Electrorepulsion Versus Electroosmosis: Effect of pH on the Iontophoretic Flux of 5-Fluorouracil

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Purpose. To delineate the contributions of electrorepulsion and electroosmosis to the iontophoretic flux of 5-FU across porcine skin *in vitro*. Also, the isoelectric point (pI) of the skin model was determined.
Methods. The electrotransport of 5-FU, anode-to-cathode ("anodal") and cathode-to-anode ("cathodal") was determined as a function of the pH of the electrolyte bathing the skin.
Results. At pH 8.5, the drug (pK_a ~8) is negatively charged and "cathodal", *viz.* electrorepulsive, transport is much greater than that in the opposite direction. At pH 7.4, where ~25% of 5-FU is charged, electrorepulsive and electroosmotic ("anodal") fluxes are balanced. Decreasing the pH to 6, and then 5, reduces the percentage of ionized 5-FU such that "anodal" electroosmosis dominates across the negatively-charged membrane. But, at pH 4, "anodal" and "cathodal" fluxes are again equal suggesting neutralization of the skin (i.e., pI ~ 4). This is confirmed at pH 3, where "cathodal" electroosmosis dominates across the now net-positively charged barrier.
Conclusions. Electrotransport is sensitive, mechanistically, to the properties of the permeant and of the skin; interactions of, for example, the drug or constituents of a formulation, that alter the barrier's net charge, can affect iontophoretic delivery. The pI of porcine ear skin is ~4.

KEY WORDS: percutaneous absorption; 5-fluorouracil; iontophoresis; electroosmosis; isoelectric point.

INTRODUCTION

Under the influence of an iontophoretic current, the total transport (J_t) of a compound across the skin is the sum of at least three distinct contributions:

$$J_t = J_p + J_{er} + J_{eo}$$

where J_p is the passive flux, J_{er} is the electrorepulsive contribution (i.e., cation repelled into the skin from the anode and anion driven into the skin from the cathode), and J_{eo} represents the electroosmotic flux, which originates due to the (usually) net negative charge on the skin, its permselectivity to cations, and the resulting induced solvent flow when the electric field is applied (1,2). It follows, therefore, under normal physiological conditions (pH 7.4), that J_{eo} is a positive contribution to J_t for cations, but is negative for anions. It should also be noted that iontophoresis can cause a significant increase in the transport of neutral, yet poorly permeable substances via the electroosmotic effect (assuming that the substance is placed in the anode chamber) even though, in these circumstances, $J_{er} = 0$ (1–4).

It is a long-term objective of our research to delineate the relative importance of electrorepulsion and electroosmosis to the overall iontophoretic flux of charged molecules across the skin. It is generally accepted that the contribution of J_{eo} becomes greater, compared to J_{er}, as the molecular size of a cation increases (5,6). Equally, for anions there will come a point, as molecular weight increases, that J_{er} and J_{eo} will cancel out one another and cathodal delivery will then cease to be useful (5). However, the exact form of the dependence of electrotransport mechanism on molecular properties has not been deduced (7).

In this work we attempt to initiate a strategy to resolve this question by asking, very simply, how the transport of a single small, negatively-chargeable compound (specifically, 5-fluorouracil, the relevant pK_a of which is ~8) is influenced by the pH of the electrolyte bathing both sides of the skin. In this way, the relative importance of J_{er} and J_{eo} was delineated as the net charge on the membrane was altered (by the changing pH). The experiments also allowed the isoelectric point of the porcine ear skin model used to be deduced.

MATERIALS AND METHODS

Chemicals

5-Fluorouracil (5-FU) was obtained from Fluka (Buchs, Switzerland) and N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) was purchased from Aldrich Chemical Co. (Lyon, France). All other reagents used were of analytical grade and were acquired from commercial sources. Deionized water (resistivity ≥18 MΩ · cm) was used to prepare all solutions.

Skin

Porcine skin, dermatomed at ~500 μm from the ear, was obtained within a few hours of slaughter of the animal at a local abattoir. The tissue was used at once, or was stored at −20°C and used within a week. Preliminary experiments revealed no difference between the permeability of fresh tissue and that of skin which had been refrigerated for up to 5 days.

