Optimizing Iontophoretic Drug Delivery: Identification and Distribution of the Charge-Carrying Species

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Purpose. To identify and quantify, *in vitro* and *in vivo* (in humans), the charge-carrying species during transdermal iontophoresis of lidocaine hydrochloride as a function of the concentration of drug relative to that of sodium chloride in the anodal solution.

Methods. *In vitro* experiments in standard diffusion cells quantified lidocaine delivery and the outward migration of chloride across the skin. Electrotransport of Na^+ was inferred by difference, allowing transport numbers of the three main charge-carrying species to be deduced. *In vivo,* outward electrotransport of Cl^- was measured and compared to the corresponding *in vitro* results.

Results. The transport number of lidocaine increased linearly with increasing mole fraction and reached 0.15–0.20 at $X_L = 1.0$. In the absence of Na^+, most of the charge was carried by Cl^- (>80%) despite the skin retaining its net negative charge and cation permselectivity. *In vivo* data correlated very well with *in vitro* results.

Conclusions. The mole faction of drug (relative to competing ions of like polarity) is the crucial determinant of the extent to which it can carry charge across the skin during iontophoresis. The outward electromigration of Cl^-, in the sense opposite to drug delivery, may offer a useful means by which to optimize iontophoretic efficiency in the absence of competing cations in the anode formulation.

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

INTRODUCTION

It is well known that iontophoresis can permit the skin permeation of charged drugs to be enhanced dramatically (1). Nevertheless, the fraction of the total charge flowing in the iontophoretic circuit that is carried by the drug itself is typically very small and rarely exceeds 10% (2). This inefficiency sometimes reflects the fact that the drug competes poorly with other electrolyte ions of similar polarity in carrying the charge flowing in from the donor electrode formulation across the skin. For instance, sodium ions are small, highly mobile species that are much more efficient charge carriers than typical drug cations (3). Alternatively, even when (for example) a cationic drug is formulated as simply as possible with no competing positive ions from a buffer or other electrolyte, there remains competition with counterions (particularly the omnipresent chloride) carrying a charge from beneath the skin's barrier into the anodal compartment.

The *in vitro* iontophoretic transport of lidocaine recently was examined as a function of concentration and of background electrolyte (4). The principal mechanism of drug transport was clearly electromigration (>90%), with lidocaine being able to carry up to 10% of the charge flowing across the skin when competing cations (primarily Na^+) were eliminated from the donor formulation. It was inferred that, in this electrolyte-free situation, the balance of charge crossing the membrane had to be carried by counterions (i.e., Cl^-) migrating in the opposite direction, cathode-to-anode.

The objective of this article was to confirm this deduction and to quantify precisely the charge being carried by lidocaine cations, Na^+, and Cl^- ions in these experiments. The study involved such characterization over a range of lidocaine to Na^+ mole fraction ratios, and the primarily *in vitro* work was extended to a selected set of observations in human volunteers *in vivo*. Finally, it is shown, for a cation such as lidocaine, delivered from an electrolyte-free anodal solution, that the outward migration of Cl^- from the skin may be used to deduce, by difference, the transport number of the drug.

MATERIALS AND METHODS

Materials

Lidocaine hydrochloride, HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanosulfonic acid) and sodium chloride were purchased from Sigma Aldrich Co. (Saint Quentin Fallavier, France). De-ionized water (resistivity > 18 Mohm.cm) was used to prepare all solutions.

In Vitro Experiments

Porcine ears were obtained fresh from the local slaughterhouse and were cleaned under cold running water. The whole skin 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 that were wrapped individually in Parafilm™ and maintained at –20 °C for no longer than 2 weeks.

Side-by-side diffusion cells were used in the iontophoresis experiments. The skin was clamped between the two half cells, with the stratum corneum side facing the anodal chamber (0.78 cm^2). Constant current (0.5 mA/cm^2) was applied via Ag/AgCl electrodes connected to a custom-made power supply (Professional Design and Development Services, Berkeley, CA) controlled by Labview™ software (National Instruments Inc., Austin, TX). The cathodal solution (3 mL) was 25 mM HEPES-buffered normal saline at pH 7.4. The anodal chamber contained 1 mL of one of the six donor solutions identified in Table I. Each of these donor solutions

