontophoresis diffusion and 5 h post-iontophoresis diffusion. As illustrated in Fig. 7, during 6 h pre-iontophoresis passive diffusion, the amount of apomorphine in the receptor chamber was below the detection limit. After switching on the current, the apomorphine flux was dramatically increased and reached steady state within a short period. Following switching off of the current the flux decreased to a final post-iontophoretic plateau. Table 1 summarizes the calculated steady-state fluxes, enhancement ratios, and lag time during 9-h iontophoresis and 5-h post-iontophoresis passive fluxes [27]. No degradation products and metabolites of apomorphine were present in either the donor or the acceptor phase. Compared with the control (PBS), the iontophoretic transport of apomorphine was substantially enhanced by a factor of about 1.4 ($P<0.01$) following the treatment with the elastic vesicle suspension in both cases of diffusion through stratum corneum and human epidermis, whereas the corresponding lag time of iontophoresis and post-iontophoresis passive fluxes remained almost unchanged. Because vesicle pretreatment did not enhance the apomorphine passive transport prior to iontophoresis, a synergistic effect was observed after

Table 1
Effect of elastic vesicle of PEG-8-L/L595/CSO_4, on iontophoretic delivery of apomorphine through human stratum corneum and epidermis

	Skin type	Iontophoretic steady-state flux (nmol cm^{-2} h^{-1})	Lag time (min)	Enhancement ratio	Post iontophoretic passive flux (nmol cm^{-2} h^{-1})	Replicates
SC	Vesicle treated	123.8±12.4*	51	1.35	7.9±0.7	5
	Control	91.9±16.0	48		8.2±1.7	16
Epidermis	Vesicle treated	100.7±8.3*	151	1.48	45.4±8.9	7
	Control	67.9±8.2	161		37.2±9.1	6

*Indicates statistically significant ($P<0.01$) than the respective PBS control.

combining elastic vesicle pretreatment with iontophoresis [27].

From these results, it can be concluded that PEG-8-L elastic vesicle treatment in combination with iontophoresis provides an additional driving force to maintain and control the target flux of apomorphine. However, the mechanism of this effect remains a subject that warrants further investigation. In the literature [29,30], several mechanisms are proposed for the synergistic effect of iontophoresis and chemical enhancers, such as reduced skin impedance, increased enhancer deposition, and reduced size selectivity.

4. Pharmacotherapeutic strategies for the treatment of Parkinson's disease

Early studies in the 1950s and 1960s established that Parkinson's disease originates from a deficiency in the dopaminergic innervation of the basal ganglia owing to the degeneration of neurons in the substantia nigra [31]. The disease affects ~1% of all adults over the age of 65 worldwide, but frequently the clinical symptoms appear at a much earlier age. The clinical syndrome comprises four cardinal features: bradykinesia, akinesia, muscular rigidity, resting tremor and abnormalities of posture and gait [32].

Current therapeutic strategies aim at restoring the balance between dopaminergic and cholinergic neurotransmission. Dopaminergic drugs have shown the best results and are therefore the preferred drug therapy. Since dopamine is not transported across the blood–brain barrier, most commonly its metabolic precursor levodopa is used. It is transported into the brain by the amino acid transporter and decarboxylated to dopamine in the striatal tissue. Since about 95% of the orally administered dose is rapidly decarboxylated in the periphery, large quantities must be frequently administered. Current administration of the peripheral decarboxylase inhibitor carbidopa, results in higher plasma levels and a longer half life of levodopa [33]. Thus the dosage of levodopa can be largely reduced and peripheral side effects diminished. Current interest has turned to the development of a triple therapy, by addition of a catechol-*O*-methyl transferase (COMT) inhibitor to the regimen. COMT inhibitors potentially have a threefold effect: (1) they prevent the degradation of levodopa to 3-*O*-methyldopa (3-OMD); (2) they enhance blood–brain barrier transport by reducing the concentration of 3-OMD that competes for the same transport mechanism; (3) they inhibit degradation of dopamine to 3-methoxytyramine [34]. After administration of the peripheral and central COMT inhibitor tolcapone, a dose-dependent increase in AUC and a longer terminal half-life of levodopa and markedly reduced plasma levels of 3-OMD were found in elderly patients [35].

