

Contributions of Electromigration and Electroosmosis to Iontophoretic Drug Delivery

Diego Marro,^{1,2} Yogeshvar N. Kalia,^{1,2}
M. Begoña Delgado-Charro,^{1,2} and Richard H. Guy^{1,2,3}

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Purpose. To determine the electromigration and electroosmotic contributions to the iontophoretic delivery of lidocaine hydrochloride, in addition to the more-lipophilic quinine and propranolol hydrochlorides, in the presence and absence of background electrolyte.

Methods: *In vitro* experiments, using excised pig ear skin and both vertical and side-by-side diffusion cells, were performed as a function of drug concentration and with and without background electrolytes in the anodal formulation. Concomitantly, the contribution of electroosmosis in each experimental configuration was monitored by following the transport of the neutral, polar marker molecule, mannitol.

Results. Electromigration was the dominant mechanism of drug iontophoresis (typically representing ~90% of the total flux). In the presence of background electrolyte, lidocaine delivery increased linearly with concentration as it competed more and more effectively with Na⁺ to carry the charge across the skin. However, iontophoretic delivery of quinine and propranolol increased non-linearly with concentration. Without electrolytes, on the other hand, electrotransport of the three drugs was essentially independent of concentration over the range 1–100 mM. Transport efficiency of lidocaine was ~10%, whereas that of the more lipophilic compounds was significantly less, with the major charge carrier being Cl⁻ moving from beneath the skin into the anodal chamber. Both quinine and propranolol induced a concentration-dependent attenuation of electroosmotic flow in the normal anode-to-cathode direction.

Conclusion. Dissecting apart the mechanistic contributions to iontophoretic drug delivery is key to the optimization of the formulation, and to the efficient use of the drug substance.

KEY WORDS: iontophoresis; electromigration; electroosmosis; transdermal delivery; electrotransport; skin.

INTRODUCTION

Iontophoresis enhances drug delivery across the skin by two principal mechanisms: electromigration and electroosmosis (1,2). Electromigration describes the direct effect of the applied electric field on the charged species present in the formulation, whereby the transport of cationic drugs is enhanced from the anode compartment into the skin, and that of anionic drugs is promoted from the cathode. The isoelectric point of mammalian skin falls within the range 3.5 to 4.8 (3–6). Therefore, at physiologic pH, the skin behaves as a negatively charged, cation-permeable membrane (7). It

follows that current passage across the skin causes a net convective solvent flow in the anode-to-cathode direction, a phenomenon generally referred to as electroosmosis (8). This current-induced flow facilitates cation transport, inhibits that of anions, and enables the enhanced transdermal transport of neutral, polar solutes by iontophoresis (1,2).

Clearly, if the principal contribution to drug transport is electromigration, then it makes sense to minimize the presence of competing ions in the applied formulation. On the other hand, for larger (cat)ions, where electroosmosis assumes the more important role, the formulation strategy may be quite different. In either case, the fraction of charge being carried in the iontophoretic circuit by endogenous ions moving from within the body into the electrode compartments may be (and often is) quite significant.

In this article, the effects of drug concentration and the presence/absence of background electrolyte in the formulation were studied. The hydrochloride salts of the cationic local anaesthetic lidocaine [an iontophoretic device for which is currently in advanced development (9)], and of two more lipophilic drugs, quinine and propranolol, have been chosen as models (Fig. 1). To separate electromigration and electroosmotic contributions to the iontophoretic delivery of lidocaine, ¹⁴C-labeled mannitol was incorporated into the experimental design as a marker of the direction and magnitude of convective flow. It was important to compare lidocaine to the more lipophilic cations because such species (e.g., certain luteinising hormone releasing hormone (LHRH) analogs, other peptides, and even propranolol itself) have the ability to alter the permselectivity properties of the skin when iontophored (10–15). The apparently tight association of these hydrophobic cations with the membrane neutralizes to various extents the intrinsic negative charge of the skin leading to a significant reduction in the normal anode-to-cathode electroosmotic flow across the barrier. It has been suggested that the close proximity of a hydrophobic surface (which acts as an “anchor”) to the positive charge on the cation is the structural motif necessary for the phenomenon to be observed (13). Here, we have chosen both propranolol and quinine to investigate whether the observed inhibition of electroosmosis, and the putative mechanism, requires a flexible linkage between the lipophilic group and the positive charge (quinine’s structure being significantly more rigid than that of the β -blocker).

MATERIALS AND METHODS

Materials

[¹⁴C]-Mannitol (specific activity 56.0 mCi/mmol) was obtained from Amersham Pharmacia Biotech (Orsay, France). Lidocaine, quinine, and propranolol hydrochlorides, D-mannitol, HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid), and sodium chloride were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). De-ionized water (resistivity > 18 Mohm/cm²) was used to prepare all solutions.

