Study of the Mechanisms of Flux Enhancement Through Hairless Mouse Skin by Pulsed DC Iontophoresis

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Enhanced iontophoretic transport using pulsed DC is usually explained by citing the observed decrease in skin resistance caused by an increase in AC pulse frequency at very small currents. Alternately, it has been suggested that the "on-to-off" nature of pulsed DC imparts an "impact energy" to the fluid, thereby increasing transport. This report provides a test of these mechanisms for enhanced delivery via pulsed iontophoresis. The DC resistance of hairless mouse skin during continuous and pulsed DC iontophoresis is measured as a function of time for selected pulse frequencies and duty cycles using current densities ranging from 0.1 to 1.0 mA/cm^2. As a test of the impact energy mechanism, the iontophoretic transport of ^{14}C-glucose measured with pulsed DC is compared with similar data obtained previously using continuous DC. It is suggested that pulsed current can yield lower resistance and enhanced drug delivery provided that (a) the "steady-state" current during the "on" phase of the pulse is very small and (b) the frequency is low enough to allow depolarization of the skin during the "off" phase of the pulse. The glucose transport results suggest that the "impact energy" concept does not apply to iontophoresis.

KEY WORDS: iontophoresis; pulsed current; electrical resistance; neutral solute flux.

INTRODUCTION

Although transdermal iontophoresis is normally carried out with a continuous, direct current (DC), the use of pulsed DC has been promoted as a method to obtain higher solute flux (1–5). Experimental studies with insulin (2–4) and formoterol fumarate (5) appear to support this view. While not always explicitly stated, the comparison between continuous and pulsed DC is presumably made under conditions of either constant time average applied voltage or constant time average current, depending on whether the study employs voltage or current control. It is pointed out (1–5) that since the impedance of skin decreases with increasing frequency of the applied current or voltage (6), a higher solute flux with pulsed current is expected theoretically. This concept is based on the inverse relation between current and impedance and an assumed direct correlation between current and flux of the solute of interest.

The stratum corneum does behave as a parallel resistance/capacitance electrical circuit (1,3–6). Specifically, at fixed applied voltage, the current is initially high due to charging the capacitor portion of the circuit, but as the capacitor charges and "polarizes," the current decreases to a value determined by the pure resistance portion of the circuit. However, since solute cannot be transported across a capacitor, the transient current into the capacitor portion of the circuit during the "on" portion of the DC pulse is irrelevant to mass transfer in iontophoresis. If the *resistance* decreases with an increase in frequency, one could logically argue that pulsed DC might be more effective for transport than continuous DC using the same time average *voltage*. If compared at constant time average *current*, pulsed and continuous DC should produce identical solute fluxes if solute flux and current are directly correlated. In the Yamamoto and Yamamoto experiments (6), the measured resistance does decrease significantly as the frequency of the voltage pulse increases (6). However, in these studies the skin samples were not exposed to significant direct current flow prior to resistance measurement, and resistance measurements were carried out using infinitesimal alternating currents (AC). The extrapolation of the Yamamoto findings (6) to resistance behavior during iontophoresis is uncertain, as iontophoresis involves the use of appreciable current densities of direct current. Indeed, the results of Burnette and Bagniefski (7) suggest that the frequency dependence of the resistance is minimal for skin previously exposed to a continuous direct current of 0.16 mA/cm^2 for 1 hr. Pulsed DC was not studied.

An alternate rationale proposed to explain the observed greater effectiveness of pulsed DC current invokes the concept of "impact energy" (2,4) in the fluid developing as a result of the rapid on:off nature of the pulsed current. While this concept was not developed in detail, the rapid change in fluid momentum was presumed to impart an additional force to fluid movement (2,4). Thus, such a mechanism should result in the greatest flux enhancement for neutral solutes which, in more conventional theory, are dependent on electroosmotic flow for enhanced transport relative to pure diffusion.

The purpose of this research is to provide experimental data which allow a test of the proposed mechanisms for enhanced iontophoretic transport via pulsed DC as compared to continuous or steady DC. The DC resistance of hairless mouse skin during continuous and pulsed DC iontophoresis is measured over a 6-hr time interval for current densities ranging from 0.1 to 1.0 mA/cm^2. Frequencies from 0 (continuous DC) to 50 kHz and duty cycles of 4:1 and 1:1 (on:off cycle) are studied. As a test of the impact energy mechanism, the iontophoretic transport of ^{14}C-glucose is studied with pulsed DC (2 kHz, 4:1 duty) and compared with similar data obtained previously using continuous DC.

