Iontophoretic Delivery of Apomorphine *In Vitro*: Physicochemic Considerations

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Purpose. To examine the mechanisms of transdermal iontophoretic delivery of apomorphine.

Methods. Anodal iontophoresis of R-apomorphine across human stratum corneum was determined *in vitro*. The effects on the flux of the following parameters were studied: stability of drug, pH of donor solution, concentration of NaCl, and type of Na^+ co-ions.

Results. Ascorbic acid was effective to prevent apomorphine degradation. The iontophoretic transport of apomorphine was strongly influenced by the pH of the donor formulation. Increasing the pH from 3 to 6 resulted in an increase in the iontophoretic apomorphine flux from 27.9 ± 4.4 nmol/cm^2*h to 78.2 ± 6.9 nmol/cm^2*h. Upon decreasing NaCl concentration from 8 to 2 g/L, the iontophoretic flux was not significantly changed. Replacing NaCl in the donor formulation by tetraethylammonium chloride or tetrabutylammonium chloride resulted in 1.3 fold greater steady-state flux.

Conclusions. For optimized apomorphine iontophoretic delivery, a constant pH of the donor formulation is of great importance. The results suggest that although flux enhancement during iontophoresis is largely due to the electrical potential gradient, secondary effects, such as convective flow and electroosmosis may also contribute.

KEY WORDS: iontophoresis; apomorphine; Parkinson's disease.

INTRODUCTION

Apomorphine as a mixed D_1 and D_2 dopamine receptor agonist has been shown to be a very potent drug for the treatment of patients with idiopathic Parkinson's disease, particularly for the random response-fluctuation at the later stages of disease progression (1,2). However, its inherent instability, negligible oral bioavailability, short elimination half life ($t_{1/2}$ = 41 min), narrow therapeutic window and the high intra- and inter-individual variation in pharmacokinetics and pharmacodynamics complicate its application in clinical practice. This puts high demands on a delivery system by which the apomorphine input rate into the systemic circulation can be accurately controlled and easily adjusted on an individual basis. So far, several alternative administration routes for apomorphine have been investigated (2). Subcutaneous injection is one of the most commonly used methods, even for outpatients. But subcutaneous nodules invariably appear at the injection sites. Nasal, sublingual, and rectal administrations in general have been found to improve pharmacologic profiles and provide therapeutic relevant input rate. However, several moderate-to-severe side effects have been claimed for each specific route. Moreover, because the fluctuating plasma level of drug is inherent to these routes, pharmacodynamic response-fluctuation is expected.

Transdermal iontophoresis offers the opportunity of external control of the drug input rate by simple adjustment of the current density (3). A series of previous studies from our laboratory has demonstrated the feasibility of iontophoresis of apomorphine *in vitro* as well as *in vivo* (4,5). The results show that the input rate of apomorphine into the blood circulation can be substantially enhanced and accurately controlled via iontophoresis. Furthermore, the observed skin irritation resulting from the application of the electric field is minor and acceptable from a clinical point of view. Nevertheless, the transport efficiency of apomorphine from such a system was still rather low. Consequently, only lower-limit therapeutical plasma levels were predicted from the original pharmacokinetic data in most of the patients (5). Therefore, systematic investigation of the affecting parameters and the mechanisms involved in the transdermal iontophoretic apomorphine delivery system are important and necessary to further enhance transdermal iontophoretic transport. The present study focused on elucidation of the impact of donor solution composition on the iontophoretic flux of apomorphine. Furthermore, this study provided insight in the mechanisms of iontophoretic transport.

MATERIALS AND METHODS

Materials

R-apomorphine hydrochloride was obtained from OPG (Utrecht, the Netherlands). Purity was tested by high-performance chromatography (HPLC) 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, sodium meta bisulphite, tetraethylammonium chloride (TEACl), and tetrabutylammonium chloride (TBACl) were purchased from Sigma Chemicals (Hilversum, the Netherlands). Dialysis membrane disks (cut-off value: 5000 Da) were obtained from Diachema (München, Germany). Trypsin (Type III, from a bovine pancreas) and trypsin inhibitor (Type II-S from soybean) were purchased from Sigma Chemicals (Zwijndrecht, 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 ≥ 18 MΩ).