Skin Preparation

Porcine ears were obtained fresh from the local abattoir and were cleaned under cold running water. The whole skin

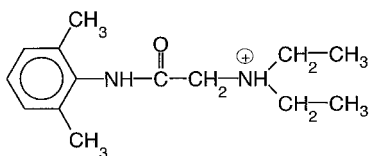
¹ Centre interuniversitaire de recherche et d’enseignement, Universities of Geneva and Lyon, “Pharmapeptides,” F-74166 Archamps, France.

² University of Geneva, Faculté des Sciences, Section de Pharmacie, 30, quai E. Ansermet CH-1211 Geneva 4, Switzerland.

³ To whom correspondence should be addressed. (e-mail: richard.guy@pharm.unige.ch)

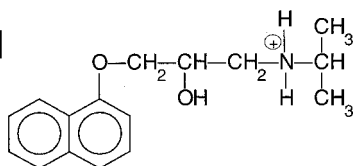
Lidocaine

MW 234
log P 2.26
pK_a 7.9



Propranolol

MW 259
log P 3.21
pK_a 9.2



Quinine

MW 324
log P 3.44
pK_a 8.8

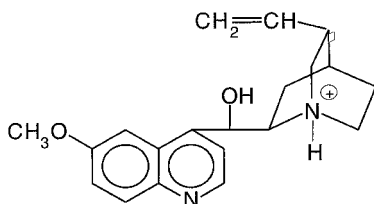


Fig. 1. Comparison of the structures, molecular weights, and lipophilicities (log(octanol-water partition coefficient)) of the unionized drug) of lidocaine, quinine, and propranolol.

was removed carefully from the outer region of the ear and separated from the underlying cartilage with a scalpel. The tissue was then dermatomed (600 μm) and cut into small squares ($\sim 9 \text{ cm}^2$), which were wrapped individually in Parafilm™ and maintained at -20°C before use for no longer than a period of 2 weeks.

Iontophoresis

The *in vitro* experimental methodology has been described previously (16,17). Both side-by-side and vertical flow-through diffusion cells were used in this study, as detailed below. In all experiments, a constant current (0.5 mA/cm²) was applied for 6 h (unless otherwise stated) via Ag/AgCl electrodes connected to a custom-made power supply (Professional Design and Development Services, Berkeley, CA). With the current held constant, the resulting voltage per iontophoresis cell over the course of the experiment fell in the range 1–5 V. All measurements were made in at least quadruplicate, using skin samples originating from no less than two different pigs.

The anodal iontophoresis of lidocaine, quinine and propranolol as a function of concentration was studied under the following two experimental situations.

Case 1: NaCl Present in the Donor Chamber

The skin (transport area = 0.78 cm²) was clamped in vertical iontophoretic diffusion cells (16), with the epidermal side facing the two electrode chambers (Fig. 2A). The lower (receptor) compartment contained 6 ml of 25 mM HEPES-buffered (pH 7.4) normal saline and was magnetically stirred and perfused at a flow rate of $\sim 4 \text{ mL/h}$. Both anodal and cathodal chambers initially were filled with 1 mL of the same electrolyte used in the receptor. After a 2-h equilibration period, the solution in the anodal chamber was replaced with either 1, 10, 40, or 100 mM lidocaine hydrochloride (LidHCl), or 0.1, 1, 4, 10, or 40 mM quinine hydrochloride, or 0.4, 1, 4,

2A. Vertical cell. Iontophoretic flux of drug (buffered donor)

(a) Anode (donor)

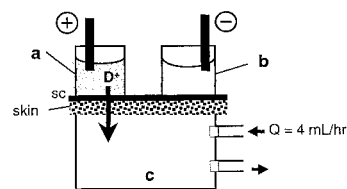
1–100 mM D⁺Cl⁻
133 mM NaCl
25 mM HEPES

(b) Cathode

133 mM NaCl
25 mM HEPES

(c) Receptor

133 mM NaCl
25 mM HEPES



2B. Vertical cell 'inverted'. Iontophoretic flux of drug (NaCl-free donor)

(a) Anode

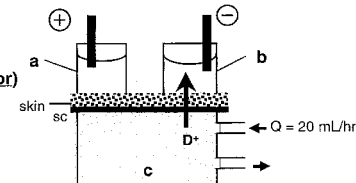
50 mM D⁺Cl⁻

(b) Cathode (receptor)

133 mM NaCl
25 mM HEPES

(c) Donor

1–100 mM D⁺Cl⁻



2C. Side-by-side cell. Effect of drug on mannitol electroosmosis in both 'anodal' (i) and 'cathodal' (ii) directions.

(a) Anode (donor)

40–100 mM D⁺Cl⁻

(b) Cathode (receptor)

133 mM NaCl
25 mM HEPES

(i) or (ii) 1 mM mannitol
(0.8 $\mu\text{Ci/mL}$ ¹⁴C-mannitol)

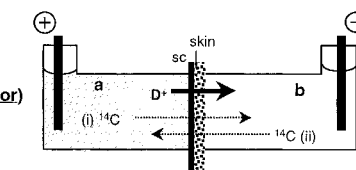


Fig. 2. Configurations of the different experimental configurations used in this work, with the corresponding compositions of the electrode and receptor solutions. D⁺ is the lidocaine cation, Q is the flow rate provided by the peristaltic pump, and SC indicates the stratum corneum side of the skin.

