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Iontophoresis of dexamethasone phosphate: Competition with chloride ions

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ABSTRACT

The objective was to study the competition of chloride released from a Ag/AgCl cathode on the iontophoretic delivery of dexamethasone phosphate (Dex-Phos). Iontophoresis of Dex-Phos was performed in side-by-side diffusion cells (0.78 cm^2) using pig skin. A 0.3 mA constant current was applied via Ag/AgCl electrodes. The amounts of Dex-Phos and dexamethasone (Dex) were also quantified in the stratum corneum (SC), using tape-stripping, after passive and iontophoretic delivery. The profiles of Dex-Phos and Dex, as a function of position in the SC, were deduced. The iontophoretic delivery of Dex-Phos from pure water was unaffected by the accumulation of Cl^- released by the donor cathode when the drug's concentration was 4.25 mM to 17 mM. At 0.85 mM, however, Cl^- competition was significant and the drug flux was significantly reduced. Formulation of the drug in the presence of Cl^- resulted in a non-linear dependence of flux on the molar fraction of the drug. Tape-stripping experiments confirmed the enhanced delivery of Dex-Phos by iontophoresis relative to passive diffusion, with Dex-Phos concentration greater inside the barrier post-iontophoresis than that in the donor. The latter observation could explain the robustness of Dex-Phos delivery to the presence of Cl^- in the donor solution.

1. Introduction

Iontophoresis is used to enhance the transdermal passage of charged and polar molecules, for both drug delivery and clinical monitoring applications [1–3], by the application of a small electrical current (<0.5 mA/cm^2). The two principal mechanisms of transdermal iontophoretic transport, electromigration and electroosmosis, have been extensively described in previous work [1,2,4]. According to Faraday's law, the iontophoretic flux (J_d) of a drug "d" transported by electromigration, the main mechanism of transport for charged drugs, is directly proportional to the intensity of the current (I) applied:

$$J_d = \frac{t_d \cdot I}{F \cdot z_d} \quad (1)$$

where t_d is the transport number of the drug, z_d its valence and F is Faraday's constant [5].

The transport number of the drug is the fraction of the electrical current applied which it transports across the skin. The drug competes with all other ions present in the system to carry the charge and it follows that, to optimize electromigration, the presence of all other ions should be minimized. Ideally, co-ions are absent from the formulation, such that only endogenous counter-ions can compete with the drug, the transport number of which is then maximized [6,7]. When competing co-ions are present, the transport number of the drug usually depends upon its molar fraction rather than its absolute concentration [5,8,9].

Electrodes are critical components of an iontophoretic system and transfer the electrical current supplied by the external circuit into ion migration across the skin [10,11]. Ag/AgCl electrodes are commonly used for iontophoretic delivery and their electrochemistry is driven at a sufficiently low potential insuring that unwanted secondary reactions are unlikely. The electrode reactions are:

$$\text{(Anode)} \quad Ag_{(M)} + Cl^-_{(aq)} \rightarrow AgCl_{(S)} + e^- \quad (2a)$$

$$\text{(Cathode)} \quad AgCl_{(S)} + e^- \rightarrow Ag_{(M)} + Cl^-_{(aq)} \quad (2b)$$

Importantly, compared to electrodes made of inert materials (platinum, stainless steel and glassy carbon for example), water electrolysis does not occur and pH shifts are avoided [11]. Ag/AgCl electrodes are well-adapted for the delivery of cationic drugs available as chloride salts provided there is a sufficient quantity of chloride in the donor to satisfy the electrochemistry at the anode. However, when negatively charged drugs are iontophoresed from the cathode, chloride ions released by the electrode will compete to transport the current and reduce the efficiency of delivery [12–15]. Strategies to circumvent this phenomenon have been described in the patent literature [16–18].