Chronic levodopa therapy often fails as a result of two interfering complications [36]: (1) the emergence of increasingly severe dyskinesias and response fluctuations, and (2) the appearance of increasingly severe cognitive impairment and psychosis, often with postural instability and falls, which are particularly prevalent in the more elderly. Peripheral and central pharmacokinetic, and central pharmacodynamic mechanisms may contribute to dyskinesias and response fluctuations. Levodopa is taken up in the small intestine and transported through the blood–brain barrier by the large neutral

amino acid transporter. Thus high levels of amino acids, for instance after a meal, may interfere with uptake from the gastro-intestinal tract and with transport across the blood–brain barrier [37]. Levodopa is widely distributed into extracerebral tissues, including the muscle sink. As a result of this, exercise can influence drug levels in plasma and the brain [38]. When the steady flow of dopamine from the nigrostriatal system gradually disappears when the disease progresses, the concentration profiles of dopamine in the central nervous system are increasingly determined by the fluctuating blood levels of orally administered levodopa. In addition, changes in the dopamine receptor response and deleterious effects of pulsatile dopamine delivery, are also likely to contribute to the observed dyskinesias and response fluctuations [39]. Due to these complexities, interest has turned in recent years to the development of directly acting dopamine agonists. Pergolide, bromocriptine, lisuride and apomorphine have been used in the clinic, of which apomorphine is the most effective one. The close structural relationship between dopamine, levodopa and apomorphine is shown in Fig. 8. This is attributed to the fact that it is both a partial D_1 receptor agonist and a full D_2 receptor agonist. The efficiency of counteracting refractory 'on–off' oscillations was found to be highly improved compared to levodopa [40]. Among the side effects that are known to occur, nausea and vomiting are most frequently reported. Domperidone, a peripheral antagonist, is able to counteract these effects. When the drug is chronically administered, some of the adverse effects are known to wane [41].

4.1. Currently used drug delivery routes

All currently used dopaminergic drugs, except for apomorphine, are dosed orally. Oral administration results in fluctuating plasma levels of the drug and for drugs with a low bioavailability, high variability in the amount absorbed is observed. The rate of absorption can furthermore be influenced by food intake, disease state, varying pH and the site of absorption [42]. As it is known that many problems associated with the treatment of Parkinson's disease originate from fluctuating and variable plasma concentrations it can be anticipated that current drug therapy in Parkinson's disease is only sub-optimal. Especially when on/off fluctuations start to occur, individual dose titration becomes a difficult task. Moreover, dysphagia is known to occur when Par-

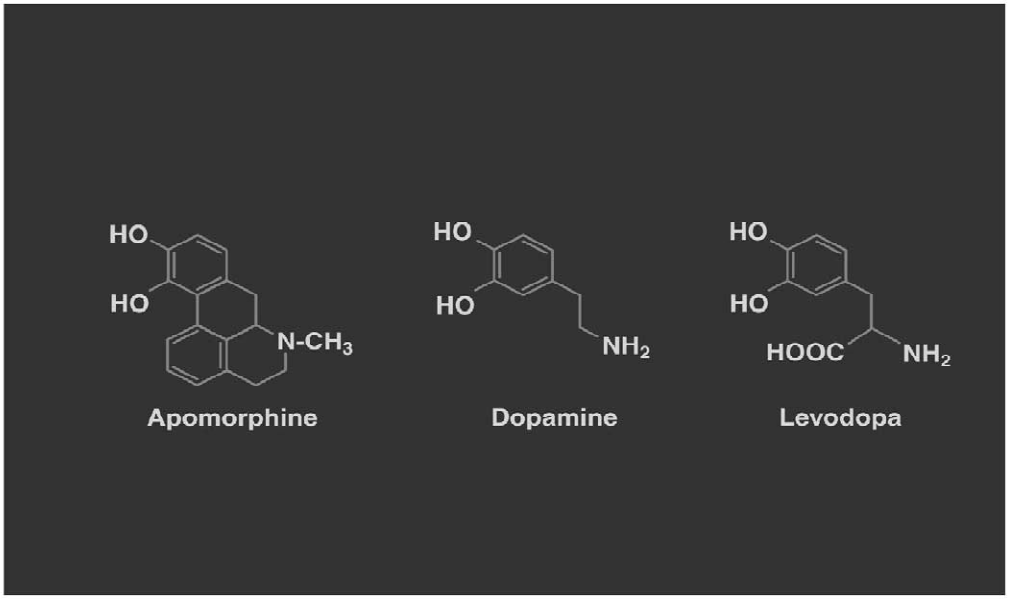

Fig. 8. Chemical structures of apomorphine, dopamine and levodopa.

kinson's disease progresses. These phenomena make the oral route highly unfavourable.