10, 40, 70, or 100 mM propranolol hydrochloride (PrHCl), dissolved in the same background electrolyte. The donor solution pH was adjusted to 7.0 (rather than 7.4) for lidocaine to ensure $\sim 90\%$ ionization (+1) (pK_a = 7.9). The current was then applied and samples were collected from the receiver compartment on an automatic fraction collector (Retriever III, Isco Inc., Lincoln, Nebraska). In separate experiments, the effect of drug concentration on mannitol electroosmosis was monitored. 1 mM mannitol (spiked with $\sim 0.8 \mu\text{Ci/mL}$ of the labeled ¹⁴C compound) was added to the anodal solution, which was either 25 mM HEPES-buffered (pH 7.0) normal saline or the same buffer containing various concentrations (see above) of the three drugs.

Case 2: NaCl Absent from the Donor Chamber

In the second series of experiments, drug iontophoresis was followed from aqueous, non-buffered, NaCl-free solutions of either LidHCl, quinine hydrochloride, or propranolol hydrochloride; the pH of these donor phases was between 5 and 6.5. The goal was clearly to avoid competition between the drug cation and the significantly more mobile Na⁺ ion to transport charge across the skin. The experimental set-up was modified to avoid the electrochemical depletion of Cl⁻ in the anodal chamber (Fig. 2B). The lower, perfused, stirred chamber of the vertical diffusion cell in this case acted as the donor, and the flow rate was set to 20 mL/h to maintain the "driving" drug concentration constant (and this was verified by assaying

the drug concentration in the donor at the end of each experiment). The membrane orientation was, of necessity, reversed in these experiments, with the epidermal side facing the lower (donor) compartment and the dermal side facing the two electrode chambers (Fig. 2B). Drug electrotransport was monitored by collecting samples manually from the cathodal chamber every hour. It should be noted that the anode compartment in these experiments contained 50 mM drug hydrochloride solution. The rationale of this choice was as follows: First, the concentration of Cl^- ions provided was high enough to ensure the necessary Ag/AgCl anodal electrochemistry for the duration of iontophoresis. Second, the use of NaCl was avoided so that Na^+ was not delivered into the donor solution, where it could compete with drug to carry charge back across the skin into the cathodal chamber. Third, because of the high flow-rate in the lower chamber, the transport of drug from the anode was not able to significantly alter the "driving concentration" (the high perfusion rate ensuring rapid dilution). Note that it was found in these experiments that steady-state fluxes had been achieved within 3 h, and the flux data are therefore reported at this time.

Once again, the effect of drug concentration on mannitol electrotransport was investigated. Side-by-side diffusion cells were used for this purpose, which allowed mannitol flux to be monitored in both the anode-to-cathode and the cathode-to-anode directions (Fig. 2C). Skin samples were clamped between the two halves of the diffusion cell (chamber volume = 3 mL; transport area = 0.78 cm^2), with the epidermal surface always facing the anodal side. The anodal solution contained either NaCl (which was used as control) or one of the three drugs, at either 40 or 100 mM. The cathodal solution was 25 mM HEPES-buffered (pH 7.4) in normal saline in all cases. 1 mM mannitol (spiked with $\sim 0.8 \mu\text{Ci/mL}$ of the labeled ^{14}C compound) was added, in two separate sets of experiments, to either the anodal or the cathodal solution to elucidate the effect of replacing Na^+ with drug ions on electroosmosis in both anode-to-cathode ("anodal" flux) and cathode-to-anode ("cathodal" flux) directions. The complete receiver solution (either cathodal or anodal, respectively) was removed every hour and replaced with the corresponding fresh electrolyte.

Assay

Lidocaine and propranolol were assayed by high-performance liquid chromatography. The mobile phase contained acetonitrile and an aqueous phase comprising 2 mL/L orthophosphoric acid and 1 mL/L triethylamine at pH 3. The ratio of organic to aqueous phases was 15:85 for lidocaine and 25:75 for propranolol. The flow rate was 1 mL/min. The column used was a Nucleosil 100-5 C18 AB (Macherey-Nagel, Hoerd, France); lidocaine and propranolol were detected via their UV absorbances at 220 and 294 nm, respectively. Quinine was quantified by fluorescence spectroscopy.

With respect to the radiolabeled mannitol experiments, the samples were mixed with 5 mL of scintillation cocktail (Ultima Gold XR, Packard Instruments SA, Rungis, France) and then analyzed by liquid scintillation counting (LS 6500, Beckman Instruments France SA, Gagny, France).

Statistical Analysis

All experiments were performed in at least quadruplicate. ANOVA followed by Bonferroni analysis was used to compare multiple data sets. When two sets of data were compared, Student *t* tests were performed. The level of statistical significance was fixed at $P < 0.05$, unless otherwise stated.