Previously, the iontophoretic delivery of dexamethasone phosphate (Dex-Phos) from the cathode was shown to be minimally affected by the release of Cl^- when the drug was present in the donor solution at concentrations between 0.2 and 0.8% w/v in water [19]. The

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objective of the present study was therefore to better understand the effects of chloride anions released from the Ag/AgCl cathode on the delivery of Dex-Phos. To this end, the iontophoresis of Dex-Phos was investigated systematically as a function of the amount of Cl^- present. In addition, tape-stripping experiments were performed post-iontophoresis to assess the accumulation of the drug in the membrane.

2. Materials and methods

2.1. Materials

Dexamethasone (Dex) (>98%) and dexamethasone sodium phosphate (Dex-Na_2-Phos) (>98%) were purchased from Sigma-Aldrich Co. (Gillingham, UK). Sodium chloride, Na_2HPO_4, KH_2PO_4 and phosphoric acid (85%) were from Acros (Geel, Belgium). Potassium citrate, methanol (HPLC grade), acetonitrile (far UV HPLC grade) were from Fisher Scientific (Loughborough, UK). NaOH 50% (ion chromatography eluent grade) was from Fluka (Buchs, Switzerland). Ag wire (>99.99% purity) and AgCl (99.999%) were purchased from Sigma-Aldrich Co. (Gillingham, UK). All regents were at least analytical grade unless stated otherwise and all aqueous solutions were prepared using high purity deionized water (18.2 MΩ·cm, Barnstead Nanopure Diamond™, Dubuque, IA). The percentage concentration of Dex-Na_2-Phos solutions is expressed in terms of the Dex-Phos concentration. For example, a 0.4% Dex-Phos solution contains 4.4 mg/ml of Dex-Na_2-Phos.

2.2. Skin preparation

Porcine ears were obtained locally. Ears were cleaned under cold, running water and the skin was dermatomed to a nominal thickness of 750 μm (Zimmer™ Electric Dermatome, Dover, OH). The pieces of tissue obtained (~9 cm^2) were wrapped individually in Parafilm™ and stored for no more than three months at −20 °C until use.

2.3. Iontophoresis

Iontophoresis experiments were performed in vitro in side-by-side diffusion cells (transport area = 0.78 cm^2) with the stratum corneum side facing the donor, cathodal chamber. In all experiments, the subdermal compartment was filled with phosphate-buffered saline (PBS — 170 mM sodium, 1.4 mM potassium, 137 mM chloride and 18 mM phosphate) at pH 7.4. Both chambers held 3.5 ml of solution and were magnetically stirred. In all experiments, a 0.3 mA constant current (0.38 mA/cm^2) was applied for 6 h via Ag/AgCl electrodes connected to a power source (Yokogawa 7651 Programmable DC source, Woodburn Green, UK). Experiments were performed at room temperature with a minimum of five replicates, using skin from at least two different pigs. Every hour for the first 2 h and every half-hour for the remaining 4 h of the experiment, 1 ml of the receptor compartment was collected and replaced by the same volume of PBS.

Two series of solutions were tested. In the first set, the donor solution consisted of Dex-Phos solutions with a concentration of 0.85 mM (0.04% w/v), 4.25 mM (0.2%), 8.5 mM (0.4%) or 17 mM (0.8%) in pure water. When the donor solution was 8.5 mM Dex-Phos in water, in addition to sampling of the receptor chamber, 0.3 ml of the donor solution was also collected every hour and assayed for chloride. In one specific experiment, the donor solution (Dex-Phos 8.5 mM in water) was continuously perfused (15 ml/h) through a 1.5 ml donor compartment, using a peristaltic pump, during iontophoresis. This ensured that the concentration of Cl^- never attained 1/10th of the drug's concentration as confirmed by ion chromatography (see sample analysis). In the second set of experiments, the donor solutions consisted of 8.5 mM Dex-Phos containing 10.3 mM (0.06%), 51 mM (0.3%), 103 mM (0.6%) or 154 mM (0.9%) NaCl as background electrolyte.

