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Research paper

Transdermal iontophoresis of dexamethasone sodium phosphate *in vitro* and *in vivo*: Effect of experimental parameters and skin type on drug stability and transport kinetics

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ABSTRACT

The aim of this study was to investigate the cathodal iontophoresis of dexamethasone sodium phosphate (DEX-P) *in vitro* and *in vivo* and to determine the feasibility of delivering therapeutic amounts of the drug for the treatment of chemotherapy-induced emesis. Stability studies, performed to investigate the susceptibility of the phosphate ester linkage to hydrolysis, confirmed that conversion of DEX-P to dexamethasone (DEX) upon exposure to samples of human, porcine and rat dermis for 7 h was limited (82.2 ± 0.4%, 72.5 ± 4.8% and 78.6 ± 6.0% remained intact) and did not point to any major inter-species differences. Iontophoretic transport of DEX-P across dermatomed porcine skin (0.75 mm thickness) was studied *in vitro* as a function of concentration (10, 20, 40 mM) and current density (0.1, 0.3, 0.5 mA cm^{-2}) using flow-through diffusion cells. Increasing concentration of DEX-P from 10 to 40 mM resulted in a ~4-fold increase in cumulative permeation (35.65 ± 23.20 and 137.90 ± 53.90 μg cm^{-2}, respectively). Good linearity was also observed between DEX-P flux and the applied current density (i_d; 0.1, 0.3, 0.5 mA cm^{-2}; J_{DEX} (μg cm^2 h^{-1}) = 237.98 i_d − 21.32, r^2 = 0.96). Moreover, separation of the DEX-P formulation from the cathode compartment by means of a salt bridge – hence removing competition from Cl^- ions generated at the cathode – produced a 2-fold increase in steady-state iontophoretic flux (40 mM, 0.3 mA cm^{-2}; 20.98 ± 7.96 and 41.82 ± 11.98 μg cm^{-2} h^{-1}, respectively).

Pharmacokinetic parameters were determined in Wistar rats (40 mM DEX-P; 0.5 mA cm^{-2} for 5 h with Ag/AgCl electrodes and salt bridges). Results showed that DEX-P was almost completely converted to DEX in the bloodstream, and significant DEX levels were achieved rapidly. The flux across rat skin *in vivo* (1.66 ± 0.20 μg cm^{-2} min^{-1}), calculated from the input rate, was not statistically different from the flux obtained *in vitro* across dermatomed porcine skin (1.79 ± 0.49 μg cm^{-2} min^{-1}). The results suggest that DEX-P delivery rates would be sufficient for the management of chemotherapy-induced emesis.

1. Introduction

Dexamethasone (DEX) is a highly potent glucocorticoid that is often used in combination therapy to treat acute and delayed emesis in patients receiving chemotherapy. Its mechanism of action is not fully understood. A report on the ability of dexamethasone to antagonize cisplatin-induced acute- and delayed emesis in the ferret suggested that inflammatory mediators may contribute to the vomiting response [1]. However, in earlier studies, no increase in prostaglandin activity was observed in patients receiving treatment with cisplatin [2]. Dexamethasone is usually administered orally – but this is not always convenient for patients who, in addition to enduring nausea and vomiting, frequently suffer from oral mucositis.

Dexamethasone sodium phosphate (DEX-P) is a water-soluble prodrug that is administered parenterally (Fig. 1). The presence of the phosphate ester means that there are two ionisable groups (pKa_1 and pKa_2 are 2.0 and 6.0, respectively). Thus, under

Abbreviations: AUC_{0-inf}, area under a curve from time = 0 to time infinity; Cl^-, chloride ion; *C*, concentration; C_{max}, maximum plasma concentration; C_p, plasma concentration; CV, coefficient of variation; DEX-P, dexamethasone sodium phosphate; HPLC, high performance liquid chromatography; *I*, current intensity; IV, intravenous administration; J_{tot}, iontophoretic flux; k_{input}, input rate; k_{10}, elimination rate constant; SB, salt bridge; SD, standard deviation.

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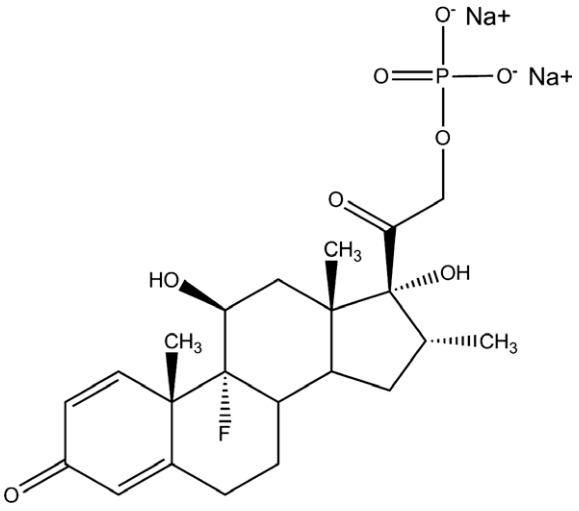