RESULTS

Iontophoretic Delivery of Lidocaine, Quinine, and Propranolol

The iontophoretic fluxes of lidocaine, quinine, and propranolol, as a function of drug donor concentration, in the presence and in the absence of background electrolyte, are compared in Figure 3. The individual data for the three cations are summarized in Table I. At 1 mM drug donor concentration, in the presence of background electrolyte, the iontophoretic fluxes of the three drugs were not significantly different from one another. However, in contrast to lidocaine, the overall concentration dependence displayed by quinine

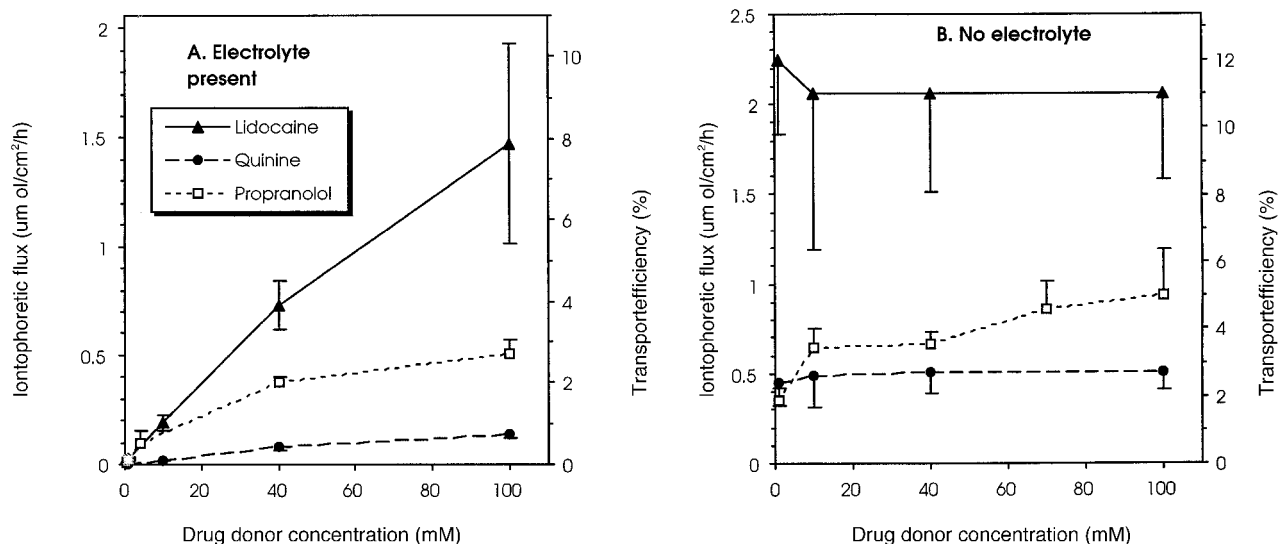


Fig. 3. Iontophoretic flux and transport efficiency (mean \pm standard deviation; $n \geq 4$) of lidocaine, quinine and propranolol as a function of drug donor concentration, in the presence (A) and absence (B) of background electrolyte.

Table I. Iontophoretic Delivery of Quinine and Propranolol and the Corresponding Transport Efficiencies in the Presence and Absence of Background Electrolyte. Data Shown are the Mean \pm SD from 4 to 10 Replicate Experiments

Drug	Concentration (mM)	Electrolyte present		No electrolyte	
		Flux \pm SD (nmol/cm ² /h ⁻¹)	Transport efficiency (%)	Flux \pm SD (nmol/cm ² /h ⁻¹)	Transport efficiency (%)
Lidocaine	1	23.1 \pm 3.2	0.13 \pm 0.02	2210 \pm 400	12 \pm 22
	10	190 \pm 38	1.0 \pm 0.2	1950 \pm 820	11 \pm 4.6
	40	730 \pm 114	4.0 \pm 0.6	1990 \pm 530	11 \pm 2.9
	100	1470 \pm 460	8.1 \pm 2.5	2050 \pm 470	11 \pm 2.5
Quinine	0.1	1.83 \pm 0.16	0.011 \pm 0.001	ND ^a	—
	1	18.8 \pm 4.9	0.11 \pm 0.03	448 \pm 127	2.39 \pm 0.68
	4	77.2 \pm 14	0.43 \pm 0.07	ND	—
	10	135 \pm 18	0.75 \pm 0.11	488 \pm 172	2.61 \pm 0.92
	40	271 \pm 81	1.44 \pm 0.43	510 \pm 122	2.72 \pm 0.65
Propranolol	100	ND	—	505 \pm 98	2.70 \pm 0.52
	0.4	10.1 \pm 1.8	0.05 \pm 0.01	ND	—
	1	26.4 \pm 2.7	0.14 \pm 0.01	347 \pm 71	1.85 \pm 0.38
	4	98.0 \pm 58	0.52 \pm 0.31	ND	—
	10	165 \pm 32	0.88 \pm 0.17	640 \pm 110	3.42 \pm 0.59
	40	374 \pm 68	2.00 \pm 0.36	667 \pm 62	3.56 \pm 0.33
	70	ND	—	858 \pm 155	4.58 \pm 0.83
	100	506 \pm 57	2.70 \pm 0.30	933 \pm 260	4.98 \pm 1.39

^a ND: not determined.

and propranolol was clearly non-linear. The iontophoretic fluxes of the two more lipophilic drugs increased only by a factor of \sim 14 with a 40-fold increase in donor concentration (1–40 mM); and, for propranolol, a further increase in concentration to 100 mM resulted in only an additional 1.3-fold increment in flux (Fig. 3A).