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ABBREVIATIONS: LHCl Lidocaine hydrochloride. L^+, lidocaine cation; Na^+, sodium cation; Cl^-, chloride anion; X_L, mole fraction of lidocaine in anodal chamber; X_{Na}, mole fraction of sodium in anodal chamber; t_L, transport number of lidocaine cations; t_{Na}, transport number of sodium cations; t_{Cl}, transport number of chloride anions; Q_0, Initial Cl^- content (moles) in the anodal solution; Q_S, moles of Cl^- in the sample; Q_J, moles of Cl^- driven across the skin by iontophoresis; Q_E, moles of Cl^- consumed at the Ag/AgCl anode during iontophoresis

Table I. Compositions (Concentration and Mole Fractions (X_i) of LHCl and NaCl) of the Different Anodal Formulations Examined in the *in Vitro* Experiments, Together with the Measured (t_{Cl} and t_L) and Deduced (t_{Na}) Transport Numbers of the Principal Charge Carriers Present

Anodal-phase composition				Transport numbers (mean ± standard deviation)		
LHCl (mM)	NaCl (mM)	X_L	X_{Na}	t_{Cl}[a]	t_L[a]	t_{Na}[b]
30.0	0.0	1.00	0.00	0.86 ± 0.07	0.16 ± 0.01	—
27.0	3.0	0.90	0.10	0.54 ± 0.06	0.19 ± 0.02	0.28
22.5	7.5	0.75	0.25	0.39 ± 0.03	0.14 ± 0.01	0.47
15.0	15.0	0.50	0.50	0.32 ± 0.02	0.07 ± 0.01	0.61
7.5	22.5	0.25	0.75	0.30 ± 0.01	0.05 ± 0.01	0.65
0.0	30.0	0.00	1.00	0.32 ± 0.02	—	0.68

[a] Determined experimentally (see text).
[b] Deduced from the experimental data, $t_{Na} = 1 - (t_L + t_{Cl})$.

contained a fixed concentration (30 mM) of electrolyte, and hence the same ionic strength; the relative amounts, expressed as mole fractions, of lidocaine cations (X_L) and Na^+ (X_{Na}) were varied systematically over the range 0 to 1.

After a 1-h equilibration period, the current was passed for 2 h. The complete anodal and cathodal phases were withdrawn every 30 min and refilled with the corresponding fresh solutions. The cathodal samples were assayed for lidocaine content by high-performance liquid chromatography. Chloride was determined in the anodal samples by mercurimetric titration. At least three replicates of each experiment were performed.

In Vivo Experiments

These experiments were approved by the University of Geneva, Département des Neurosciences cliniques et Dermatologie, Commission d'Ethique and were performed in four normal, healthy volunteers (one male and three females aged from 25 to 30 years), from which informed consent had been obtained.

A cylindrical glass chamber (area = 0.78 cm^2) was fixed to the ventral forearm surface using double-sided adhesive tape and served as the anode compartment. After a 20-min equilibration period with distilled water, the chamber was charged with 0.5 mL of either 30 mM LHCl in water or with 0.5 mL of a solution containing 7.5 mM LHCl and 22.5 mM NaCl. An Ag/AgCl electrode was carefully positioned in the chamber, avoiding contact with the skin surface, and was connected to the positive output of a commercially available iontophoretic power supply (Phoresor II, Iomed, Salt Lake City, UT). The return (cathode) electrode (Iogel™, Iomed) was connected to the negative pole of the power supply and attached to the skin at a distance of 4 cm from the anode chamber. A constant current of 0.5 mA/cm^2 was applied for 25 min at the end of which the entire contents of the anode compartment were removed and analyzed for Cl^- by mercurimetric titration.

Assay

Lidocaine was assayed by high-performance liquid chromatography using the method described (4). Chloride was assayed by mercurimetric titration (5). Reagents from the Aquamerck™ Chlorid-Test (Merck, Darmstadt, Germany) were used. Two-hundred microliters of sample were placed in an Eppendorf™ tube, and one drop each of diphenylcarbazone indicator solution and of nitric acid was added. Mercury nitrate was then titrated into the sample using a 50-μL microsyringe (Hamilton Inc., Reno, NV) while shaking the tube in a Vortex-Genie 2 (Scientific Industries, Inc., Merck, Argau, Switzerland). The end-point was indicated by formation of a blue-violet complex. One microliter of titration solution corresponded to 33.3 nmol of Cl^-.