Preparation of Stratum Corneum

Human abdomen skin was obtained after cosmetic surgery and processed on the same day. To avoid interference with sebaceous lipids or contamination of subcutaneous fat, the skin surface was carefully wiped with 70% ethanol. Extraction of skin lipids and penetration enhancement by ethanol is minimal during this cleaning procedure (6). Subcutaneous fat was removed, after which the skin was dermatomed (Padgett Dermatome, Kansas City, MO). The dermatomed

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skin was incubated on filter paper soaked in a 0.1% trypsin solution in isotonic phosphate-buffered saline (PBS; pH = 7.4) for one night at 4°C and 1 h at 37°C. By this splitting technique, the stratum corneum (SC) is separated from the underlying epidermis. Because the remaining trypsin might interfere with the integrity of stratum corneum and also affect the transdermal penetration, the stratum corneum was treated subsequently with a 0.1% solution of trypsin inhibitor in PBS. Then the stratum corneum was washed with Millipore water, blotted dry, and stored at room temperature in a silica gel containing desiccator filled with N_2 gas to inhibit oxidation of SC lipids. SC less than 4 months old was used in the experiments.

In Vitro Iontophoretic Studies

A 9-channel computer controlled power supply was used to provide constant current (Electronics Department, Gorlaeus Laboratories, Leiden University, the Netherlands). This instrument is capable of delivering both constant and constant-pulsed current of varying current intensity, frequency and on:off ratio. In addition, it can also measure the resistance across the skin while current supply is on. A silver plate electrode was used as an anode, a silver/silver chloride electrode as a cathode.

All diffusion experiments were carried out at a constant current density of 500 $\mu A/cm^2$, at room temperature, and in three-chamber continuous flow-through diffusion cells (4). Human SC (∅18mm) was hydrated for 1 h in PBS (pH = 7.4) before to mounting in the cells. One piece of human SC were placed between the donor and intermediate chamber and another piece of human SC between intermediate and acceptor chamber with the epidermal sides of the SC facing the intermediate cell. At least three skin specimens were used for every experimental condition studied. Dialysis membrane (cut-off, 5000 Da) was used as a support membrane. R-apomorphine hydrochloride was applied in the anodal compartment. The donor formulation contained 15 mM apomorphine and 5 mM citrate buffer. The pH and electrolyte composition of the donor solutions varied as outlined below. The receiver chamber was filled with PBS (pH = 7.4). The intermediate chamber was continuously perfused using a peristaltic pump with PBS buffer pH 7.4 at 7 mL/h. The total duration of the iontophoretic delivery was 5 h. Samples were collected with a fraction collector at half an hour interval. During the experiments, both anodal and cathodal chambers were magnetically stirred at 375 rpm. Precautions were taken to prevent oxidation of apomorphine by using N_2 gas, keeping the system in dark (fraction collector was covered by a box) and adding 200 μL of anti-oxidant solution (0.05% EDTA, 0.5% sodium meta bisulphite dissolved in 25% HPO_4) in the collecting tubes. No degradation of apomorphine was detected in the donor chamber as checked by HPLC analysis (data not shown).

Effect of Ascorbic Acid as an Antioxidant

The donor solution was citric buffer (5 mM, pH 5) containing 1 g/L of ascorbic acid as anti-oxidant instead of the 1 g/L of sodium meta bisulphite used in the control formulation. pH shifts in the donor formulations were measured during the 1-week storage at room temperature. The stability of apomorphine in the donor formulations was assessed under the following two experimental conditions: during 1 week of storage in the dark at room temperature and after 5 h of incubation under an electric field with a constant current density of 500 $\mu A/cm^2$. The amount of intact apomorphine left in the solutions was quantified by HPLC assay as described below (analytical method).