Drug delivery strategies that aim to achieve long lasting and less fluctuating drug levels may have some potential therapeutic advantages [43]: (1) minimization of peak levels and thereby reduction of side effects; (2) extended and predictable duration of drug release and thereby extended and more predictable duration of action; (3) reduction of the inconvenience of repetitive dosing and thereby enhancement of patient compliance. The advantages of continuous infusion were established for levodopa in the late 1980s. Mouradian et al. [44] showed that conversion from the oral to the intravenous route resulted in sustained stable plasma concentrations. As a result of this, a gradual reduction of motor fluctuations was observed. At present, intravenous infusion is only used in the clinic, for acute or short-term dosing. Due to the optimal control of the input rate, it is the preferred method for investigating PK/PD relationships. Kurlan et al. [45] showed that sustained stable plasma concentrations could also be achieved by continuous duodenal infusion of levodopa. They also demonstrated an improved mobility of the treated patients. However, for the long-term treatment of Parkinson's disease on an out-patient basis, these delivery strategies are not preferred due to the inherent risk of bleeding and inflammation and/or the overall tolerability to the system. These studies have nonetheless initiated the search for oral controlled release formulations. The search for a suitable drug delivery system has been most intense for apomorphine, since it was established early on, that the oral bioavailability of this drug is negligible. Continuous subcutaneous infusion and intermittent subcutaneous injections are the currently used therapy and can be used on an out-patient basis. Frankel et al. [41] reported, for the first time, the effects of long-term treatments in large groups of patients. The onset time of the effect was reported to be <10 min. Despite the observed beneficial effects, visual hallucinations and confusion occurred in three out of 21 patients on continuous infusion. In one patient the hallucinations disappeared on dose reduction. Continuous subcutaneous infusions invariably result in the development of subcutaneous nodules at the injection sites. The severity of these nodules is related to the total daily dose of apomorphine and can be reduced by diluting the infused formulations.

Alternatively, nasal, sublingual and rectal delivery have been tested by various investigators [46–51]. For these routes of administration, the surface areas have shown to be sufficiently permeable to apomorphine to allow therapeutically relevant input rates into the systemic circulation. Pharmacological responses were comparable in magnitude and onset time to subcutaneous administration of apomorphine. These delivery routes can be used for short to medium duration delivery depending on the properties of the specific dosage form. However, other factors have inhibited their use on a routine basis. For sublingual administration, systemic side effects occurred more frequently and inconsistency of dissolution and unpleasant taste were noted [46]. In a follow-up study, 50% of the patients developed stomatitis [47]. No local toxicity was observed following rectal administration [49]. The limited acceptability of this route will probably limit its widespread use. A reduction of systemic side effects was observed upon nasal administration [50]. Prolonged use resulted in moderate to severe nasal irritation in all patients [51]. Apart from the problems related to the specific transport route, fluctuating plasma levels are expected to result in suboptimal therapy for similar reasons as previously discussed for levodopa. Other delivery strategies for drug used in the treatment of Parkinson's disease, that have not been tested in patients so far are: pulmonary, ocular and buccal delivery and implants. For pulmonary and ocular delivery similar concerns with respect to side effects and fluctuating drug levels, as previously discussed for the delivery of levodopa and apomorphine, are to be expected. The buccal route has received increased attention of researchers over the last decade. New buccal drug delivery systems have been developed which can be used for both short, intermediate and long duration dosing. This route of administration bears in it all the advantages of the sublingual route, but the precision of delivery is increased, due to the fact that the exposed membrane area and its environment are better defined. Moreover, the release rate can be controlled by the use of release controlling membranes in the dosage form. Bioavailability can be improved by the co-application of absorption en-

hancers [52]. Application may be limited due to the available surface area and the early need for repeated application during chronic treatment. Implants may have the advantage that the input profile of a drug can be zero, preprogrammed or externally controlled. The bioavailability is potentially high. This may (depending on the drug applied) compensate for the higher production and implantation costs of these systems. Intra-cerebroventricular delivery of apomorphine from an implanted minipump was reported in a primate model (MPTP-treated monkeys). Motor activity improved on some occasions, however severe local brain toxicity was observed [53].