THEORY

Figure 1 shows schematically the mass balance of Cl^- in the anodal chamber at the end of each sampling period, both *in vitro* and *in vivo*. The amount of Cl^- in the sample (Q_S) is given by the following equation:

$$Q_S = Q_0 + Q_J - Q_E \quad (1)$$

where Q_0 is the initial Cl^- content in the anodal solution at the beginning of the sampling period, Q_E is the Cl^- consumed

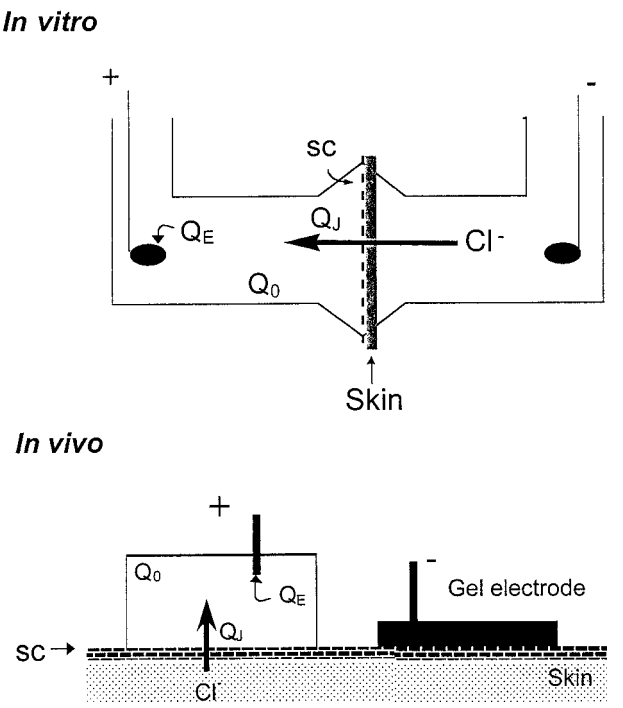

Fig. 1. Schematic diagrams of the *in vitro* and *in vivo* experiments performed illustrating the mass balance of Cl^- ions in the system (see text for details).

electrochemically at the Ag/AgCl anode, and Q_J is the amount of Cl^- driven across the skin by iontophoresis during the sampling period. As Q_S and Q_0 are measured and known quantities, respectively, and Q_E is calculable from the total current passed during the iontophoresis period, it follows that Q_J can be directly determined. Hence, the transport number of Cl^- (t_{Cl}), the fraction of the applied current (Q_E) that is transferred by Cl^- ions (Q_J) across the skin, can be found in the following:

$$t_{Cl} = Q_J/Q_E \quad (2)$$

In the *in vitro* experiments, it is reasonable to suppose that there are three ions responsible for carrying most, if not all, of the charge flowing across the skin, i.e., L^+, Na^+, and Cl^-. The assays performed in the electrode compartments (L^+ in the cathode, Cl^- in the anode) allow direct assessment of t_{Cl} (see above) and t_L (6). Hence, the Na^+ transport number can be deduced by difference:

$$t_{Na} = 1 - (t_L + t_{Cl}) \quad (3)$$

RESULTS AND DISCUSSION

In Vitro Experiments

The measured transport numbers of lidocaine cations (t_L) and chloride anions (t_{Cl}), and those deduced for Na^+ (t_{Na}), as a function of the initial anodal chamber composition are in Table I. The values of t_L increase linearly with the mole fraction of lidocaine in the donor solution (Fig. 2). This relationship is not only true for the data generated in this work at fixed total ionic strength but also holds for the results of our earlier work in which the donor phase contained lidocaine hydrochloride at concentrations between 1 and 100 mM and NaCl at 133 mM (open symbols in Fig. 2). The line of linear regression through the combined data sets has $r^2 = 0.94$.