Effect of pH

The donor solution pH was varied from 3 to 6 by changing the composition of citric buffer, which has an ionic strength of 5 mM.

Effect of Co-Ions

Two studies on the effect of Na^+ co-ions in the donor formulation on iontophoretic delivery of apomorphine were performed. In the first study, the concentration of NaCl was stepwise decreased from 8 to 2 g/L whereas the proper amount of mannitol was added in the donor solution to keep the osmolarity constant. The lowest concentration, 2 g/L, was still sufficient amount to allow an oxidation reaction at the Ag plate anode during the iontophoresis. In the second study, NaCl was replaced by an equimolar concentration of TEACl or TBACl.

Analytical Method

Samples were injected directly into an HPLC system consisting of 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/0.1M phosphate buffer (25/75 v/v). The phosphate buffer of pH 3 contained 0.1 M NaH_2PO_4, 20 mg/mL 1-octanesulfonic acid and 10 mg/mL EDTA. Freshly made standard solutions were used to obtain calibration curves for each experiment (4). The calibration curves were linear ($r > 0.999$) in the concentration range of 10–1000 ng/mL. The intra- and inter-assay variations were <5% for all concentrations tested. The detection limit under these conditions was 50 fmol.

Data Analysis

The cumulative amount of apomorphine permeated per unit skin area was plotted against time and the slope of the linear portion of the plot was estimated as the steady-state flux. All results were expressed as mean values ± standard deviations. Statistical comparisons were made using Student's *t* test. The probability value of less than 0.05 was considered to be significant.

RESULTS

Stability of Apomorphine and Effect of Ascorbic Acid as an Antioxidant

No degradation of apomorphine hydrochloride in the donor solution was observed in the presence of either sodium meta bisulphite or ascorbic acid after both 1 week of storage in dark at room temperature and 5 h of iontophoresis at a current density of 500 $\mu A/cm^2$. It was therefore concluded

that apomorphine is rather stable when 1% antioxidant is present in the donor formulation.

However, adding sodium meta bisulphite caused a large pH shift in the donor solution, varying from 5.0 to 4.28 (shown in Fig. 1). The increased concentration of H^+ may diminish the current efficiency as a result of ion competition with apomorphine to carry the current during the process of iontophoresis (see below). In contrast, the pH of the donor solution containing 1g/L ascorbic acid was at a fairly constant level around original pH 5.0.

pH Effect

The influence of pH on the iontophoretic apomorphine steady-state flux is illustrated in Figure 2. From pH 3 to 4, a dramatic increase in flux ($P < 0.01$) was observed, whereas an increase in pH from 4 to 5 increased the apomorphine transport to a smaller extent. This is consistent with a positive charge in the human SC at pH 3 and charge reversal around pH 4.75. No subsequent increase was observed upon further increase in pH from 5 to 6.

Effect of Co-Ion in the Donor Formulation

Two studies were performed to provide insight in the effect of Na^+ co-ions on the apomorphine delivery. The first study was conducted to determine the effect of NaCl concentration, which was stepwise decreased from 8 g/L to 2 g/L. The iontophoretic steady-state fluxes of apomorphine are illustrated in Figure 3A. These data show that apomorphine flux varied in a range from 78.0 ± 3.8 nmol/cm^2*h to 82.3 ± 5.2 nmol/cm^2*h. Thus no significant change in the apomorphine steady-state flux is observed when reducing the NaCl concentration.

In the second study, Na^+ in the donor formulation was replaced by either TEA^+ or TBA^+ at the same molar concentration. The effect of both TEA^+ and TBA^+ on the iontophoretic apomorphine delivery is illustrated in Figure 3B. This replacement resulted in greater steady-state fluxes of 106.0 ± 12.3 nmol/cm^2*h and 95.9 ± 13.7 nmol/cm^2*h for TEA^+ and TBA^+, respectively, compared to the Na^+ containing donor solution (78.0 ± 3.8 nmol/cm^2*h).