2.4. Tape-stripping

In three experiments, for which the donor solution was Dex-Phos 8.5 mM in water, the amount of drug in the stratum corneum (SC) was assessed by tape-stripping following (a) passive diffusion for 3 h (n=4), (b) iontophoresis for 30 min (n=5), and (c) iontophoresis for 3 h (n=7).

At the end of the experiment, any solution remaining on the skin surface was removed using absorbent paper. The skin was then pinned to a dissecting board and a polypropylene foil template with a circular aperture (8 mm diameter) was positioned over the treated area. The SC was then removed by repeated adhesive tape-stripping (Scotch Book Tape, 3M, St. Paul, MN). Between 15 and 28 tapes were required to completely ablate the barrier. Each tape was weighed before and after stripping on a 0.1-μg precision balance (Satorius SE2-F, Epsom, UK) to determine the mass and thickness of the SC layer removed [20,21]. Dex and Dex-Phos were completely extracted from the tapes by overnight shaking with 1 ml of 30:70 acetonitrile: pH 2 phosphate buffer. Validation of the extraction procedure involved spiking tape-stripped samples of untreated skin with a known amount of Dex and/or Dex-Phos; recovery was (98±2)% (n=6).

2.5. Sample analysis

The concentrations of Dex-Phos and Dex in the receptor chamber and tape extracts were assayed by high-performance liquid chromatography (ASI-100 automated sample injector, P680 pump, TCC-100 thermostated column compartment, PDA-100 diode array detector, Dionex, Sunnyvale, CA) under isocratic conditions. A mobile phase consisting of 30:70 (v:v) acetonitrile:phosphate buffer (0.15 M, pH 2) was pumped (0.75 ml/min) through a Lichrospher® 100 RP-18 (4× 125 mm) reverse-phase column (HiChrom, Reading, UK) fitted with its guard column and thermostated at 25 °C. Dex-Phos and Dex concentrations were quantified via their UV absorbance at 240 nm using their respective linear calibration curves (correlation coefficient >0.999, relative standard deviation <5%) obtained from a minimum of five standard solutions (made from 500 ppm stock solutions in methanol appropriately diluted in PBS pH 7.4) covering the entire range of experimental concentrations. The retention times for Dex-Phos and Dex were ~3.5 and ~10.5 min respectively; the detection limit for both was 0.02 μg/ml for a 25 μl sample injection (used for analysis in receptor compartment) and 0.01 μg/ml for a 50 μl sample injection (used for the analysis of tape extracts).

Ion chromatography (AS50 autosampler and thermal compartment, GP50 gradient pump, ED50 electrochemical detector, Chromeleon software, Dionex, Sunnyvale, CA) was used to measure the concentrations of chloride. The 35 mM NaOH mobile phase was pumped under isocratic conditions (1 ml/min) through an IonPac™ AS16 column (Dionex, 250×4 mm) thermostated at 30 °C and the ASRS ULTRA II suppressor (Dionex, 4 mm) set at a current of 90 mA. Quantification was performed against the linear calibration curve (correlation coefficient >0.999, relative standard deviation <2%) obtained from at least five sodium chloride solutions.

2.6. Data analysis and statistics

Linear regressions, Lowess curves and statistics were performed using Graph Pad Prism V.4.00 (Graph Pad Software Inc., San Diego, CA, USA). Statistical differences within multiple data sets were assessed by one-way ANOVA, followed by a Tukey's multiple comparison test. The level of statistical significance was fixed at $p<0.05$. The fluxes were obtained from the slope of the cumulative amount delivered as a function of time for each replicate and are expressed as mean±SD. The reported Dex-Phos flux is the sum of the Dex-Phos and Dex fluxes into the receptor phase to account for the partial dephosphorylation of the prodrug that occurred during transdermal passage or in the receptor

phase. Transport numbers were calculated using these fluxes and Eq. (1). The pH of the donor solution did not significantly shift during the experiments, and was measured in the range 7.2–7.6 in all cases. As a consequence, the valence used for Dex-Phos in all calculations was 2, as at least 85% of the Dex-Phos in solution was in the di-anionic form.