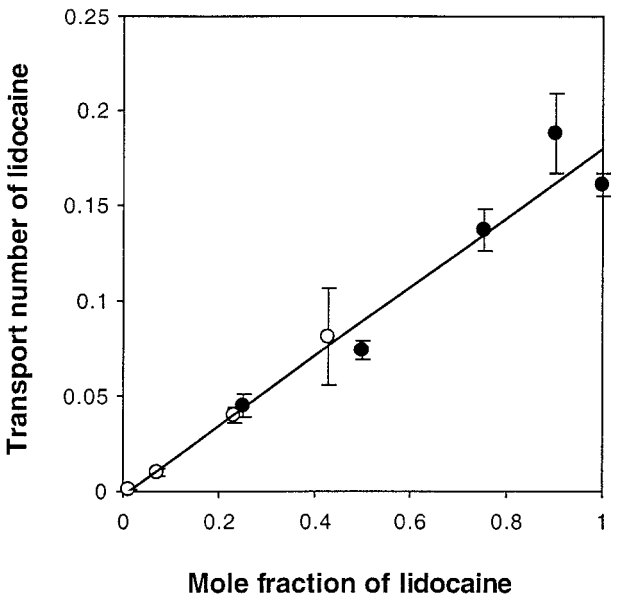

Fig. 2. Transport number of lidocaine as a function of its mole fraction in the anode formulation. The *in vitro* data obtained in this work (•) are compared to those determined previously (○), for which the lidocaine concentration was allowed to vary between 1 and 100 mM, with NaCl always present at 133 mM (4). Each data point is the mean (± standard deviation) of at least three measurements. The line of linear regression through the data has a slope of 0.18 (intercept = –0.002) with $r^2 = 0.94$; ($F_{(1,30)} = 437$; $P < 0.01$).

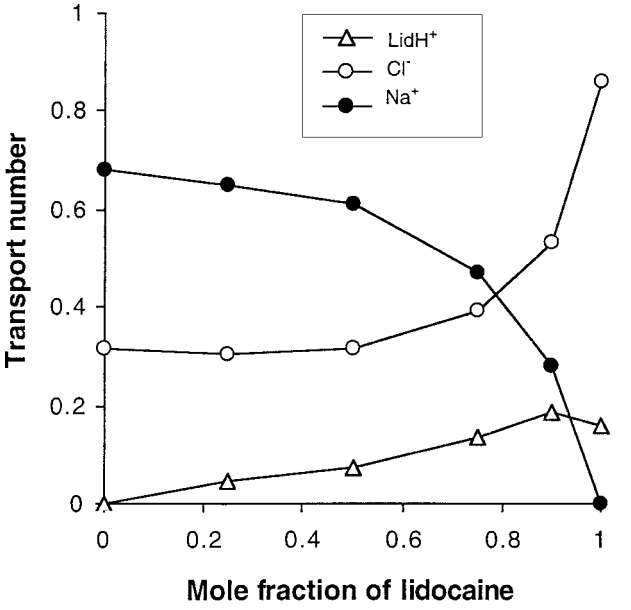

Fig. 3. Transport numbers of lidocaine (△), chloride (○), and sodium (•) ions as a function of the drug's mole fraction in the anode formulation; t_L and t_{Cl} were experimentally measured *in vitro,* whereas t_{Na} was deduced from Equation 3.

Figure 2 emphasizes that the efficiency of drug transport by iontophoresis depends upon its concentration relative to that of the competing species present in the donor (i.e., to the mole fraction of the drug). Hence, we can see, at $X_L = 0.25$, that the value of t_L (0.05) determined in this work ([LHCl] = 7.5 mM, [NaCl] = 22.5 mM) is essentially identical to that

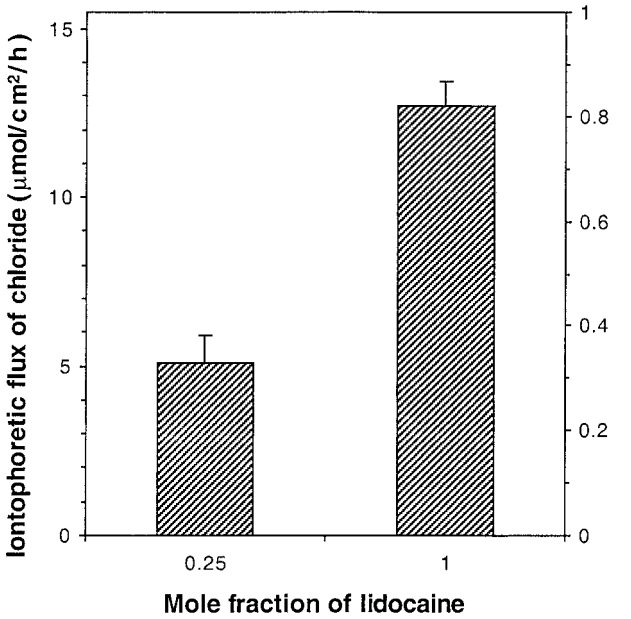

Fig. 4. *In vivo* electromigration of Cl^- across the skin into the anodal chamber and the deduced transport numbers (mean ± standard deviation; n = 4). The anode chamber contained either 7.5 mM LHCl and 22.5 mM NaCl (mole fraction of drug = 0.25) or simply 30 mM LHCl (mole fraction = 1).