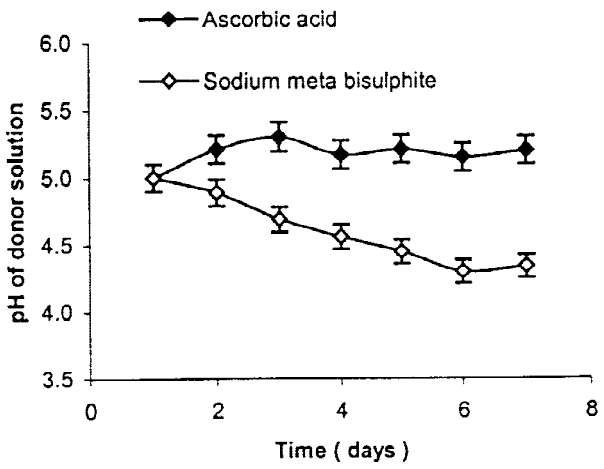

Fig. 1. pH shift in the donor solution during storage at room temperature. The donor solutions contained 15 mM apomorphine, 8 g/L NaCl, and 1 g/L of either ascorbic acid or sodium meta bisulphite in a 5 mM citrate buffer, pH 5. Data are presented as mean ± standard devation (n = 3).

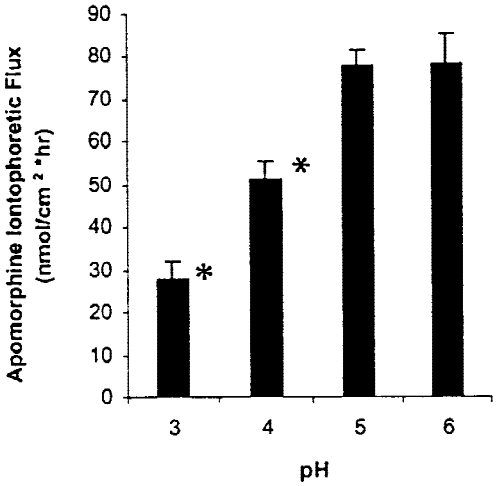

Fig. 2. Effect of donor solution pH on the iontophoretic transport of apomorphine across human stratum corneum at a current density of 500 μA/cm^2 (room temperature). All donor solutions contained 15 mM apomorphine, 8 g/L NaCl, and 1 g/L sodium meta bisulphite in a 5 mM citrate buffer, pH 5. Data are presented as mean ± standard deviation (n = 4). *Indicates significant difference over the control donor formulation at pH 5.

DISCUSSION

One of the difficulties in apomorphine administration is its inherent instability. It is known that aqueous solutions of apomorphine rapidly undergo spontaneous oxidative decomposition and turn green (7). This process is accelerated by oxygen and high pH. Normally, the degradation of about 2% (1 mg in 100 mL of the solution) of the available apomorphine results in a very dark and green solution. Therefore, it is very important to keep sufficient antioxidant in the iontophoretic donor formulation, especially because the presence of an electrical current may facilitate the degradation process. It was shown that 1 g/L of antioxidant in the donor solution, either sodium meta bisulphite or ascorbic acid, could effectively prevent the degradation of apomorphine. However, ascorbic acid is more suitable for the iontophoretic delivery of apomorphine than sodium meta bisulphite because the latter induced a large pH decrease.