3. Results and discussion

3.1. Effect of Cl^-

Cl^- ions are released from the Ag/AgCl electrode into the cathode compartment at a constant rate determined by the electrical current flowing in the circuit (Eq. (2b)). Hence, the concentration of the competing co-ion gradually increases in the donor solution even when it initially contained only Dex-Phos in water. Fig. 1 compares the theoretical evolution of the Cl^- concentration in the 3.5 ml donor solution, as a function of time, for a current of 0.3 mA, assuming that all the chloride formed remains in the electrode compartment, with the experimentally measured values when the Dex-Phos concentration was 0.4%. The figure also shows the Cl^- concentrations predicted (dashed line) when 20% of the charge is carried by these ions liberated at the cathode. The measured values suggest that when Dex-Phos is present at a reasonably high concentration in water, the contribution of Cl^- to the transport of current is relatively modest and is less than 20% as had been previously deduced from the measurement of the transport of the counter-ions [19].

Earlier results showed that the Dex-Phos fluxes from 4.25 mM, 8.5 mM and 17 mM solutions in water were statistically indistinguishable, despite the fact that the times and current doses at which the Cl^- concentration reached a level equivalent to that of Dex-Phos were quite different. Respectively, these can be read from Fig. 1: for 4.25 mM Dex-Phos, Cl^- reached the same concentration at ~80 min (current dose of about 24 mA·min); for 8.5 mM at 160 min (48 mA·min); for 17 mM at 320 min (96 mA·min). The same was true when the 8.5 mM Dex-Phos in water was perfused continuously to maintain the level of Cl^- at 1/10th of this concentration (Fig. 2). Only for 0.85 mM is the Dex-Phos flux markedly lower (see Table 1 and Fig. 2); in this case Cl^- matches the Dex-Phos level after 16 min (4.8 mA·min).

The impact of Cl^- in the drug formulation was systematically studied and the combined results of the new experiments reported here together with those from before are summarized in Fig. 2 and Table 1. Clearly, the transport of the drug is progressively hampered by an increase in the presence of the competing halide.

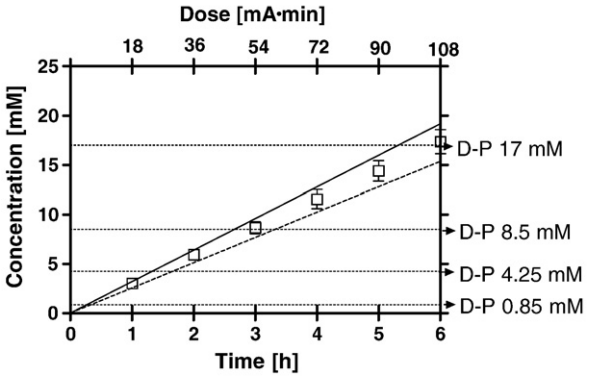

Fig. 1. Theoretical evolution of Cl^- concentration in the cathode compartment over a 6-hour period of iontophoresis at 0.3 mA when either all the ions remain in the donor (solid line) or when 20% are subsequently 'delivered' across the skin (dashed line). Experimentally measured values when the donor solution was initially 8.5 mM Dex-Phos in water (mean ± SD, $n = 11$) are shown for comparison. Also shown, are the different Dex-Phos donor concentrations used (dotted lines).