Fig. 1. Chemical structure of dexamethasone sodium phosphate (MW 516.4 Da; pKa 2.04 and 6).

physiological conditions, DEX-P exists principally as a di-anion. Its aqueous solubility and charge render DEX-P a much better candidate for transdermal iontophoretic delivery than the parent molecule (DEX) [3]. Iontophoresis is ideally suited to the delivery of hydrophilic charged molecules, and its major advantage, when compared to other transdermal technologies, is that modulation of the intensity and duration of current application enables precise control of drug delivery kinetics [3,4]. Different iontophoretic devices and "fill-on-site patches" are currently used – in particular, in physical therapy – to deliver corticosteroids locally [5,6]; prefilled iontophoretic patch systems for lidocaine and fentanyl have also been approved by the US FDA [3,4,7,8]. The first administration of DEX-P by anodal iontophoresis was accomplished in the 1980s for the treatment of localized tissue inflammation [9]. Drug levels were measured in deep underlying structures following anodal delivery at a current density of 0.94 mA cm^{-2}. Petelenz et al. compared anodal and cathodal DEX-P iontophoresis in the presence of lidocaine [10]. Surprisingly, their results indicated that although cathodal iontophoresis was more efficient *in vitro*, there was no difference between anodal and cathodal delivery *in vivo*. More recently, Sylvestre et al. evaluated the effect of different experimental variables on the transdermal iontophoresis of DEX-P *in vitro* [11,12].

The main objective of the present work was to investigate cathodal iontophoretic delivery of DEX-P *in vitro and in vivo* using an electrode configuration where the electrode and formulation compartments were separated in order to optimize DEX-P transport. Cathodal iontophoresis using Ag/AgCl results in the liberation of Cl^- ions from the electrode that compete with drug anions to transport current; the progressive increase in the Cl^- concentration during current application results in a decrease in drug anion delivery – as reported for different nonsteroidal anti-inflammatory drugs [13–16] – hence the interest in separating the formulation and electrode compartments.

The specific aims of the investigation were as follows: (a) to examine the *ex vivo* stability of DEX-P using different skin species – in order (i) to identify any inter-species differences and (ii) to confirm that the ester linkage and hence the charged moiety would be retained during transport (early hydrolysis would produce the neutral DEX molecule, which cannot be delivered by cathodal iontophoresis), (b) to study cathodal iontophoresis of di-anionic DEX-P as a function of current density and formulation composition and (c) to investigate the feasibility of delivering therapeutic amounts of drug *in vivo* for the treatment of chemotherapy-induced nausea and vomiting (CINV).

2. Materials and methods

Dexamethasone 21-disodium phosphate salt, dexamethasone, agarose, silver wire and silver chloride used for the fabrication of electrodes were purchased from Sigma–Aldrich (Buchs, Switzerland). HEPES buffer and sodium chloride were obtained from Fluka (Buchs, Switzerland). Silicon tubing (1.6 mm ID, 3.2 mm OD, and 0.8 mm wall thickness) for collecting samples and PVC tubing (3 mm ID, 5 mm OD, 1 mm wall thickness) used to prepare salt bridge assemblies were obtained from Fisher Bioblock Scientific SA (Illkirch, France). Chromatographic reagents were obtained from VWR (Dietikon, Switzerland). All solutions were prepared using deionised water (resistivity > 18 MΩ cm). All other chemicals were at least of analytical grade.

Porcine ear skin was obtained from two abattoirs (STAC; Chambery, France and CARRE; Rolle, Switzerland). The skin was excised (thickness 750 μm) with an electro-dermatome (Zimmer; Etupes, France). Rat skin was obtained from the Animal Experimental Research Centre at the University of Valencia (Spain). Human skin was acquired from the Department of Plastic, Reconstructive and Aesthetic Surgery, Geneva University Hospital (Geneva, Switzerland). All tissue samples were wrapped in Parafilm™ and stored at −20 °C for a maximum period of 2 months.

2.1. Experimental protocol in vitro

2.1.1. Evaluation of DEX-P stability

The influence of skin metabolism on DEX-P stability was examined *in vitro* using Franz diffusion cells and three different types of skin. A 200 μM DEX-P solution (in 25 mM HEPES/133 mM NaCl, pH 7.4) was placed in contact with the dermal surfaces of samples of human, rat and porcine skin for 7 h. Aliquots were collected after 30, 60, 90, 120, 180, 240, 300, 360 and 420 min.

The stability of DEX-P in the presence of dermatomed porcine skin and heat-separated porcine epidermis was also compared in a separate study. Briefly, heat-separated epidermis was prepared by first heating skin samples to 60 °C for 30 s in water; then, the epidermis was carefully separated from underlying tissue using forceps and transferred to Petri dishes filled with phosphate buffer saline solution (pH 7.4). The internal surfaces of dermatomed porcine skin and heat-separated porcine epidermis were placed in contact with a solution containing 20 mM DEX-P in water (∼pH 7.8). Samples were withdrawn over a 7 h period at the same time-points mentioned earlier.

The stability experiments were performed in triplicate.