Experimental

Vertical diffusion cells, in which both electrode chambers are located on the same, epidermal side of the skin, were used (8). The area of skin exposed in each electrode chamber was 0.7 cm^2. To increase the efficiency of the methodology, 5-FU (at 50 mM) was placed in the lower chamber of the diffusion cell and, when the iontophoretic current was passed, its simultaneous transport into the anodal and cathodal chambers (i.e., its 'cathodal' and 'anodal' fluxes, respectively) in the upper half of the diffusion cell was followed. The background electrolyte in the lower compartment, and in the upper electrode chambers, was 25 mM HEPES and 133 mM NaCl buffered at one of the following pH values: 3, 4, 5, 6, 7.4 and 8.5.

After assembly of the diffusion cell and insertion of Ag/AgCl electrodes, prepared in the usual manner (6), into the electrode chambers, constant current (0.5 mA/cm^2) was passed

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Table I. Transport of 5-FU Across Porcine Ear Skin *In Vitro* as a Function of the pH of the Solutions Bathing the Tissue. Flux Values After 6 Hours of Transport are Reported (Mean ± SD; n ≥ 3)

	5-FU flux (nmol/cm²/h)				
pH	Passive	Anodal	Corrected anodal[a]	Cathodal	Corrected cathodal[b]
3	24 ± 13	72 ± 10	49	303 ± 54	280
4	29 ± 7	122 ± 25	93	127 ± 12	98
5	41 ± 10	154 ± 41	113	58 ± 17	17
6	44 ± 7	166 ± 36	122	49 ± 9	5
7.4	65 ± 27	144 ± 43	79	146 ± 34	81
8.5	101 ± 31	44 ± 17	−57	321± 37	220

[a] Absolute anodal flux minus the corresponding passive value.
[b] Absolute cathodal flux minus the corresponding passive value.

for six hours. The current was generated by a custom-built power supply (Professional Design & Development Services, Berkeley, California) interfaced to a Macintosh IIfx computer (Apple Computer Inc., Cupertino, California) running Labview software (National Instruments, Inc., Austin, Texas). Every hour, the entire contents of the anodal and cathodal chambers (0.8 mL each) were removed for 5-FU analysis (see below) and replaced with fresh electrolyte. All experiments were performed in at least triplicate, together with the corresponding no-current controls.

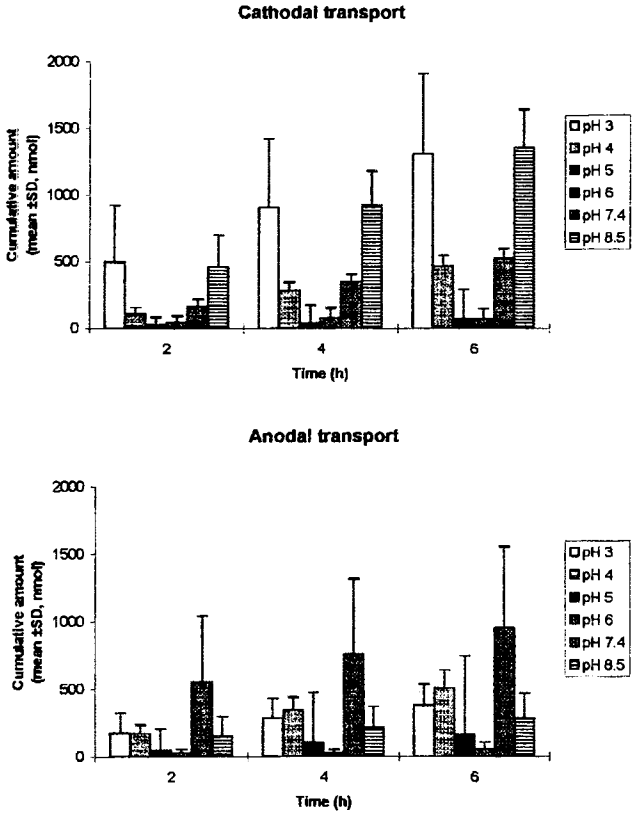

Fig. 1. Evolution of the permeation profiles of 5-FU with time in the 'anodal' and 'cathodal' directions, as a function of pH (mean values + one standard deviation; n ≥ 3).