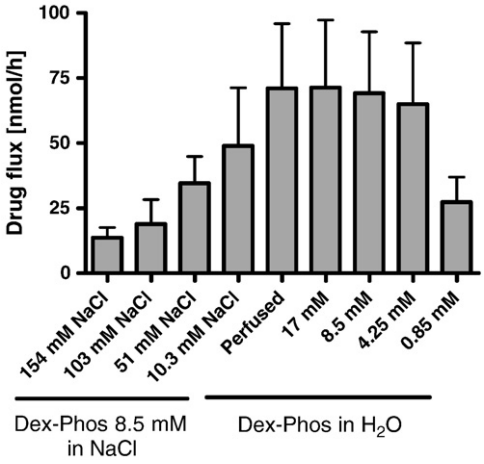

Fig. 2. Dex-Phos fluxes from a donor solution containing drug at 8.5 mM in various concentrations of saline, compared to those obtained when the drug was dissolved in water alone. The intensity of current applied was 0.3 mA in all cases.

Table 1 also includes the theoretically calculated molar fractions of Dex-Phos as a function of the time of iontophoresis assuming that all Cl^- released from the cathode remained in the donor solution. It is apparent that, whenever the drug's molar fraction was less than 50% after 1 h of current passage, the Dex-Phos flux was compromised. In some cases, it is worth noting, the molar fraction of drug dropped precipitately during the 6 h of iontophoresis; for example, from 1 to 0.18 and to 0.04, respectively, for 4.25 mM and 0.85 mM Dex-Phos in water. Nevertheless, it is intriguing to note that, despite the decreasing Dex-Phos molar fraction, the drug's iontophoretic flux over the 6-hour duration of the experiment first increased over the initial period of current passage before remaining relatively constant. This phenomenon is illustrated in Fig. 3 for the two donor solutions mentioned above. In other words, the anticipated falling off of the flux with increasing time (as has been observed for other anions, such as amino acids and peptides [12,13], piroxicam [14] and diclofenac [15] under similar conditions) was not observed.

The transport numbers reported for Dex-Phos in Table 1 do not vary linearly with the initial molar fraction of Dex-Phos in the donor solution (Fig. 4). In this respect, the results diverge from those reported for small cations (Na^+, K^+, Li^+ and NH_4^+), and for drugs like

Table 1
Dex-Phos fluxes and transport numbers under various conditions

Experimental condition	Dex-Phos flux [nmol/h]	100*Transport number[a]	Dex-Phos molar fraction			
			$t=0$	$t=1$ h	$t=3$ h	$t=6$ h
Dex-Phos 8.5 mM in 154 mM NaCl	14±4	0.25±0.07	0.05	0.05	0.05	0.05
Dex-Phos 8.5 mM in 103 mM NaCl	19±9	0.34±0.16	0.08	0.07	0.07	0.07
Dex-Phos 8.5 mM in 51 mM NaCl	37±10	0.66±0.18	0.14	0.13	0.12	0.11
Dex-Phos 8.5 mM in 10.3 mM NaCl	49±22	0.88±0.39	0.45	0.39	0.30	0.22
Dex-Phos 8.5 mM in H_2O. Perfused	73±25	1.3±0.4	1	>0.9	>0.9	>0.9
Dex-Phos 17 mM in H_2O	71±26	1.3±0.4	1	0.84	0.64	0.47
Dex-Phos 8.5 mM in H_2O	69±24	1.2±0.4	1	0.73	0.47	0.31
Dex-Phos 4.25 mM in H_2O	65±24	1.2±0.4	1	0.57	0.31	0.18
Dex-Phos 0.85 mM in H_2O	27±10	0.48±0.18	1	0.21	0.08	0.04

The initial molar fractions of the drug in the donor solutions ([Dex-Phos]/([Dex-Phos]+[Cl^-])) and their evolution with the duration of iontophoresis (t) are also shown.

[a] Multiplication of the transport number by 100 yields a value equal to the percentage of the charge being transported across the skin.