In this study, it was shown that the iontophoretic transport of apomorphine was strongly influenced by the pH of the donor solution. Increasing pH from 3 to 6 induced a significant increase in the apomorphine flux. In general, the pH of the donor solution influences delivery in several ways, which have a complex interrelationship. In the case of iontophoretic delivery of apomorphine, varying pH from 3 to 6 in the donor formulation induces the following three contradictory changes: (1) the fraction of apomorphine in its ionised form decreases from 100 to 94.3% (pKa of apomorphine is 7.2); therefore, the electromigration contribution to the overall apomorphine flux will be slightly reduced. However, this will only play a role between pH 5 and 6; (2) upon increasing pH, the concentration of H^+ in the donor solution is decreased from 10^{-3} to 10^{-6} M, thus H^+ competition will be decreased. However, because the H^+ concentration is more than 140

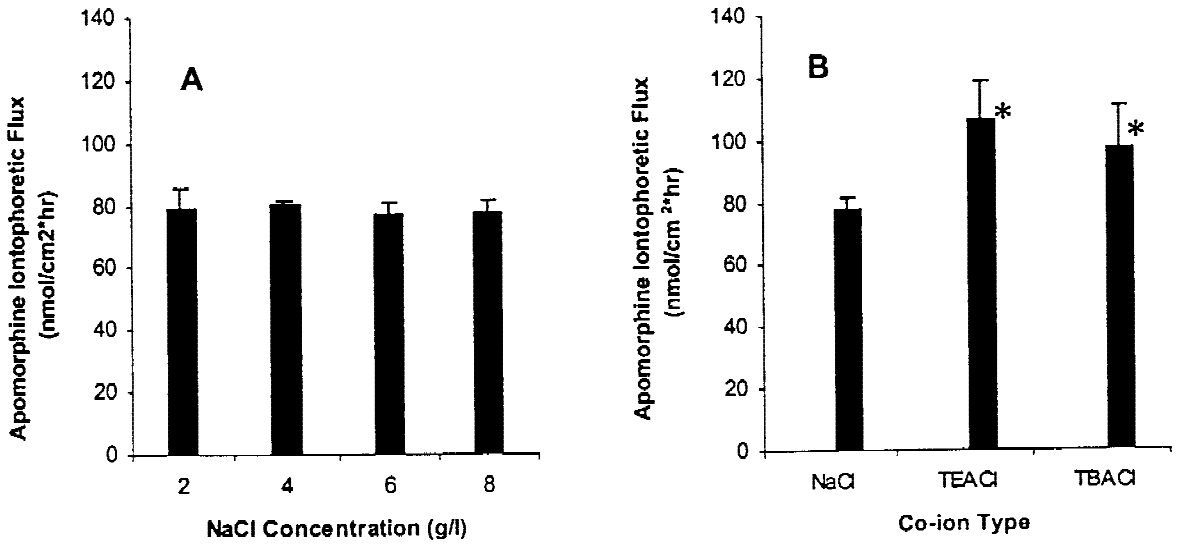

Fig. 3. Effect of co-ion Na^+ in the donor forumulation on the iontophoretic transport of apomorphine across human stratum corneum at a current density of 500 $\mu A/cm^2$ (room temperature). All donor solutions contained 15 mM apomorphine and 1 g/L sodium meta bisulphite in a 5 mM citrate buffer, pH 5. (A) NaCl concentration is varied from 8 to 2 g/L; (B) 8 g/L NaCl is replaced by equimolar concentration of either tetraethylammonium chloride or tetrabutylammonium chloride. The results are mean values of 3 to 5 experiments. *Indicates significant difference over the control donor formulation with 8 g/L NaCl.

times lower than the Na^+ concentration in the donor solution, the Na^+ ions are expected to contribute much stronger to the ion competition than the H^+, despite the higher mobility of the latter. Therefore, the strong change in apomorphine flux cannot only be caused by a decrease in H^+ concentration; (3) the degree of fixed negative charge within the skin increases with increasing pH as shown by Pikal and Shah (8,9). Accordingly, apomorphine transport due to the electroosmosis force will be increased with increase pH.