2.1.2. Effect of concentration

Dermatomed porcine skin was clamped in three-compartment vertical flow-through diffusion cells (A = 0.95 cm^2). The electrode and receptor compartments were filled with buffer solution (comprising 25 mM HEPES + 133 mM NaCl, pH 7.4). After equilibration, 1 ml of unbuffered DEX-P solution 10, 20 or 40 mM (pH ∼ 7.8) was placed in the cathodal compartment; at this pH, the molecule exists principally in the di-anionic form. A constant current density of 0.3 mA cm^{-2} was applied for 7 h via Ag/AgCl electrodes connected to a power supply (APH 1000 M, Kepco, Flushing, NY). Samples were collected hourly from the receptor compartment using a syringe pump (SP220IZ, WPI, Sarasota, FL), which provided a continuous flow of buffer solution (1 ml h^{-1}).

2.1.3. Effect of current density

This was investigated in separate studies at 0.1, 0.3 and 0.5 mA cm^{-2} using a 40 mM unbuffered DEX-P solution. Salt bridges (SB) were included to separate the cathodal and donor compartments and to eliminate competition from Cl^- ions. Current

was applied for 7 h. All other aspects of the protocol were the same as those outlined earlier.

2.2. Experimental protocol in vivo

Male Wistar rats (260–280 g) were supplied by the Animal Experimental Research Centre at the University of Valencia, Spain. Experimental protocols were approved by the Ethical Committee for Animal Experimentation at the University of Valencia. Twenty four hours before iontophoresis, the rats were anesthetized by intraperitoneal administration of pentobarbital sodium (Dolethal solution, 40 mg kg^{-1}; Vetoquinol, Madrid, Spain). The jugular vein was cannulated using medical-grade silicon tubing (Silastic, Dow Corning Co.; ID 0.5 mm; OD 0.94 mm).

For iontophoretic administration of DEX-P, animals were anesthetized (pentobarbital sodium, 40 mg kg^{-1}) and mounted on a plastic support. Two glass chambers ($A = 1.33\ cm^2$) were then placed 2.5 cm apart on the animal's abdomen (shaved beforehand) and fixed with glue. The anodal and cathodal compartments contained buffer solution (25 mM HEPES/133 mM NaCl, pH 7.4). The DEX-P formulation (0.7 ml of unbuffered 40 mM DEX-P solution; pH ~ 7.8) was separated from the cathode by a SB. A power supply (APH 1000 M, Kepco, Flushing, NY) delivered a constant, direct current of 0.66 mA (0.5 mA cm^{-2}) for 5 h. Blood samples (1.0 ml) were withdrawn at hourly intervals and immediately centrifuged at 10,000 rpm; the plasma collected was separated and stored at −20 °C until analysis by HPLC. After each sampling time, the blood volume was replaced with the same volume of saline solution. At the end of each experiment, the animals were sacrificed using pentobarbital sodium at a dose of 200 mg kg^{-1}.

2.3. Analytical method

A P680A LPG-4 pump equipped with an ASI-100 autosampler (Dionex; Voisins LeBretonneux, France) and a UV detector (UVD 170/340-U) was used to quantify both the prodrug (DEX-P) and dexamethasone (DEX). Isocratic separation was performed using a 125 mm × 4 mm column packed with 5 μm C_{18} end-capped silica reversed-phase particles (Lichrocart, Merck KGaA, Germany). The flow-rate was 1.0 ml min^{-1}, and the column temperature was kept at 30 °C. The mobile phase consisted of 50% 0.01 M oxalic acid and 50% methanol (pH = 6.0). The injection volume ranged from 5 to 100 μl. The UV absorbance at 240 nm was used for detection. The elution times for DEX-P and DEX were 4.8 and 10.4 min, respectively; the total run time was 12 min. The limits of detection (LOD) and quantification (LOQ) for DEX were 3.48 and 10.44 μg/ml, and for DEX-P, 0.48 and 1.44 μg/ml, respectively.

2.4. Skin extraction

DEX-P was extracted from the skin by soaking the samples in 5 ml of mobile phase for 4 h at ambient temperature. The extraction suspensions were membrane-filtered (nylon membranes 0.22 μm, Whatman, Maidstone, England). DEX-P content was analyzed by HPLC (see above). Experiments were typically performed in sextuplicate. The method was validated and the data are shown in Table 1. Validation was performed by spiking the stratum corneum surface of dermatomed skin samples (thickness 750 μm; area 1.06 cm^2) with known amounts of drug (10, 50 and 125 μl of 20 mM DEX-P standard solution in mobile phase). After solvent evaporation, skin samples were subjected to extraction with 5 ml of mobile phase and then analyzed. The recovery of DEX-P was determined by calculating the ratio of the amount extracted from the skin samples to the amount added, determined by direct injection of spiking solution in the absence of skin. However, it should be noted that the recovery obtained after a simple application of the drug in a volatile solvent to the skin surface followed by extraction may not be an accurate predictor of recovery following iontophoresis using aqueous solutions for several hours, where the objective is to extract drug from within the membrane. Thus, although the recovery data presented below demonstrate that drug is retained within the skin during iontophoresis, they may underestimate the total amounts present since extraction efficiency following iontophoresis may be <100%.