In the absence of background electrolyte, the iontophoretic fluxes of lidocaine and quinine were independent of concentration over the range 1–100 mM (Fig. 3B). For propranolol, a small, not quite 3-fold, increase in flux was observed over the same 1–100 mM increase in donor concentration (i.e., once again, a very low sensitivity to the anodal concentration); nevertheless, the differences between fluxes at certain concentrations did sometimes achieve statistical significance. Overall, these results contrast dramatically with the behaviour observed when NaCl was present in the donor solution.

Mannitol Transport

The effect of drug concentration, in the presence of background electrolyte, on electroosmotic flow in the “normal” anode-to-cathode direction is summarized in Table II. In the presence of electrolyte, mannitol transport was determined in the vertical cell configuration (Fig. 2A). For lidocaine, analysis of variance on the data in the anode-to-cathode direction revealed that only at the highest drug concentration used (100 mM) was electroosmosis significantly reduced. Anodal fluxes of mannitol when either quinine or propranolol were iontophoresed across the skin in the presence of background electrolyte revealed that neither 1 mM quinine nor 4 mM propranolol had a significant effect on mannitol transport compared to the no-drug control. However, when increasingly higher levels of both drugs were introduced into the anodal chamber, a concentration-dependent attenuation of mannitol electrotransport was observed (Table II).

In the absence of NaCl, mannitol transport was mea-

sured in side-by-side diffusion cells (Fig. 2C), which allowed electroosmosis in both anode-to-cathode and cathode-to-anode directions to be evaluated (Fig. 4). As controls, mannitol flux was first determined (in both directions) when the anode solution was either 40 or 100 mM NaCl in water ([drug] = 0). As expected, electroosmosis from anode-to-cathode was dominant, and transport from anode-to-cathode (and in the opposite sense) was not sensitive to NaCl concentration in the anode (data not shown). When the anode chamber contained 40 mM LidHCl and, in particular, 100 mM LidHCl, the anode-to-cathode electroosmosis was significantly attenuated; cathode-to-anode transport was not changed. More remarkable still, however, was the observation, when the

Table II. Inhibiting Effect of Drug Donor Concentration on Mannitol Electrotransport (in the Anode-to-Cathode Direction) in the Presence of Background Electrolyte.

Drug	Concentration (mM)	Mannitol flux ^a (mean \pm SD)	Inhibitor factor ^b
Control	0	2.14 \pm 0.58	1
Lidocaine	1	2.43 \pm 0.78	0.9
	10	2.35 \pm 0.36	0.9
	40	2.64 \pm 1.05	0.8
	100	0.89 \pm 0.17 ^c	2.4
Quinine	1	2.55 \pm 1.09	0.8
	10	0.95 \pm 0.24 ^c	2.3
	40	0.41 \pm 0.30 ^c	5.2
Propranolol	4	2.65 \pm 1.53	0.8
	10	0.88 \pm 0.29 ^c	2.4
	40	0.35 \pm 0.13 ^c	6.1
	100	0.21 \pm 0.11 ^c	10.2

^a Steady-state values after 6 hours iontophoresis. Experiments were performed in at least quadruplicate (and, typically, 5 to 7 times).

^b Inhibition factor = mannitol flux in the absence of drug (control) divided by mannitol flux in the presence of drug.

^c Value significantly smaller ($p < 0.05$) than the control flux.