Overall, the dependency of the total iontophoretic flux of apomorphine on the pH is determined by the relative importance of these contributions. The trend of pH effect observed in this study implies that next to the potential gradient, electroosmosis plays an important role in the apomorphine transport across the skin. A similar trend was also reported for verapamil hydrochloride iontophoretic transport (10). The authors attributed the pH effect to the skin permselectivity. In another case with lignocaine hydrochloride (11), the decreased flux was investigated while the pH of the donor solution was increased from 4 to 8. This is because the volume flow was found to be negligible.

Na^+ ions are the primary co-ions in the donor formulation of apomorphine iontophoretic system. They are expected to carry an appreciable amount of the total current. In this study, a complex effect of NaCl on iontophoretic apomorphine transport was demonstrated. Upon decreasing NaCl concentration from 8 to 2 g/L, the apomorphine iontophoretic flux was not significantly changed. However, replacing NaCl in the donor formulation by TEACl or TBACl resulted in about 1.3-fold greater steady-state fluxes of apomorphine.

According to the mathematical model derived by Phipps and Gyory (12), which described the co-ion effect under an assumption that no volume flow is involved, a decrease in the concentration of Na^+ ions in the donor solution will result in an increase in apomorphine iontophoretic flux. In addition to that a decrease in Na^+ concentration from 8 to 2 g/L will increase the zeta potential, resulting in an increase in the double layer thickness within the negatively charged pore pathway in the skin. This will increase the electroosmosis force and, therefore, promote the apomorphine transport across the skin. In contrast, a reduction in NaCl concentration will reduce the convective flow of Na^+ ions. Thus the amount of apomorphine delivered in this way, possibly as ion-pair, will be decreased. Overall, NaCl effect is the summation of the above three influences. The result of NaCl concentration effect observed in this study suggests that the convective effect may counterbalance the cumulative effects of electroosmosis and electromigration in the case of apomorphine transport.

When Na^+ ions are replaced by TEA^+ or TBA^+ in the donor solution electroosmosis and convective flow is expected to be affected to a lesser extent. Therefore, the increased iontophoretic flux of apomorphine resulting from this replacement must be due to a reduction in ion competition and therefore an increased contribution of the electromigration of apomorphine. Yoshida and Roberts have demonstrated both theoretically and experimentally that a compound's molecular weight, calculated molar volume, as well as solute radius, are inversely related to iontophoretic mobility (13). Because TEACl and TBACl are bigger molecules than NaCl, the fractions of current carried by them will be diminished due to their less mobility. Therefore overall fluxes of apomorphine will be increased.

Other investigators have also studied the complex effect of NaCl on iontophoretic drug transport. Gangarosa *et al.* (14) have reported increased transport of non-conductive compounds during anodal iontophoresis in dilute sodium chloride solutions. These investigators postulated that increased transport results from water movement (iontohydrokinesis) associated with sodium ion transfer. Also Wearley and Chien (15) have observed that with the addition of an electrolyte, such as NaCl, the skin permeation of azidothymidine, a neutral compound, was observed to first increase proportionally up to 0.1 M NaCl and then reach a plateau. The authors suggested a

mechanism of convective flow due to the movement of NaCl. In another study, the iontophoretic permeation rate of verapamil ions (positively charged) was noted to decrease at low concentration of NaCl due to the decrease in the electrical transference number of verapamil; after the minimum value (equal to the passive diffusion rate of verapamil) was reached the skin permeation rate of verapamil was observed to increase, possibly again due to the convective flow of Na^+ ions (10).

In summary, among all physicochemic parameters tested in this study, pH variation from 3 to 6 demonstrated a relatively larger effect on the iontophoretic flux. Therefore, it is important to keep the donor formulation pH stable by using ascorbic acid instead of sodium meta bisulphite as an antioxidant. Moreover, although the relative contributions of electromigration and volume flow to the total iontophoretic flux is difficult to quantify, their relative importance can be estimated by the above experimental approaches. The flux enhancement of apomorphine during iontophoresis is due principally to the electrical potential gradient, secondary effects, both convective flow and electroosmosis contribute also to flux. This effect is dependent on physicochemical conditions of the donor formulation.

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