Table 1
Validation of the DEX-P extraction method used to quantify drug retained within the skin during experiments *in vitro*.

Control concentration (μg ml^{-1})	Sample concentration (μg ml^{-1})	Recovery (%)	CV (%)
2.45	2.70	110.317	8.47
10.43	9.93	95.188	8.14
27.23	26.77	98.398	2.74

2.5. Plasma extraction

The extraction procedure was based on a one-step liquid–liquid technique [17]. Diethyl ether (1 ml) was added to 200 μl of plasma, and the resulting mixture was vortex-mixed for 15 min. After centrifugation at 12,000 rpm and separation, the organic layer was evaporated at 30 °C. The residue was dissolved in 200 μl of mobile phase, and finally 50 μl injected into the chromatographic column. Calibration curves ($n = 6$) that spanned the range of DEX concentrations in the plasma samples were prepared in triplicate in order to validate the analytical method. A linear correlation was obtained, and the intercept was not significantly different from zero ($r^2 = 0.999$). The mean recovery of DEX averaged 84.0 ± 6.1% ($n = 8$); the LOQ was 10.44 μg/ml. The relative error and coefficient of variation were the parameters evaluated for accuracy and precision and are shown in Table 2 (US Food and Drug Administration, 2001). The stability of DEX in plasma stored at −20 °C during 3 weeks was also evaluated. The relative variation was ~5% over a concentration range from 0.035 to 0.750 μg ml^{-1}.

2.6. Pharmacokinetic analysis

Pharmacokinetic parameters for the iontophoretic delivery of DEX were calculated using a one-compartment model with zero-order absorption and first-order elimination (WinNonlin software version 5.2.1; Pharsight, Inc., Apex, NC) as follows:

Table 2
Accuracy and precision values for the extraction method used to quantify DEX in rat plasma samples from the *in vivo* studies.

Theoretical concentration (μg ml^{-1})	Experimental concentration (mean ± SD) (μg ml^{-1})	CV (%)	Deviation from theoretical value (%)
Intra-day (n = 3)			
0.075	0.072 ± 0.009	11.892	4.617
0.350	0.370 ± 0.008	2.168	5.726
0.750	0.812 ± 0.009	1.087	8.244
2.000	2.242 ± 0.130	5.812	12.090
Inter-day (n = 3)			
0.075	0.068 ± 0.002	3.658	9.467
0.350	0.338 ± 0.032	9.584	3.543
0.750	0.750 ± 0.048	6.415	0.020
2.000	2.261 ± 0.150	6.612	13.064

$$C_p = \left(\frac{k_{input}}{V_d k_{10}}\right) * (1 - e^{-k_{10}t}) \quad \text{if } t \le t_{ionto} \tag{1}$$

$$C_p = \left(\frac{k_{input}}{V_d k_{10}}\right) * (1 - e^{-k_{10}t}) * (e^{-k_{10}(t - t_{ionto})}) \quad \text{if } t \ge t_{ionto} \tag{2}$$

where C_p is the plasma concentration of DEX at time (t), V_d is the volume of distribution, k_{input} is the input rate and k_{10} is the elimination rate constant.

Plasma profiles were fitted using a Gauss–Newton algorithm with Levenberg–Hartley modification. Non-compartmental parameters such as C_{max}, half-life and AUC_{0-inf} were calculated in Microsoft Excel. The volume of distribution of the drug was obtained from literature [18].

2.7. Data analysis and statistics

Values were expressed as mean ± SD. Outliers determined using the Grubbs test were discarded [19]. Statistical analysis was conducted using analysis of variance (ANOVA) to evaluate the difference between multiple data sets. Student's *t*-test was used to compare two data sets. The level of significance was fixed at $\alpha = 0.05$.

3. Results and discussion

3.1. Skin metabolism of DEX-P

Fig. 2 shows the extent of metabolism of the prodrug when in contact with human, porcine and rat dermis; the average recovery of intact DEX-P after 7 h was 82.2 ± 0.4%, 72.5 ± 4.8% and 78.6 ± 6.0%, respectively. The HPLC chromatograms showed only one additional peak, which increased with time and was identified (also by HPLC) as the hydrolysis product, dexamethasone (DEX). Overall, DEX-P displayed good stability, and there was little inter-species variation. It has previously been reported that both animal and human skins possess esterase activity and can hydrolyse corticosteroid 21-monoesters – although typically the ester (e.g., valerate or (di)propionate) is used as a means to increase the lipophilicity of the molecule and facilitate partitioning into the stratum corneum rather than introducing a hydrophilic charged moiety as is the case here [20]. More generally, there are several studies that point to the ability of cutaneous esterases to hydrolyse many different ester prodrugs [20–25] – including valaciclovir, the charged amino-ester derivative of aciclovir [26] – although the rate and extent of hydrolysis can vary depending on the structure and physicochemical properties of the molecules. It should also be borne in mind that there may be differences between the cutaneous activities and distribution of esterases and phosphatases. When DEX-P was placed in contact with the external and internal surfaces of porcine epidermis, the drug appeared to be essentially unaffected (96.46 ± 1.92% and 97.96 ± 8.22%, respectively; Student's *t*-test, $P < 0.05$). This is significant since electromigration through the epidermis and subsequent entry into the dermis depends on the presence of the negatively charged phosphate group – thus, cleavage of this moiety should only occur in the dermis or once the molecule is in the bloodstream itself.