reported in a previous experiment (4) for which [LHCl] was 40 mM and [NaCl] was 133 mM (i.e., $X_L = 0.23$). That is, simply increasing the drug's concentration may not improve the efficiency of delivery—what is important is that the amount of drug relative to the "competition" is enhanced. Such a conclusion, of course, is not novel and was articulated several years ago in an excellent review by Phipps and Gyory (6). These results are quite consistent with their deductions.

A further conclusion to be drawn from Figure 2 is that, at $X_L = 1$, a maximum transport number of the drug between 0.15 and 0.20 will be achieved in the absence of background electrolyte, and that this value will be independent of the drug's absolute concentration; that is, the only requirement is that X_L in the donor equals 1.0. This deduction is consistent with experimental observations in the literature, reported for different cations (4,7,8). The maximum transport number of the drug, it is believed, will be determined by its mobility across the skin relative to that of the counterion charge carriers (in particular, Cl^-) migrating in the opposite direction (9).

The measured transport numbers of Cl^- vary from 0.32 (±0.02), when the anode solution was simply 30 mM NaCl [a value consistent with that in the literature for human skin under similar *in vitro* conditions (3)], to 0.86 (± 0.07) when the anode formulation was 30 mM LHCl with no background salt. In the latter case, we first note that the sum of experimentally measured t_{Cl} and t_L values is statistically indistinguishable from unity, implying a negligible participation of other ions in