Taking all these delivery systems and delivery routes into account, the advantages of transdermal application of apomorphine by means of iontophoresis without any doubt justify the development of this application route.

Another already mentioned advantage of iontophoresis with respect to other delivery systems is its potential to deliver the drug apomorphine 'on-demand' not only by measuring blood levels (pharmacokinetics, PK) but especially by the possibility to measure its pharmacodynamic (PD) action and to directly correlate it to the disease parameters. Hence the establishment of a PK/PD relationship is of great value for optimized and individualized apomorphine therapy.

5. Pharmacokinetic–pharmacodynamic studies on apomorphine

At present pharmacokinetic considerations still play an important role in developing dosing regimens of new drugs. Important progress has been made, however, with a more comprehensive approach based on modelling of the relationship between pharmacokinetics and pharmacodynamics [54]. Such modelling allows for the characterization and the prediction of the time course of drug action rather than concentration and provides a scientific basis for development of the dosing regimen. Initial pharmacokinetic–pharmacodynamic models focused on the characterization of rapidly reversible direct drug effects. These models typically consist of three components: (1) a pharmacokinetic model characterizing the time course of drug (and metabolite) concentrations in blood or plasma; (2) a pharmacodynamic model, characterizing the relationship between drug concentration and pharmacological response intensity; and (3) often a link model which serves to account for the delay of the effect relative to the plasma concentration [55]. More recently, emphasis has been given to the development of models for drugs with an indirect mechanism of action, where the delay between drug concentration and effect is largely determined by the processes in the pharmacodynamic phase, rather than by slow distribution to the site of action [56]. The models for drugs with direct and indirect mechanisms of action are complementary, and have been applied successfully to the effects of a large number of drugs [57].

5.1. Concentration–effect relationship [56]

Characterization of the concentration–effect relationship is a central issue in integrated pharmacokinetic–pharmacodynamic investigations. Typically in this kind of study, a single intravenous dose of a drug is administered and the time course of the pharmacological response is determined in conjunction with the time course in concentration in plasma. By linking the effects to the concentration, concentration–effect relationships are obtained in individual patients. In the case of apomorphine, however, the application of this approach is complicated by its elimination half-life. As a result, the plasma concentrations following an intravenous bolus administration change rapidly, thereby not allowing sufficient time for a meaningful quantification of the pharmacological response intensity. In order to overcome this problem, a stepwise intravenous infusion protocol was developed, which results in multiple relatively stable plasma concentrations within each individual patient. According to this protocol the pharmacokinetic–pharmacodynamic correlation of apomorphine was studied in 10 patients with idiopathic Parkinson's disease. In these studies intravenous infusion of apomorphine was started at a rate of 10 $\mu g\ kg^{-1}\ h^{-1}$, and increased every 20 min by 10 $\mu g\ kg^{-1}\ h^{-1}$, until the maximum tolerated infusion rate or the infusion rate of 100 $\mu g\ kg^{-1}\ h^{-1}$ was reached. Thereafter the infusion rate was decreased every 20 min by 10 $\mu g\ kg^{-1}\ h^{-1}$ until baseline. At the end of each infusion interval a blood

sample was obtained for determination of the concentration of apomorphine and the pharmacological effect was measured. The primary effect parameters were tapping score and tremor assessment. To determine the tapping score, the patient had to tap two buttons, with a distance of 30 cm over 30 s, with the right hand as well as the left hand at maximal speed.

Fig. 9 shows the time course of the plasma concentration of apomorphine and of the effect on the tapping score in a representative patient. When the plasma concentration increases gradually beyond the minimally effective concentration, a sharp increase in the effect to its maximum value is observed. This maximum effect is maintained until, with a decrease in the infusion rate, the concentration drops to subtherapeutic values and the effect returns to baseline. Within each individual patient the concentrations at onset of effect were generally quite similar. Upon examination of the concentration–effect relationships it appeared that the effect was quantal rather than continuous. Therefore the pharmacodynamics were parameterized in terms of minimal effective concentration (MEC) at the onset of the beneficial effect (25% tap score increase), the minimal dyskinesia concentration (MDC) as the onset concentration of the increase in dyskinesia and finally, the minimal toxic concentration (MTC) as the onset concentration of the adverse effects (nausea, vomiting, sleepiness, dizziness, hypotension). Between patients wide differences were observed with respect to the minimal concentrations for each of the effects. Of the 10 patients, eight responded to apomorphine and two were non-responders. In the responders the values of the minimally effective concentration (MEC) varied between 1.4 and 10.7 ng ml^{-1}, whereas the concentration at which dyskinesia was observed (MDC) varied between 2.7 and 20.0 ng ml^{-1}. The minimal toxic concentration varied between 8.5 and 24.5 ng ml^{-1}. Between patients there is a wide variability in the therapeutic window (=the difference between the MEC on one hand and the MDC on the other) of apomorphine, which is typically quite narrow. The wide interindividual variability in both pharmacokinetics and pharmacodynamics, in combination with the narrow therapeutic window in many patients underlines the need of individualized and carefully controlled delivery of apomorphine in the management of Parkinson's disease.