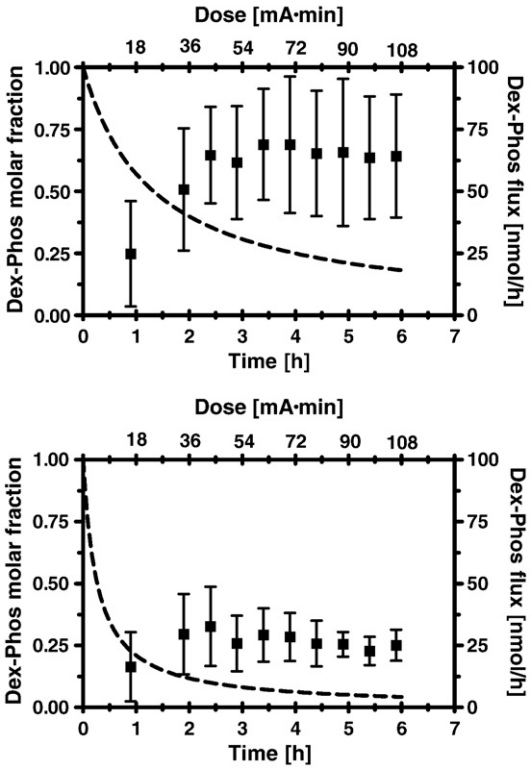

Fig. 3. Measured Dex-Phos fluxes as a function of time of iontophoresis (square symbols, mean ± SD) compared to the calculated change in the molar fraction of the drug in the donor solution (dotted line) (upper panel: 4.25 mM Dex-Phos in water; lower panel 0.85 mM Dex-Phos in water). The intensity of current applied was 0.3 mA in all cases.

lidocaine [8], but parallel with those seen for quinine and propanolol, two relatively lipophilic cations believed to interact significantly with the fixed charge on the skin and to change its permselective properties [7]. The results reported here raise the question, therefore, as to whether Dex-Phos is perhaps altering the membrane in some way and/or accumulating in the tissue.

In attempting to predict the effect of co-ion competition, Phipps and Gyory derived an expression for the transport number of a drug across a homogeneous uncharged membrane as a function of its molar fraction in the donor solution [5]:

$$t_d = \frac{\frac{t_d^0}{1-t_d^0}}{\frac{t_d^0}{1-t_d^0} + \frac{BZX}{1-t_a^0} + 1} \quad (3)$$

where t_d^0 and t_a^0 are the transport numbers of the drug and the co-anion (Cl^-) when they are the only species present (the single anion situation), B is a proportionality constant that relates the ratio of the anion concentrations in the membrane to that in the donor solution, Z is the valence ratio of the anions and X their molar fraction ratio in the donor solution. In practice, this approach has found little practical use because there is no validated method to predict the value of the parameter B [8]. In theory, when $B=1$, the concentration ratio of the co-anion and the drug is conserved inside the membrane; a value of $B<1$ suggests a higher concentration of the drug in the membrane relative to that of the co-anion when compared to the donor solution. The data from the experiments described here were fitted to the model assuming $t_a^0=0.4$, the reported transport number of Cl^- in the single ion situation [6]. The B value derived from the fit was 0.3 (Fig. 4) suggesting that the apparent concentration of the drug relative to Cl^- inside the skin is more important relative to that in the donor solution.

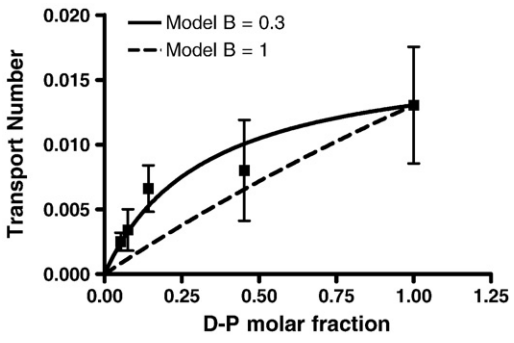

Fig. 4. Transport numbers of Dex-Phos as a function of its initial molar fraction in the donor solution compared to predictions of the Phipps and Gyory model [5].