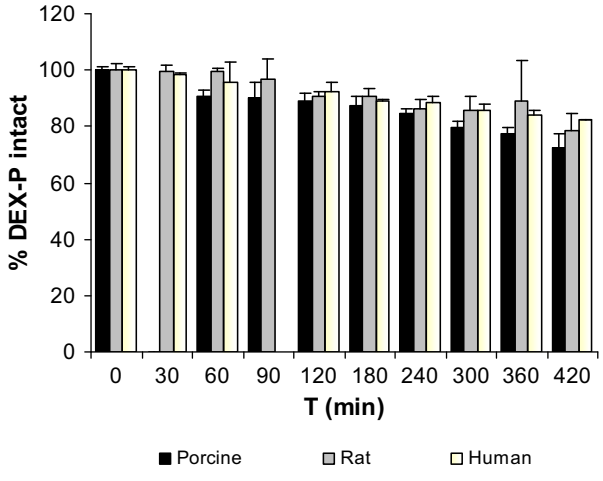

Fig. 2. Stability of DEX-P when placed in contact with porcine, rat and human dermis *in vitro* (mean ± SD; $n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Iontophoretic delivery of DEX-P

3.2.1. In vitro transport kinetics

Control passive diffusion experiments with DEX from a commercial formulation (Dexolan, 0.1% ointment; G Streuli & Cie. SA Uznach, Switzerland) during 7 h resulted in very poor permeation and skin retention; DEX levels were below the limit of detection.

In contrast, cathodal DEX-P iontophoresis using a 10 mM solution (~0.5%) resulted in significant drug transport; cumulative permeation after 7 h was 35.65 ± 23.20 μg cm^{-2}. The effect of drug concentration on DEX-P iontophoresis from unbuffered solutions is shown in Fig. 3; an increase from 10 to 40 mM resulted in a ~4-fold increase in cumulative permeation (at 40 mM, DEX-P delivery was 137.90 ± 53.90 μg cm^{-2}). Drug extraction from the skin carried out at the end of the iontophoretic experiments using 10, 20 and 40 mM DEX-P showed that significant amounts of DEX-P were also retained in the membrane (171.56 ± 109.20, 484.67 ± 290.40 and 520.92 ± 409.75 μg cm^{-2}, respectively). The calculated delivery efficiencies based on the sum of the cumulative amount permeated and retained within the skin using the 10, 20 and 40 mM formulations were 0.93%, 2.99% and 3.58%, respectively.

The impact of increasing current density on DEX-P transport is illustrated in Fig. 4. Good linearity was observed between the cumulative amount of DEX-P permeated and the applied current density (i_d; 0.1, 0.3, and 0.5 mA cm^{-2}) (J_{DEX} (μg cm^2 h^{-1}) = 237.98i_d − 21.32, $r^2 = 0.96$). Although an increase in current density from 0.1 to 0.5 mA cm^{-2} resulted in a ~12-fold increase in DEX-P permeation following 7 h of iontophoresis, there was no statistically significant difference between the amounts of DEX-P extracted from the skin at 0.1, 0.3 and 0.5 mA cm^{-2} (Fig. 4). The absence of any effect of current density on the amount of DEX-P retained within the membrane was somewhat surprising. However, given that the skin is negatively charged under physiologic conditions, accumulation of anionic and cationic drugs may occur by different mechanisms. For example,

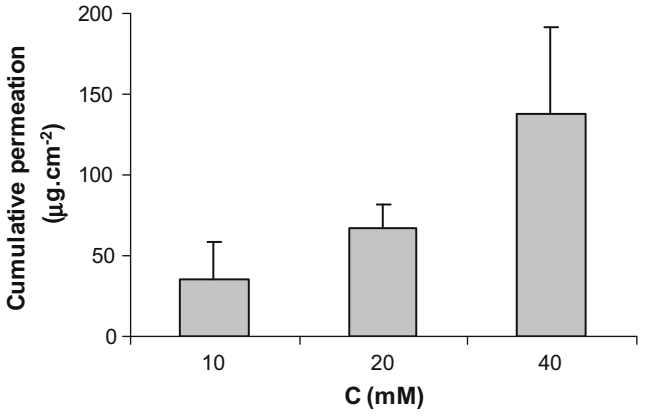

Fig. 3. Cumulative permeation of DEX-P after 7 h of iontophoresis at 0.3 mA cm^{-2} across porcine skin *in vitro* as a function of concentration (mean ± SD; $n \geq 3$).