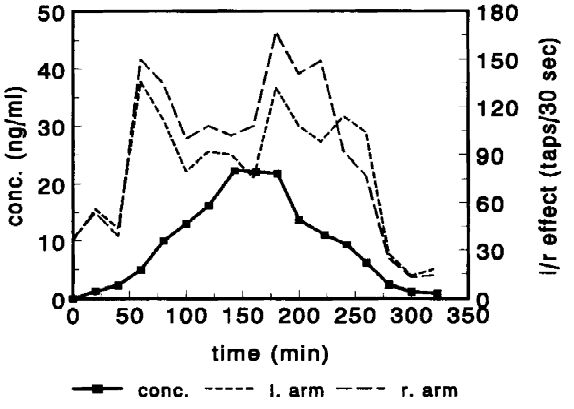

Fig. 9. The concentration–time profile following a stepwise intravenous infusion with apomorphine (left *y*-axis), combined with the clinical effect score (number of taps/30 s) of both the left and the right arm of one individual patient (right *y*-axis).

6. Iontophoretic delivery of apomorphine to Parkinson patients [56]

In order to determine the feasibility of transdermal iontophoresis of apomorphine in vivo, a study was conducted in 10 patients with idiopathic Parkinson's disease. According to a randomized cross-over design the patients received apomorphine by transdermal iontophoresis at a current density of either 250 or 375 μA cm^{-2} over 1 h on one occasion.

At another occasion 30 μg kg^{-1} were administered intravenously over 15 min. In none of the patients was significant passive transdermal transport of apomorphine observed. Upon current application, increasing plasma concentrations were observed in all patients. The observed maximum concentrations were directly related to the applied current density: 1.3±0.6 ng ml^{-1} at 250 μA cm^{-2} and 2.5±0.7 ng ml^{-1} at 375 μA cm^{-2}. When the current was switched off all concentrations returned to baseline values in ~90 min (Fig. 10). The calculated individual transdermal transport rates of apomorphine at an applied current are depicted in Fig. 11. These results show that also in the in vivo situation the transdermal transport rate of apomorphine is determined by the applied current density. By mathematical deconvolution it was demonstrated that the

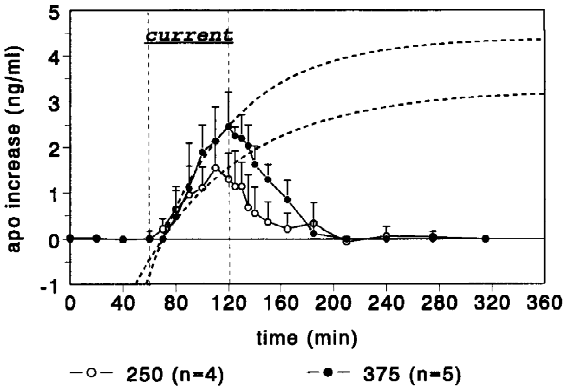

Fig. 10. Average plasma concentration–time profiles of apomorphine upon transdermal iontophoretic delivery at current densities of 250 μA cm^{-2} (○) ($n=4$) and 375 μA cm^{-2} (●) ($n=5$) for 1 h, starting at $t=60$ min. Steady state plasma concentrations were predicted by fitting the plasma concentration–time profiles between 60 and 120 min.