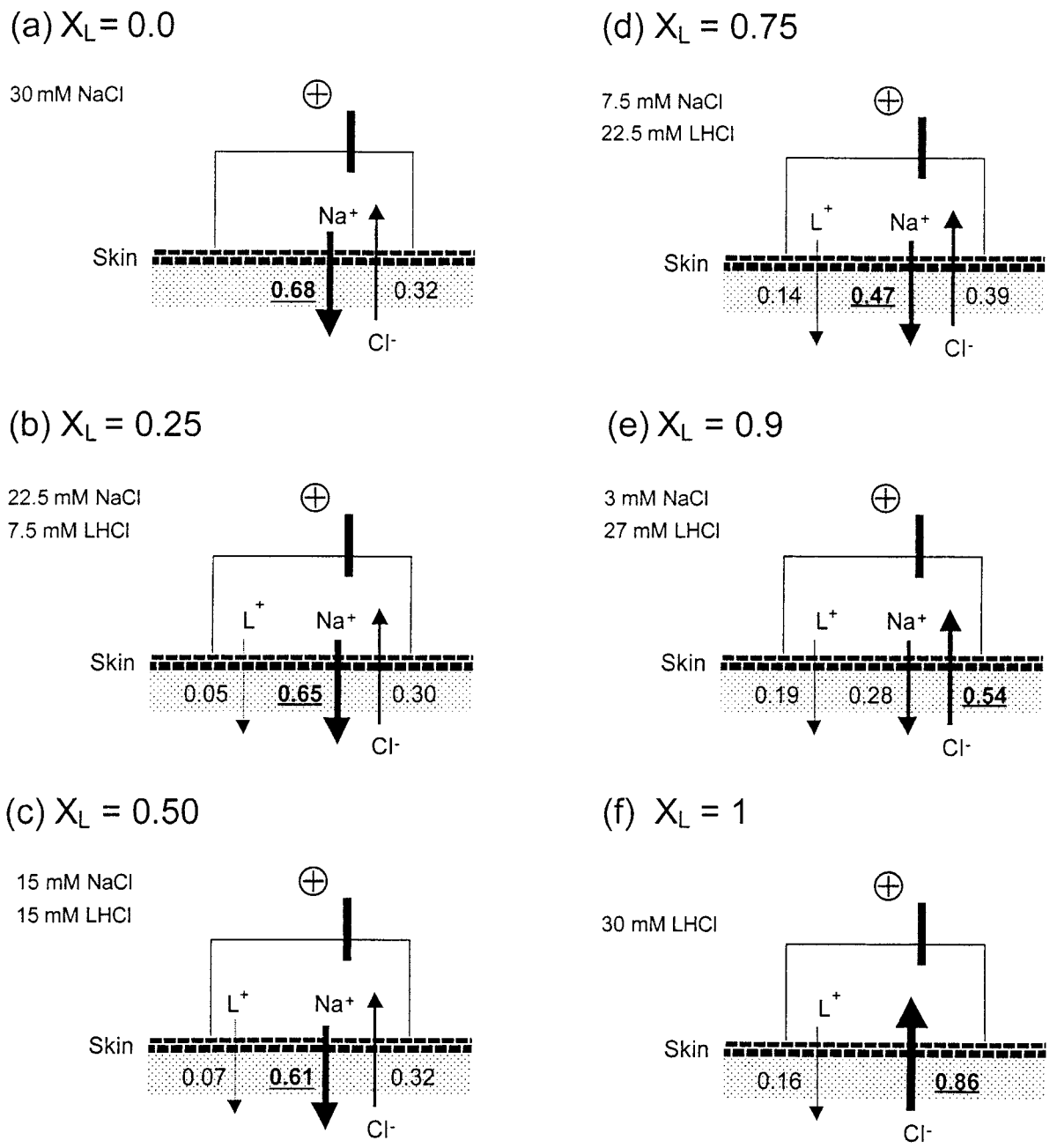

Fig. 5. Contributions of the major charge-carrying species (L^+, Cl^-, Na^+) in lidocaine iontophoresis as a function of the drug's mole fraction in the anode solution.

carrying charge across the skin under these circumstances. This also lends support, when the mole fraction of lidocaine is reduced by adding NaCl, to the attribution of the difference $[1 - (t_L + t_{Cl})]$ to charge transfer by Na^+. We therefore deduce $t_{Na} = 0.68$ when the donor contains only NaCl at 30 mM, again a result not inconsistent with the literature (3).

Figure 3 summarizes the measured (t_L, t_{Cl}) and deduced (t_{Na}) values of the transport numbers from this work as a function of the mole fraction of lidocaine in the donor solution. The striking observation, as t_{Na} diminishes to zero and the mole fraction of lidocaine increases to unity, is that although t_L is increasing linearly, it is Cl^- that assumes the principal charge-carrying role for the system. This is true despite the skin's cationic permselectivity and the fact that it retains a net negative charge over all experimental conditions explored in this work [as shown by our previous measurements of electroosmotic flow during lidocaine iontophoresis as a function of concentration in the presence and absence of background electrolyte (4)]. In other words, the much higher mobility of Cl^- relative to that of the bulkier drug cation permits the former to assume responsibility for transporting more than 80% of the charge flowing across the skin, even though the membrane remains cation-permselective.

In Vivo Experiments

The Cl^- transport numbers measured *in vivo* in human volunteers are presented in Figure 4, for two values of X_L. With only 30 mM LHCl in the anode formulation, t_{Cl} was 0.82 ± 0.05, i.e., not significantly different from the value determined under similar conditions *in vitro* (0.86 ± 0.07). When X_L was reduced to 0.25, t_{Cl} fell to 0.33 ± 0.05 (as Na^+ assumed the more important charge-carrying role), a result again indistinguishable from that obtained *in vitro* (0.30 ± 0.01).

It follows that the *in vitro* experiments performed were reliable predictors of the *in vivo* situation. Although the *in vivo* study performed was not exhaustive, the parallel behavior observed at high and low values of lidocaine mole fraction suggests that the favorable comparison is valid. The result also leads to an interesting hypothesis for the optimization of the anodal formulation of a cationic drug in the absence of competing ions: The simple measurement of Cl^- electrotransport out of the skin can be used to predict the *in vivo* delivery of the drug due to electromigration.

CONCLUSION

Figure 5 summarizes the manner in which the contributions of the charge-carrying species (L^+, Cl^-, Na^+) depend upon the mole fraction of lidocaine cations present in the anode solution. Replacement of Na^+ by L^+ increases the drug's transport number to between 0.15 and 0.20. However, despite the persistent cation permselectivity of the skin in these experiments, Cl^- becomes the predominant charge carrier at high X_L.

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