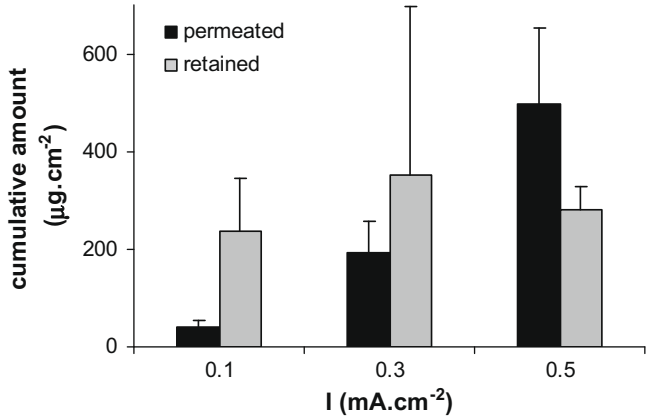

Fig. 4. Cumulative permeation and skin deposition of DEX-P into and across porcine skin *in vitro* as a function of current density after 7-h iontophoresis at 0.1, 0.3 and 0.5 mA cm^{-2} (mean ± SD; $n \geqslant 3$).

lipophilic cations may bind to the fixed negative charges in the skin and form additional van der Waals' interactions with neighbouring regions. However, negatively charged drugs may not be able to bind to as many sites in the skin due to electrostatic repulsion. Hence, if there is a limited number of binding sites, these may become saturated. Thus, increasing current density would no longer produce any effect on the amount retained within the membrane.

The production of mobile chloride ions at the Ag/AgCl cathode during iontophoresis impacts upon the transport efficiency of anionic drugs [13–16]. The theoretical evolution of chloride ion concentration in the cathodal compartment over 7 h at the different current densities used in these experiments is depicted in Fig. 5. Although this is an oversimplification since there would be some loss of chloride ions into the receiver compartment, it is clear that DEX-P transport would be progressively diminished by increasing levels of more mobile chloride ions. Isolation of the DEX-P formulation from the cathode by means of a SB can reduce competition from chloride ions. This is illustrated in Fig. 6, which shows the doubling of iontophoretic fluxes (and hence transport number) when using the SB assembly ($P < 0.05$).

3.2.2. Delivery kinetics in Wistar rats in vivo

The *in vivo* data confirmed that, in contrast to its relative stability in contact with skin, DEX-P was rapidly hydrolysed by phosphatases in the blood and only the active corticosteroid (DEX) was

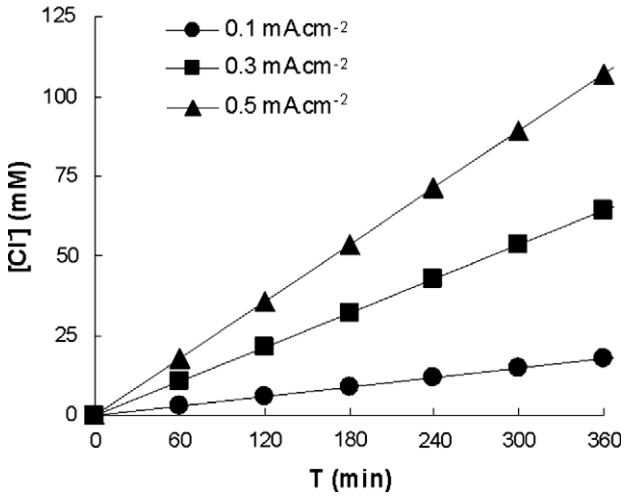

Fig. 5. Concentration profile of chloride ions generated at the cathode compartment (1 cm^3) during 7 h of iontophoresis as a function of current density.

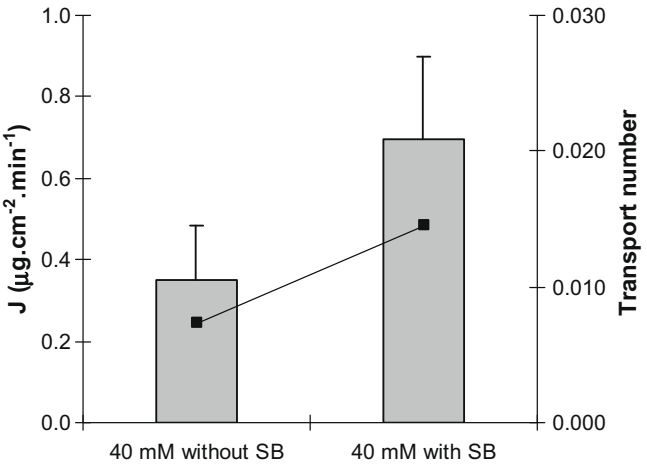

Fig. 6. Improvement in DEX-P delivery (flux (*J*) and transport number) upon eliminating competition from Cl^- ions by using a salt bridge (SB) (mean ± SD; $n > 3$).