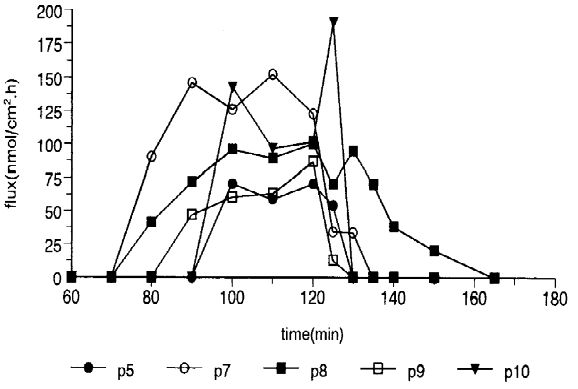

Fig. 11. Calculated individual transdermal transport rates of apomorphine at an applied current density of 375 μA cm^{-2} for 1 h. Mathematical deconvolution (Loo–Riegelman method) was applied on the plasma concentration–time profiles, using the individual pharmacokinetic parameters.

steady-state transdermal flux values are reached within 1 h of current application. The values of the steady-state flux were 69±30 nmol cm^{-2} h^{-1} at 250 μA cm^{-2} and 114±34 nmol cm^{-2} h^{-1} at 375 μA cm^{-2}, indicating that variation in the applied current density may be used to control the delivery. The delivery of apomorphine at the aforementioned rates results in steady-state plasma concentrations of ~3–4.5 ng ml^{-1} which is at the lower end of the therapeutic concentration range.

7. Skin barrier integrity and skin irritation after iontophoresis [58]

Electric fields may affect the skin at three levels: (a) at the level of the stratum corneum barrier, e.g. through a modification of the lipid structure; (b) in the viable epidermis, e.g. through direct interaction with the epidermal keratinocytes; (c) in the dermis, where vascular events may take place [59]. Each of these types of interaction may develop into an irritant response.

One of the parameters which largely reflects the electrical properties of the skin and has been used as a marker of changes induced at the level of the stratum corneum, is its electrical resistance. When skin is exposed in-vitro to current for a longer period of time, a drop in its electrical resistance is usually observed. Only in skin samples that are exposed to currents in the low microamperic range, the resistance fully recovers. Partial recovery of the electrical resistance is found after 5–10 h following current exposure of a few hundred microamperes. Irreversible resistance changes take place when applying currents higher than that [60].

With regard to electrically-induced effects on skin barrier properties and on the viable strata of the skin, only limited quantitative data are available [61,62]. The scarce literature available on this subject is usually limited to occasional burn reports, qualitative pain scores and erythema scores. Furthermore, it has been reported that the irritation potential of an electric current is not only dependent on the current strength but also on the current profile: claims have been made that pulsed depolarized current wave forms are less irritating to the skin than the comparable constant current levels [63].

To assess possible local skin irritation and toxicity it is a prerequisite to also monitor electrically-induced skin barrier modulation and skin irritancy in humans by comparing two different current profiles: constant DC and pulsed DC.

Transepidermal water loss (TEWL) was measured as a marker of skin barrier integrity [64,65]. Changes in TEWL have frequently and successfully been used to monitor stratum corneum barrier modulation by skin irritants [66]. Laser Doppler flowmetry (LDF) was used to quantify the erythematous response following current application. The study was de-

signed in such a way that both the initial response after the current had been switched off, and the time-dependent relaxation of these parameters to normal values, could be assessed.

Five male and four female healthy volunteers (average age 22 years, range 19–25) not associated with a history of skin disorders enrolled in a study. Both arms were kept out of contact with excessive sun and creams for 3 days and with detergents for 1 day prior to the experiment. On all treated sites and occluded controls, TEWL returned to baseline in about 30 min. This is the time usually needed for occluded skin sites to return to normal values. Irrespective of whether current was applied or not, the relaxation profile followed identical patterns for all sites on which application chambers had been applied. The average TEWL values ($n=9$), for the immediate response, calculated for nine volunteers are given in Fig. 12. Subjecting the skin sites to current, either positive or negative, did not result in significantly higher values than the occlusion of the control sites.