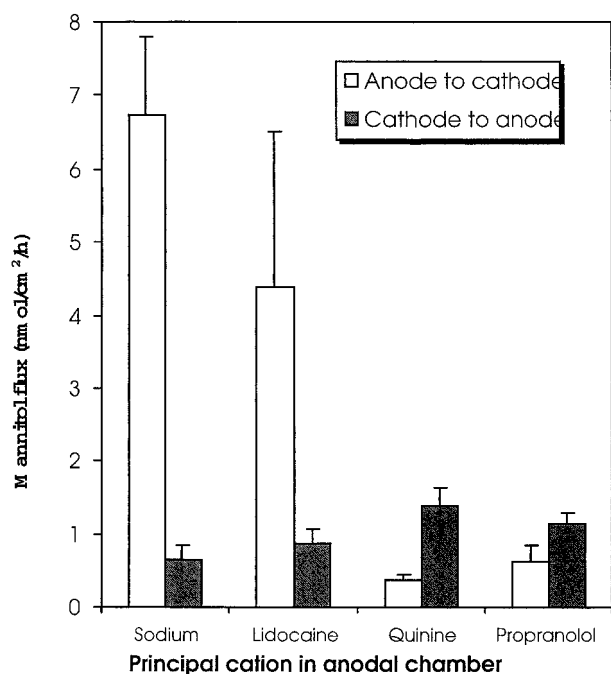


Fig. 4. Mannitol electrotransport in both anode-to-cathode and cathode-to-anode directions as a function of the principal cationic charge carrier (present at 40 mM) in the anodal chamber in the absence of background electrolyte. The steady-state flux values (mean \pm standard deviation; $n \geq 4$) after 6 h, iontophoresis was presented. For sodium and lidocaine, electroosmosis is significantly greater ($P < 0.01$) in the anode-to-cathode direction; for quinine and propranolol ($P < 0.05$), the opposite is true.

principal anodal cation was quinine or propranolol, that electroosmosis in the cathode-to-anode direction became significantly greater than that in the opposite sense.

DISCUSSION

Effect of the Presence/Absence of Background Electrolyte

In the absence of background electrolyte the iontophoretic fluxes of the three drugs are essentially independent of donor concentration over a 100-fold range (Table I). Lidocaine, the smaller and least lipophilic cation, however, is transported at a significantly higher flux (and efficiency) than either quinine or propranolol. With the experimental configuration used, the drug ions are the only cationic species in the conductive medium between the skin and the anode. At this electrode, therefore, the drug ions can be assumed to be the only cationic charge carriers transferring current across the skin, the remaining electric charge presumably being transported by counterions (i.e., Cl^- principally) in the opposite sense. Kasting and Keister (18) theoretically analyzed an equivalent experimental situation to that described here and predicted that the efficiency of drug delivery (i.e., the fraction of the iontophoretic current transported by the drug ions), in the absence of competing species, would be determined by the ratio of drug diffusivity in the skin to that of the predominant counter-ion (i.e., Cl^-) on the opposite side of the membrane. As the skin diffusivities of drug and Cl^- can be expected, certainly to a first approximation, to be independent of their respective concentrations, the efficiency of drug de-

livery is thus predicted to be unaffected by the drug donor concentration. Our results fully support this analysis, with the transport efficiencies of lidocaine, quinine and propranolol remaining constant (at $\sim 11\%$, $\sim 2.6\%$ and $\sim 4\%$, respectively) despite a 100-fold change in drug concentration (Table I).

The implied deduction that $\sim 90\%$ of the current flowing beneath the anode is transferred by anionic charge carriers (predominantly Cl^-) migrating in the cathode-to-anode direction is remarkable, particularly in light of the facts that: (a) the skin is intrinsically negatively charged and therefore cation-permeable, and (b) electroosmosis (as measured by mannitol flux) is 5 to 7 times higher in the anode-to-cathode direction than that in the opposite sense. Specifically, when the lidocaine hydrochloride concentration was 40 mM, the anode-to-cathode and cathode-to-anode fluxes of mannitol were $4.38 (\pm 2.14)$ and $0.64 (\pm 0.19)$ nmol/cm²/h, respectively; with the drug driving concentration at 100 mM, the corresponding values were $2.14 (\pm 0.53)$ and $0.42 (\pm 0.28)$ nmol/cm²/h. Hence, even though the principal charge carrier has the same charge as the membrane, the fact that the skin remains cation-selective means that electroosmotic flow continues to be from anode-to-cathode; that is, it is the charge on the membrane that determines the direction of convective solvent flow, not the polarity of the principal charge carrier. Confirmation of these conclusions, and quantification of Cl^- transport, is addressed in detail in the accompanying article (19).

In the presence of electrolyte in the anode, the efficiency of drug delivery is strongly dependent on concentration (Table I and Fig. 3). Now, as well as Cl^- moving from the receptor side, the drug has to compete with Na^+ on the donor side to carry charge across the skin. At low drug concentrations (up to 10 mM), Na^+ is present at much higher levels and, being a smaller and mobile ion, it carries a significant fraction of the charge; under these circumstances, the iontophoretic fluxes of the three drugs are not significantly different. However, at 40 mM and above, the electrotransport of quinine and propranolol is much less than that of lidocaine. Only for this most polar drug, as its concentration is increased to higher values, can its transport efficiency arrive at the level achieved in the absence of electrolyte and, as predicted in such cases (18), its delivery is proportional to concentration.