detected in the bloodstream. The mean DEX concentration–time profile upon iontophoretic administration at 0.5 mA cm^{-2} and the fitted curve are shown in Fig. 7. Significant DEX levels (~1 µg ml^{-1}) were achieved at 1 h, reflecting the fast input kinetics of the phosphate prodrug (Table 3). Furthermore, the DEX concentration remained significant throughout the post-iontophoretic period between $t = 5$ and 8 h. The flux across rat skin *in vivo*, calculated from the input rate, 1.66 ± 0.20 µg cm^{-2} min^{-1}, was not statistically different from the flux obtained *in vitro* across dermatomed porcine skin (1.79 ± 0.49 µg cm^{-2} min^{-1}). Although experiments in pigs would be required to obtain a true *in vitro*/*in vivo* comparison, it may be that electrically assisted transport reduces inter-

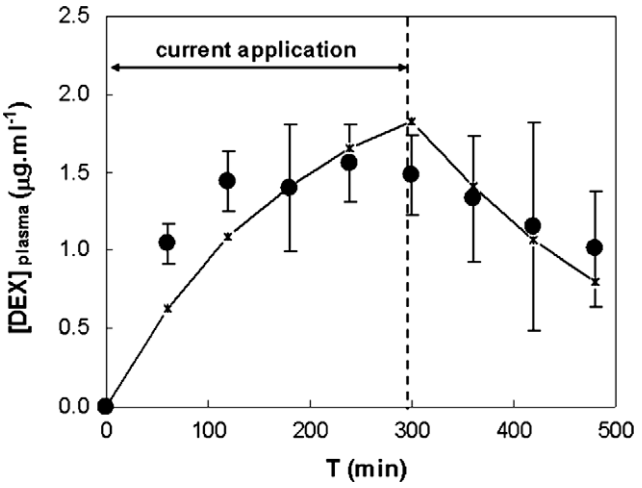

Fig. 7. DEX plasma concentrations in Wistar rats after iontophoretic administration of DEX-P for 5 h (40 mM; 0.5 mA cm^{-2} with a SB). The solid line represents the fitted values (mean ± SD; $n = 4$).

Table 3
Pharmacokinetic parameters for DEX after cathodal iontophoresis of DEX-P in Wistar rats for 5 h at 0.5 mA cm^{-2} ($n = 4$).

Parameter	
C_{max} (µg ml^{-1})	1. 62 ± 0.36
AUC_{0-inf} (µg min ml^{-1})	914.33 ± 378.27
Half-life (min)	235.12 ± 165.83
Input rate (k_{input}) (µg min^{-1})	2.21 ± 0.27
Elimination rate constant (k_{10}) (min^{-1})	0.006 ± 0.002

species variations when compared to passive diffusion across the membrane. Since ion transport is through aqueous channels, this may decrease possible interactions with skin components. However, this needs to be investigated more thoroughly before general conclusions might be drawn. The estimated pharmacokinetic parameters after iontophoretic delivery are given in Table 3, and although these will certainly be different from those seen in larger mammals or in humans, it is worth noting that iontophoretic delivery of DEX-P resulted in an almost 2-fold increase in DEX half-life when compared to that seen after IV or IM administration (3.9 h cf. 2.3 h) [18].

4. Conclusions

The results presented here suggest that DEX-P can be delivered efficiently by cathodal iontophoresis and that transport is further enhanced by eliminating competition with Cl^- ions generated at the cathode. Moreover, DEX-P flux increased linearly as a function of donor concentration and current density. This offers a significant advantage in modulating delivery rates. Pharmacokinetic analysis using a one-compartment model estimated the *in vivo* flux for DEX at 0.5 mA cm^{-2} as 1.66 ± 0.20 μg cm^{-2} min^{-1}. The usual initial adult dosing of dexamethasone for treating delayed CINV ranges from 4 to 12 mg *per os*, depending on patient response. Based on our results *in vivo*, it should be possible to deliver therapeutic amounts of DEX for the treatment of CINV using moderately sized iontophoretic systems containing DEX-P with application areas of 5–10 cm^2 at reasonable current densities.