Average LSF data ($n+9$) for the immediate response are given in Fig. 13. Both protocols and both anodal and cathodal currents resulted in a significantly higher erythematous response than the occluded and the untreated controls. No difference in response depending on the current protocol was found. The variability in LDF values measured directly after chamber removal and drying at the sites

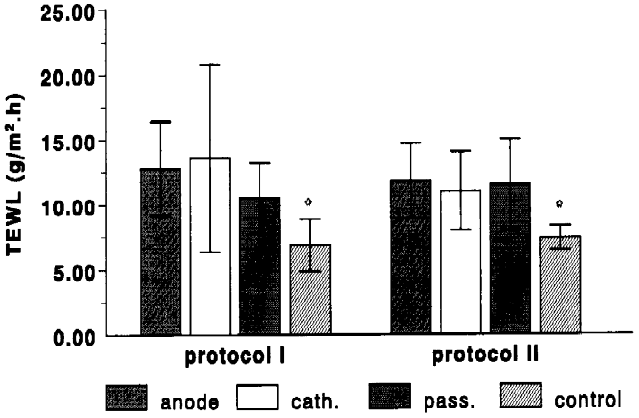

Fig. 12. Average TEWL values measured directly after patch removal. *Indicates that anodal, cathodal and passive TEWL values were significantly higher than the control values ($n=9$, mean±S.D.).

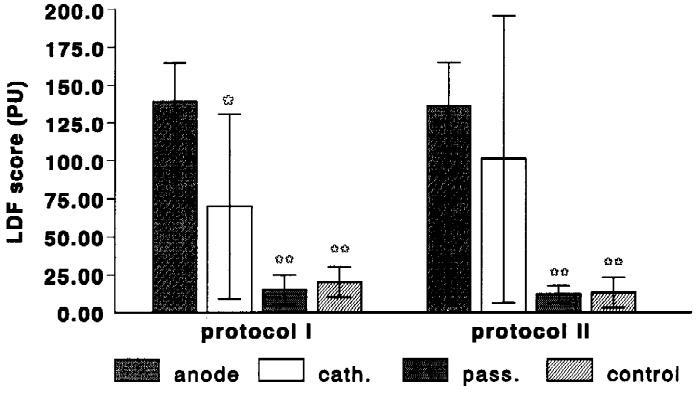

Fig. 13. Average LDF values measured directly after patch removal. *Indicates that anodal values were significantly higher than cathodal ones, **indicates that anodal and cathodal values were significantly higher than passive and control values ($n=9$, mean±S.D.).

exposed to cathodal currents was higher that at the site exposed to anodal currents. This difference was not associated with a gender-related differential response at the cathodal sites. All LDF values correlated with the visual erythema scores. Upon removal of the transdermal iontophoretic patch, mild transient erythema was observed which was reflected in evaluated LDF values. No local adverse effects such as prolonged inflammation and green colouring (which is typically seen upon subcutaneous apomorphine administration) were observed. These findings indicate that the transdermal delivery of apomorphine is feasible at acceptable levels of skin irritation.

8. Conclusion and future perspectives

Transdermal drug delivery by means of iontophoresis can be an excellent tool to deliver suitable drugs 'on-demand' especially for suitable multi-factorial diseases such as Parkinson's disease. With apomorphine it could 'be shown' in a first study in Parkinson patients that with a tolerable patch size between 50 and 80 cm^2, therapeutic plasma levels can be achieved albeit at the lower level of therapeutic effect. The use of suitable chemical penetration enhancers or vesicular formulations may be an interesting tool in combination with the electrically enhanced transdermal delivery of apomorphine to both increase the therapeutic efficacy of apomorphine and to reduce the patch size. A second study with Parkinson patients with iontophoretic delivery of apomorphine is currently being performed using optimized surfactant formulations for pretreatment before iontophoresis occurs.

The most interesting feature of using iontophoresis for controlled drug delivery is that the Parkinson patient can directly control the needed amount of apomorphine by increasing or decreasing the drug input in order to achieve optimal drug therapy with a minimum of toxic side effects. Furthermore, the typical features of Parkinson's disease such as bradykinesia, akinesia and tremor could be used to directly monitor the needed drug input by means of chip sensors which are able to directly measure the most important Parkinson disease parameters and to regulate the drug input. Such a system would be the very first closed-loop system monitoring not pharmacokinetic data (blood levels) but more importantly the pharmacodynamic effects of Parkinson's disease. This scenario is the more feasible as skin irritation and toxicity studies have proven that iontophoresis is a safe route of treatment.

In more general terms, the future perspectives of iontophoresis are the miniaturization of the devices, reducing the costs of the treatment by producing disposable drug-containing patches, developing high energy batteries for the microcomputer to be used as hardware. Also the design of a suitable charged drug of high potency will increase the future use and the versatility of application of iontophoresis devices including targeting of antigens to the Langerhans cells in the skin.

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