Analysis. The anodal and cathodal fluxes of 5-FU, with and without iontophoresis, were evaluated respectively from the quantities of the compound determined in the cathode and anode chambers by a high-pressure liquid chromatography method (9). The column was μbondapak C-18 (Waters, Saint Quentin, France) and the eluent was a 30:70 mixture of acetonitrile and pH 3 phosphate buffer, at a flow rate of 1 mL/min. Ten μL of sample were injected and 5-FU was detected by its UV absorbance at 268 nm (Waters 481, Waters, Saint Quentin, France,). The retention time was 2.85 minutes.

RESULTS AND DISCUSSION

Table 1 presents the passive, 'anodal' and 'cathodal' fluxes of 5-FU determined after six hours of contact between the driving 5-FU solution and the skin, as a function of pH. Passive transport is relatively constant, except at the highest pH values where an unexpectedly significant increase is apparent (for which no explanation has been identified). The evolution of the iontophoretic permeation profiles with time are summarized in Fig. 1. To separate electrotransport from the passive diffusion of 5-FU, Table 1 also includes "corrected" values of the 'anodal' and 'cathodal' fluxes (i.e., the corresponding absolute values minus the passive contribution) at each pH. The dependence of the corrected electrotransport on pH is shown in Fig. 2. It should be noted that these corrected fluxes ignore any small increases in skin permeability induced by the application of current; such changes are relatively minor and are not dependent upon pH.

The results exhibit several important features about the dominant mechanism(s) of iontophoresis and the charge on the skin as a function of pH, and the state of ionization of the permeant. 5-FU behaves as a weak acid, with a pK_a of approximately 8 (i.e., at pH values above 8, the molecule is predominantly negatively-charged) (9). The corrected iontophoretic flux values determined at pH 8.5 are entirely consistent, therefore,

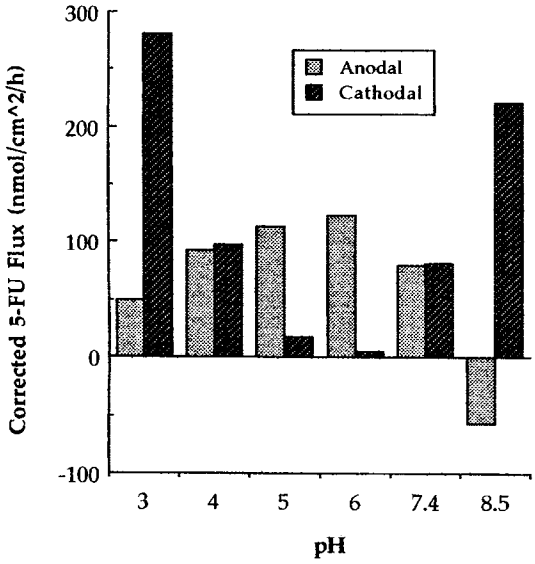

Fig. 2. Corrected cathodal and anodal fluxes of 5-FU as a function of the pH of the solutions bathing the skin tissue(n ≥ 3). The corrected values represent the absolute iontophoretic fluxes minus the corresponding passive transport rates (see Table I).