3.2. Tape-stripping

Tape-stripping experiments were performed to test directly whether Dex-Phos accumulated in the stratum corneum (SC) during iontophoretic delivery from a donor solution containing Dex-Phos 8.5 mM in water. The results are in Table 2. When no current was applied (passive control), the amount of drug present in the SC was an order of magnitude less than that achieved post-iontophoresis. The duration of current passage influenced the amount of drug (Dex-Phos + Dex) uptake into the SC, with about 70% more being found in the SC after 3 h compared to 30 min. The higher level of Dex recovered accounted for nearly all of this difference. However, the absolute drug level in the SC, even with iontophoresis, was small compared to the measured flux (~69 nmol/h) implying that "accumulation" was not a significant factor.

As the tape-stripping experiment involved individual analysis of drug on all the strips, and quantification of the amount of SC removed (achieved gravimetrically as previously described [20]), it was possible to derive concentration profiles of Dex-Phos and Dex across the SC after passive diffusion and following either 30 min or 3 h of iontophoresis (Fig. 5). When no current was applied, the profiles decayed rapidly from the skin surface, intercepting the concentration axis at a level similar to that of Dex-Phos in the donor solution (8.5 mM). With iontophoresis, on the other hand, while the surface level of the drug was similar, its concentration (and that of Dex as well) then increased over the first one-third or so of the SC, before falling off towards the inner boundary of the barrier (although not always returning to zero). Dex-Phos levels were obviously higher than Dex at 30 min but had become quite similar (as well as of greater absolute magnitude) by 3 h of current passage.

The pattern observed in Fig. 5 has been predicted theoretically from the Nernst–Plank equation (with the electroneutrality assumption) [22,23], and shows that iontophoresis can enhance a drug's concentration in the membrane relative to that in the delivery formulation. Whether the increased level of Dex-Phos can account for the negligible impact of Cl^- released from the cathode in some of the experiments reported here cannot be unambiguously deduced. Certainly, it could be argued that an elevated presence of anions inside the already negatively charged membrane would be

Table 2
Dex-Phos, Dex and {Dex-Phos + Dex} uptake into the SC after 3 h of passive diffusion or following 30 min or 3 h of iontophoresis

	Iontophoresis	Iontophoresis	Passive diffusion
	3 h	30 min	3 h
Dex-Phos in SC [nmol]	4.9 ± 2.0	4.6 ± 0.9	0.41 ± 0.17
Dex in SC [nmol]	5.0 ± 1.6	1.4 ± 0.2	0.28 ± 0.06
Dex-Phos + Dex [nmol]	9.9 ± 3.3	6.0 ± 1.1	0.69 ± 0.24