Electromigration and Electroosmosis Contributions to Iontophoresis

Mannitol is a neutral, hydrophilic molecule typically used as a marker for electroosmosis in iontophoretic studies (1,3,6). Passive diffusion of mannitol across the skin is negligible and its transport by iontophoresis may be attributed exclusively to electroosmosis (1). During iontophoresis, the velocity (V_w) of the current-induced water flow across the skin can be estimated from (8):

$$V_w = J_{\text{mannitol}} / C_{\text{mannitol}} \quad (1)$$

where J_{mannitol} is the mannitol flux and C_{mannitol} is the mannitol concentration in the donor phase. It follows that, if V_w has been quantified in the presence of drug, then the convective component of its iontophoretic transport (J_{EO}) can be calculated multiplying V_w by the concentration of drug in the donor solution (C_{drug}):

$$J_{EO} = V_w \times C_{\text{drug}} \quad (2)$$

This analysis implies two assumptions: (a) that drug and mannitol are transported in a similar fashion by convective solvent flow, and (b) that electroosmotic transport of the marker molecule is proportional to its concentration in the solvent. The first assumption is reasonable in that the drug cations and mannitol are both polar and of reasonably similar molecular size (differing by less than a factor of two in molecular weight). The second assumption was specifically tested in a separate series of experiments, which showed that electroosmotic transport was linear ($r^2 = 0.99$) with concentration over the range 1–100 mM. Specifically, mannitol electrotransport ($\text{nmol}/\text{cm}^2/\text{h}$), with six or seven measurements per concentration, was 2.14 ± 0.58 ; 159 ± 15 ; and 346 ± 21 with donor concentrations of 1, 50, and 100 mM, respectively.

It was then possible to estimate the relative contributions of electroosmosis and electromigration to the total iontophoretic flux of the three drugs, as a function of their donor concentrations, both in the presence and absence of background electrolyte (Table III). The “electroosmotic fluxes” are displayed graphically in Figure 5. The electromigration component was simply deduced from the difference between the total flux and the flux due to electroosmosis.

In the presence of background electrolyte, the calculated “electroosmotic flux” of lidocaine increased proportionally with concentration up to 40 mM. However, when the LidHCl concentration was increased to 100 mM, a significant reduction in water flow in the anode-to-cathode direction was observed, with the concomitant decrease in electroosmosis contribution to the total iontophoretic flux (from ~14% to ~6%) (Table III). On the other hand, the electroosmotic fluxes of quinine and propranolol increased with increasing concentration (as predicted by theory) but in a manner, which was far from linear (Fig. 5). The percentage contribution from electroosmosis, in fact, decreased with increasing concentration. Furthermore, at 40 mM, quinine and propranolol induced a 5-

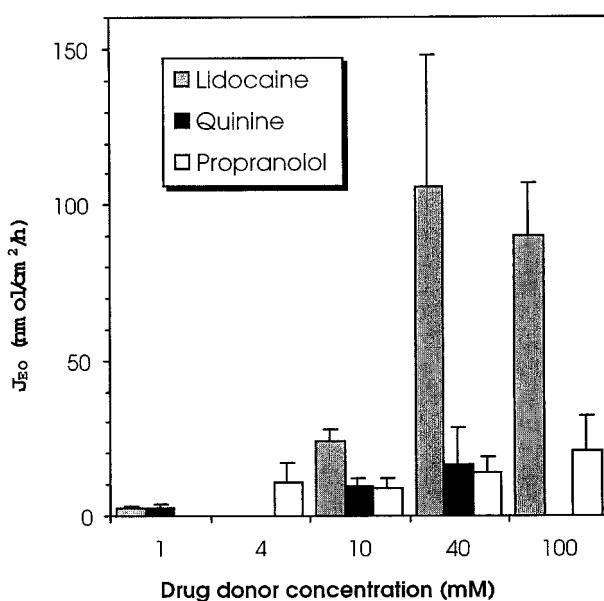


Fig. 5. Comparison of the deduced electroosmotic fluxes of lidocaine, quinine, and propranolol, as a function of anodal donor concentration, in the presence of background electrolyte.

to 6-fold inhibition of the normal level of electroosmosis and, at this concentration and above, effectively caused the direction of convective solvent flow across the skin to be reversed (see Fig. 4). This observation, of course, has been made before for propranolol (10), for the LHRH analogues, leuprolide and nafarelin (11,12,15), and for other lipophilic, cationic peptides (13,14).

The current hypothesis to explain this behavior is that the close juxtaposition of a lipophilic surface to the centre of positive charge in these lipophilic cations provides a structural

Table III. Electromigration and Electroosmotic Contributions (J_{EM} and J_{EO} , Respectively) to the Iontophoretic Flux of Lidocaine, Quinine, and Propranolol across the Skin, and the Relative Contributions of EM and EO to the Drug's Total Electrotransport, as a Function of Drug Donor Concentration and the Presence/Absence of Background Electrolyte