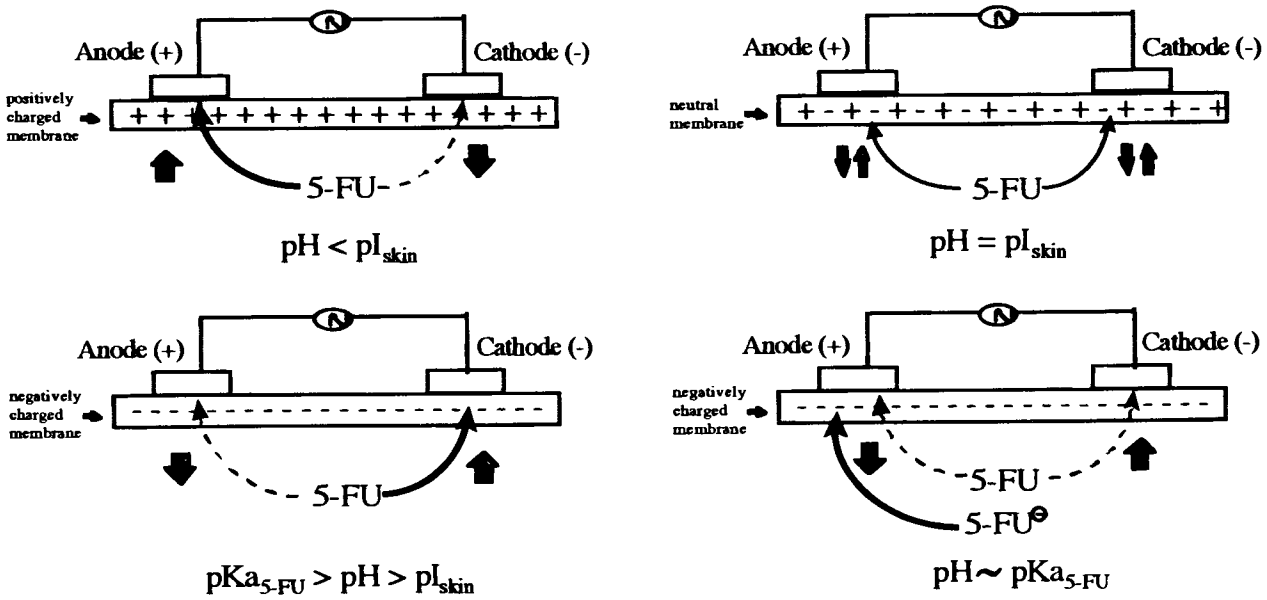

Fig. 3. Schematic illustration of the directions and mechanisms of 5-FU electrotransport as a function of pH, and relative to the pK_a of the drug and to the pI of the skin. The solid arrows, of course, represent the dominant direction of 5-FU and/or 5-FU-transport, while the dashed arrows indicate minor or negligible contributions. The ⇑⇓ arrows show the directions of electroosmotic flow.

with the electrotransport of an anion, with electrorepulsion completely overwhelming the electroosmotic contribution flowing in the opposite direction.

As the pH is lowered to 7.4, however, the corrected 'anodal' and 'cathodal' fluxes become statistically indistinguishable. Now, on average, only about 25% of 5-FU is ionized; so, while there remains a cathodal electrorepulsive component, its magnitude is clearly reduced (relative to that at pH 8.5) and electroosmosis, which carries uncharged 5-FU in the opposite, 'anodal' direction, assumes a significant role. Like other mammalian skin models employed in iontophoresis research, therefore, the porcine barrier must also support a net negative charge at physiological pH (1,10).

At pH 6, there is an exaggeration of the result at pH 7.4. Now, with only 1% of 5-FU in the ionized state, 'anodal' electroosmosis of the neutral species dominates completely. A similar result at pH 5 confirms that the skin barrier remains net negatively-charged at this point. However, at pH 4, 'cathodal' transport increases significantly and the 'anodal' flux concomitantly falls, such that the corrected values are once again statistically indistinguishable. In this case, however, there has been essentially no change in the ionization state of 5-FU (which remains resolutely uncharged); it follows, therefore, that it must be the charge on the skin which has altered. That is, there has been a neutralization, at least in part, of the 'normal' net negative charge on the membrane. The equivalence of 'cathodal' and 'anodal' transport, in fact, suggests that, at a pH value close to 4, one has arrived at the isoelectric point (pI) of the membrane. The fact that the corrected 'anodal' and 'cathodal' fluxes are still about three-fold the passive value suggests that, while the net charge on the skin at the pI approximates zero, there is probably co-existence of about an equal distribution of positively and negatively charged pathways across which electroosmotic flow is possible. The inferred pI of porcine skin of about 4 is a little lower than that recently reported for hairless mouse skin (9), but quite consistent with the value that has been estimated for human skin (1).

At pH 3, with a further injection of hydronium ions into the system, the skin attains a net positive charge and the direction of counterion, and hence electroosmotic flow, reverses to the cathodal-to-anodal direction. 'Cathodal' transport of 5-FU now significantly exceeds that in the 'anodal' direction.

It follows, then, that over the range of pH conditions examined in this series of measurements, the sign and magnitude of the net charge on the membrane has been dramatically manipulated, and the degree of ionization of the permeant has been systematically reduced, with the result that the dominant mechanism and the predominant direction of electrotransport has been strikingly altered. Figure 3 summarizes diagramatically these changes.

The results from this study provide information of mechanistic relevance, and offer insights valuable to the practical applications of iontophoresis. The "teasing apart" of the electrorepulsive and electroosmotic contributions to iontophoretic transport remains an important goal as optimization of formulations for drug delivery and for reverse iontophoretic extraction is undertaken.

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