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References

[1] T.S. Sam, S.W. Chan, J.A. Rudd, J.H. Yeung, Action of glucocorticoids to antagonize cisplastin-induced acute and delayed emesis in the ferret, Eur. J. Pharmacol. 417 (2001) 231–237.
[2] S.L. Curry, J. Rine, C.W. Whitney, W.A. Nahhas, R. Mortel, L.M. Demers, The role of prostaglandins in the excessive nausea and vomiting after intravascular cisplatinum therapy, Gynecol. Oncol. 12 (1981) 89–91.
[3] Y.N. Kalia, A. Naik, J. Garrison, R.H. Guy, Iontophoretic drug delivery, Adv. Drug. Deliv. Rev. 56 (2004) 619–658.
[4] J.A. Subramony, A. Sharma, J.B. Phipps, Microprocessor controlled transdermal drug delivery, Int. J. Pharm. 317 (2006) 1–6.
[5] A.K. Banga, P.C. Panus, Clinical applications of iontophoretic devices in rehabilitation medicine, Crit. Rev. Phys. Rehab. Med. 10 (1998) 147–179.
[6] H. Hamann, M. Hodges, B. Evans, Effectiveness of iontophoresis of anti-inflammatory medications in the treatment of common musculoskeletal inflammatory conditions: a systematic review, Phys. Ther. Rev. 11 (2006) 190–194.
[7] W.T. Zempsky, J. Sullivan, D.M. Paulson, S.B. Hoath, Evaluation of low-dose lidocaine iontophoresis system for topical anesthesia in adults and children: a randomized controlled trial, Clin. Ther. 26 (2004) 1110–1119.
[8] E.R. Viscusi, M. Siccardi, C.V. Damaraju, D.J. Hewitt, P. Kershaw, The safety and efficacy of fentanyl iontophoretic transdermal system compared with morphine intravenous patient-controlled analgesia for postoperative pain management: an analysis of pooled data from three randomized, active-controlled clinical studies, Anesth. Analg. 105 (2007) 1428–1436.
[9] J.M. Glass, R.L. Stephen, S.C. Jacobson, The quantity and distribution of radiolabeled dexamethasone delivered to tissue by iontophoresis, Int. J. Pharm. 19 (1998) 519–525.
[10] T.J. Petelenz, J.A. Buttke, C. Bonds, L.B. Lloyd, J.E. Beck, R.L. Stephen, S.C. Jacobsen, P. Rodriguez, Iontophoresis of dexamethasone: laboratory studies, J. Control. Release 20 (1992) 55–66.
[11] J.-P. Sylvestre, R.H. Guy, M.B. Delgado-Charro, *In vitro* optimization of dexamethasone phosphate delivery by iontophoresis, Phys. Ther. 88 (2008) 1177–1185.
[12] J.-P. Sylvestre, C. Díaz-Martín, M.B. Delgado-Charro, R.H. Guy, Iontophoresis of dexamethasone phosphate: competition with chloride ions, J. Control. Release 131 (2008) 41–46.
[13] C.L. Gay, P.G. Geen, R.H. Guy, M.L. Francoeur, Iontophoretic delivery of piroxicam across the skin *in vitro*, J. Control. Release 22 (1992) 57–67.
[14] X. Hui, A. Anigbogu, P. Singh, G. Xiong, N. Poblete, P. Lieu, H.I. Maibach, Pharmacokinetic and local tissue disposition of [^{14}C] sodium diclofenac following iontophoresis and systemic administration in rabbits, J. Pharm. Sci. 90 (2001) 1269–1276.
[15] Y. Katori, M. Erickson, J.A. Subramony, R. Padmanabhan, J.B. Phipps, Transdermal iontophoretic delivery of ketoprofen, in: 34th Annual Meeting and Exposition of the Controlled Release Society. Long Beach, CA, July 7–11, 2007.
[16] A. Romand, J. Cázares-Delgadillo, Y.G. Bachhav, Y.N. Kalia, Optimizing iontophoretic delivery kinetics of diclofenac *in vitro*, in: 6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology. Barcelona, April 7–10 2008.
[17] A. Chmielewska, L. Konieczna, A. Plenis, H. Lamparczyk, Sensitive quantification of chosen drugs by reversed-phase chromatography with electrochemical detection at a glassy carbon electrode, J. Chromatrogr. B 839 (2006) 102–111.
[18] M.N. Samtani, W.J. Jusko, Comparison of dexamethasone pharmacokinetics in female rats after intravenous and intramuscular administration, Biopharm. Drug. Dispos. 26 (2005) 85–91.
[19] J.E. De Muth, Basic Statistics and Pharmaceutical Statistical Applications, Marcel Dekker, New York, 1999.
[20] U. Taüber, K.L. Rost, Esterase activity of the skin including species variation, in: B. Shroot, H. Schaefer, B. Shroot, H. Schaefer (Eds.), Pharmacology and the Skin, Skin Pharmacokinetics, vol. 1, Karger, Basel, 1987, pp. 170–183.
[21] A.L. Stinchcomb, A. Paliwal, R. Dua, H. Imoto, R.W. Woodward, G.L. Flynn, Permeation of buprenorphine and its 3-alkyl-ester prodrugs through human skin, Pharm. Res. 13 (1996) 1519–1523.
[22] H. Bundgaard, N. Mork, A. Hoelgaard, Enhanced delivery of nalixidic acid through human skin via acyloxymethyl ester prodrugs, Int. J. Pharm. 55 (1989) 91–97.
[23] G. Nicolau, A. Yacobi, Transdermal absorption and skin metabolism of vipristol, a synthetic prostaglandin E2 analogue, Drug Metab. Rev. 21 (1990) 401–425.
[24] T. Seki, T. Kawaguchu, K. Juni, Enhanced delivery of zidovidudine through rat and human skin via ester prodrugs, Pharm. Res. 7 (1990) 948–952.
[25] O. Pillai, M.O. Hamad, P.A. Crooks, A.L. Stinchcomb, Physicochemical evaluation, *in vitro* hum skin diffusion and concurrent biotransformation of 3-O-alkyl carbonate prodrugs of naltrexone, Pharm. Res. 21 (2004) 1146–1152.
[26] N. Abla, A. Naik, R.H. Guy, Y.N. Kalia, Topical iontophoresis of valaciclovir hydrochloride improves cutaneous aciclovir delivery, Pharm. Res. 23 (2006) 1842–1849.