Drug	Concentration (mM)	Donor formulation	J_{EM}^a (nmol/cm ² /h ⁻¹)	J_{EO}^b (nmol/cm ² /h ⁻¹)	EM (%)	EO (%)
Lidocaine	1	HEPES-buffered normal saline	20.7 ± 3.2	2.4 ± 0.8	89	11
	10		166 ± 38	24 ± 3.6	87	13
	40		624 ± 114	106 ± 42	86	14
	100	Water	1380 ± 460	90 ± 17	94	6
	40		1815 ± 530	175 ± 86	91	9
	100		1836 ± 470	214 ± 53	90	10
Quinine	1	HEPES-buffered normal saline	16.3 ± 4.9	2.6 ± 1.1	86	14
	10		126 ± 18	9.5 ± 2.4	93	7
	40		255 ± 81	16 ± 12	94	6
	40	Water	496 ± 122	14 ± 3.6	97	3
	100		471 ± 98	34 ± 21	93	7
	100		1836 ± 470	214 ± 53	90	10
Propranolol	4	HEPES-buffered normal saline	87 ± 58	11 ± 6.1	89	11
	10		156 ± 32	8.8 ± 2.9	95	5
	40		360 ± 68	14 ± 5.2	96	4
	100	Water	485 ± 57	21 ± 11	96	4
	40		643 ± 62	24 ± 10	97	3
	100		896 ± 260	37 ± 23	96	4

^a J_{EM} = total flux (see Table I) - J_{EO} .

^b J_{EO} is the product of convective water flow (deduced from the mannitol experiments—see data in Table II) and the corresponding drug concentration.

motif optimal for strongly associating these species with negative charges in the skin. This leads to neutralization of the membrane (or, ultimately, a reversal of its charge), loss of its cation permselectivity and eventual attenuation and/or reversal of the normal direction of electroosmotic flow. That lidocaine (at 100 mM) would elicit the same effect is a little surprising as it is less lipophilic [$\log P$ of the unionized form being 2.26 (20)]. Also, no compensatory increase in electroosmosis from cathode-to-anode was detected, as is the case for the lipophilic cations mentioned above, and would be expected mechanistically. Perhaps, because lidocaine is delivered so well by iontophoresis, it is simply a question of there being, at any time, a considerable amount of the drug in the skin and that this is enough to inhibit the normal electroosmotic flow. It should also be said that the solution of 100 mM LidHCl in HEPES-buffered normal saline has a very high ionic strength, something that has been seen before (21) to elicit lower-than-normal convective flow from the anode.

It should also be mentioned that the data in Table II reveal that the electroosmotic flow-inhibiting abilities of quinine and propranolol are not statistically different at any concentration tested. The question of the effect of the relative flexibility of the linkage between hydrophobic surface and positive charge cannot be answered, therefore, by these experiments.

In the absence of background electrolyte, a similar 2-fold reduction in water flow is also observed when LidHCl concentration is raised from 40 to 100 mM. As a consequence, no statistical difference (Student's *t* test, $P < 0.05$) is found between the corresponding calculated "electroosmotic fluxes" of the drug at these concentrations (Fig. 4). However, in this case, in contrast to the with-electrolyte situation, the relative contributions of electroosmosis and electromigration remain constant (~10% electroosmosis), presumably because the transport efficiency (and thus the electrorepulsive component of transport) is independent of concentration. In general, electromigration accounts for about 90% of the measured iontophoretic flux of lidocaine, and it is clear that an efficient formulation would strive to avoid the presence of competing species, to maximize the efficiency of drug delivery. The data also show that, under these circumstances, because of the independence of flux upon drug driving concentration, that optimal delivery can be achieved using something less than maximal drug loading in the anode compartment. The contribution of electroosmosis to the total iontophoretic transport of quinine and propranolol, at 40 or 100 mM, was low (on average, about 5% or less). It follows (and this was also true when electrolyte is present) that the principal mechanism of iontophoretic transport of these lipophilic cations, just like lidocaine, is electromigration. Likewise, one is drawn to the conclusion, in the electrolyte-free experiments, that the principal charge carrier at the anode is, in fact, Cl^- moving from the receptor out across the skin. Presumably, the neutralization, and eventual charge reversal, caused by association of the quinine and propranolol cations with the skin would favor this important role for Cl^- .

In conclusion, the results of this work reveal how the presence of background electrolyte can impact iontophoretic drug delivery. Ions, for which electromigration is the principal mechanism (such as those examined here), are best served by minimizing as far as possible the presence of competing species; and, as just mentioned, it may not be necessary to use the

highest drug concentration possible in order to achieve effective delivery. On the other hand, when the principal contribution to electrotransport comes from the electroosmotic flow [as is the case for larger peptides, for example (2,17)], the presence or absence of background electrolyte is less important and drug delivery, in general, improves as the driving concentration is increased (Eq. 2). These deductions provoke a number of questions, which should now be addressed experimentally. First of all, for example, what will happen as the size of the cationic drug increases? If it is lipophilic and, because of its greater bulk, it carries less charge across the skin, does this mean that its delivery will be significantly (self)-impeded, given that electroosmosis will play a more important role? Second, is the conclusion that Cl^- can assume such a central role in carrying charge across the (usually negatively-charged) skin correct? Is it possible to verify this inference *in vitro* and/or *in vivo*? And, if this is indeed the case, can one use the measured transport efficiency of Cl^- to deduce the efficiency of electrotransport of the drug itself? Responses to these questions represent the focus of our ongoing work.

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