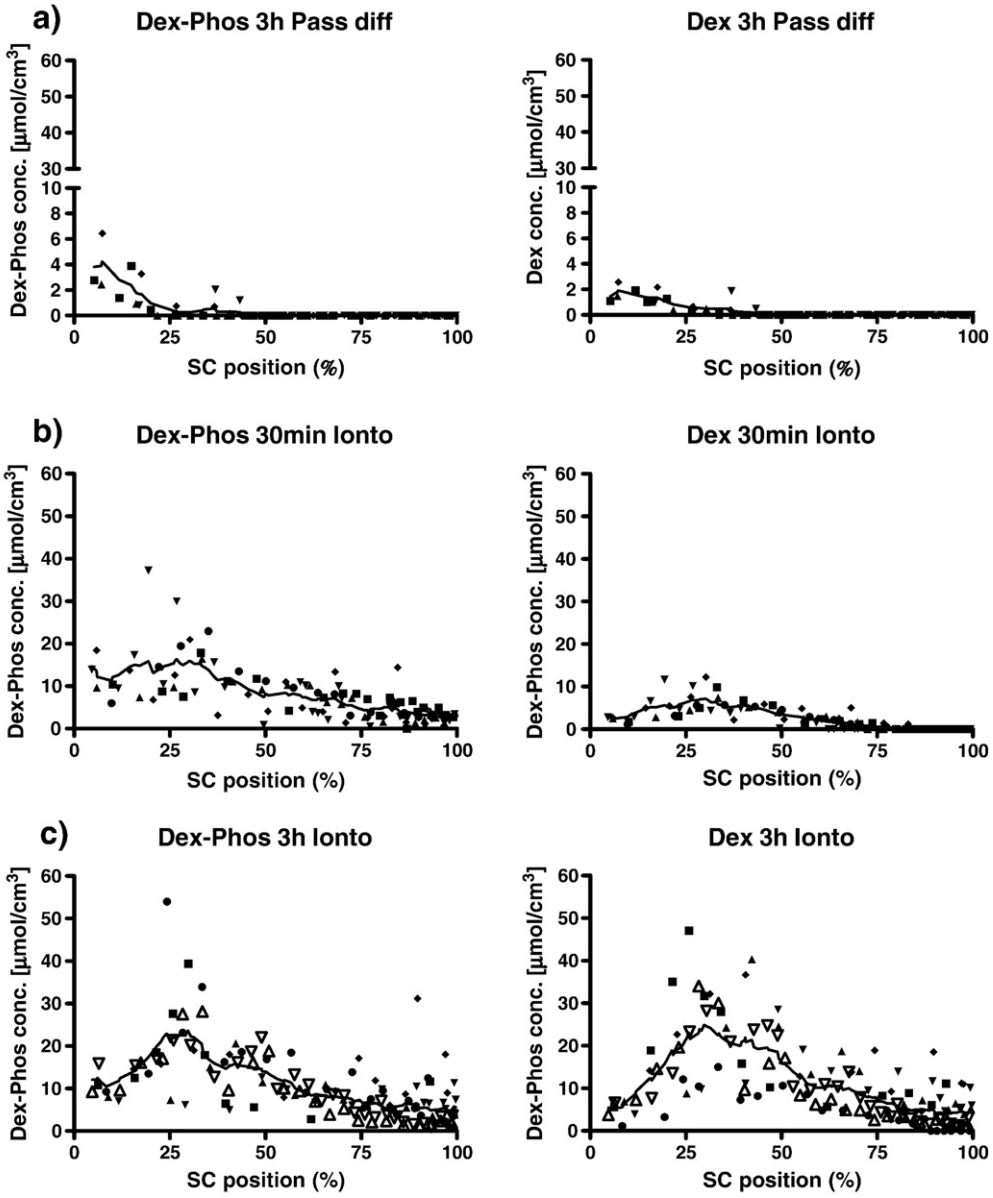

Fig. 5. Dex-Phos (left panel) and Dex (right panel) concentration profiles as a function of relative position in the stratum corneum for (a) 3 h of passive diffusion ($n=4$), (b) 30 min of iontophoresis ($n=5$), and (c) 3 h iontophoresis ($n=7$) of a solution of 8.5 mM Dex-Phos in H_2O. Each piece of skin is represented by a different symbol and the full line tracks the trend of the data without following a specific model (Lowess curve).

more likely to raise the transport number of Na^+ (moving from the receiver solution into the cathode chamber) and disadvantage the migration of Cl^-, at least over the relatively short duration of these experiments.

4. Conclusions

In summary, the results presented here demonstrate that the delivery of Dex-Phos from a Ag/AgCl cathode is relatively robust to the presence of Cl^- in the donor solution. From a practical standpoint, the results suggest that optimal delivery of the drug should be obtained if the cathode compartment contains sufficient Dex-Phos to ensure that its molar fraction remains >50% for the first ~20 mA·min of the dose. The exact reasons for this phenomenon are still unclear, but quantification of the drug in the SC by tape-stripping revealed that it was not due to a significant absolute accumulation of the drug in this barrier. The concentration profile of the Dex-Phos inside the SC could, on the other hand, bring some new insight to this problem.

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