EXPERIMENTAL

All materials and methods were as described in earlier studies (8,9) except when specifically noted otherwise later in this section. Fully hydrated (soaked overnight at 5°C) excised hairless mouse skin was employed in all experiments. Previous experience with electroosmotic flow experiments, which are sensitive to establishment of hydration equilibrium (8), suggest that this hydration procedure gives skin samples closer to hydration equilibrium than soaking for several hours at room temperature. Ag/AgCl electrodes were

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used for both the working electrodes and the probe or measurement electrodes (8,9). The buffer solution is 0.1 *M* NaCl, 0.05 *M* glucose, 0.01 *M* Tris buffer at pH 8.6 for all measurements. The electrical resistance studies varied slightly from the previously described procedure (8), in that, in the present study, the polarity of the current was not reversed in the resistance determination. Since polarity is not reversed during an iontophoresis experiment, the present procedure better mimics the electrical history of a skin sample during iontophoresis. However, the bias potential of the probe electrodes was measured at the beginning, middle, and end of the experiment by briefly turning off the current. Bias potentials were <0.5 mV and were not significant.

The apparatus used for the pulsed experiments employed a voltage pulse generator (Wavetek Modek 187) in series with a precision 1000-Ω resistor, the iontophoresis or resistance cell, and a digital ammeter (Fluke Model 8020B). An oscilloscope (Tektronix Model 2230) was attached across the precision resistor to allow measurement of the current pulse resulting from the square-wave voltage pulse. The pulse generator amplitude was periodically adjusted throughout the experiment to maintain constant "time average" current, as measured by the ammeter. Verification of the accuracy of the ammeter under these conditions was obtained by comparison of the net DC current displayed on the oscilloscope with the reading of the ammeter.

RESULTS

The current pulse resulting from the application of a square-wave voltage mirrors the applied potential when only buffer solution is between the electrodes (Fig. 1A), but when a skin sample is placed between the electrodes, the current flow is significantly higher during the initial 0.1 msec of the voltage pulse than during the last 0.3 msec of the "on"phase. After the voltage is turned off (i.e., 0.4–0.5 msec), the current reverses direction and then decays at roughly the same rate as noted during the first 0.1 msec of the pulse sequence. This behavior is qualitatively the same as observed by Okabe and coworkers (1) and is consistent with the model which treats the stratum corneum as electrically equivalent to a parallel capacitor and resistor circuit and describes the remainder of the skin as a pure resistor in series with the stratum corneum (3). In terms of this model, the initial higher current and the current reversal after the voltage is turned off is due to charging and discharging, respectively, of the capacitor within the circuit. Note that at high current density (Fig. 1C), the relative magnitude of the time dependence is less than at low current density (Fig. 1B). The data in Fig. 1 refer to the "initial" current pulse profile. After passing current for a few hours, the magnitude of the time dependence is noticeably less than shown in Fig. 1. The time constant (i.e., time for difference between initial and steady-state current to decrease by the factor $1/e$) calculated from the data in Fig. 1 is significantly greater (≈50 μsec) than the time constant calculated from the data of Okabe and co-workers (≈7 μsec). The data in Fig. 1 refer to a frequency of 2 kHz, while the data of Okabe and co-workers (1) refer to a frequency of 50 kHz. Thus, a quantitative comparison of data from Fig. 1 with Okabe and co-workers is not possible. However, we note that our data at 50 kHz are not consistent

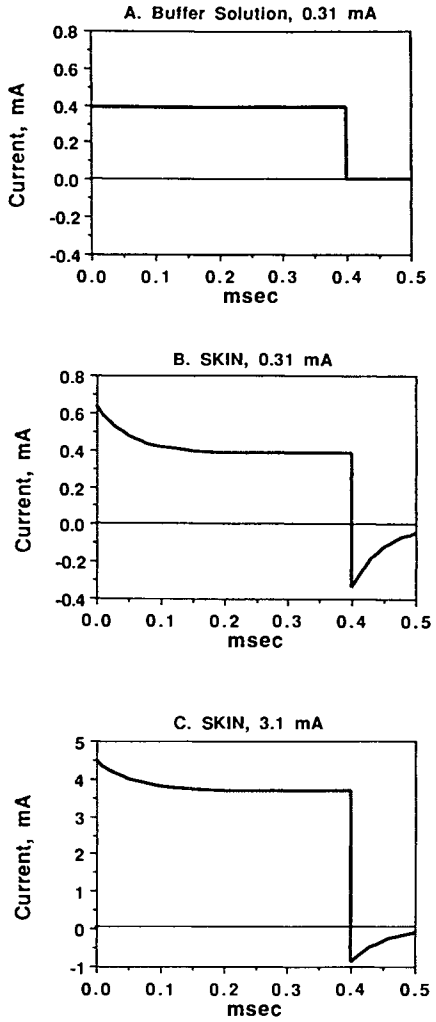

Fig. 1. Current pulse form resulting from square-wave DC voltage pulse at 2 kHz and a 4:1 on:off duty cycle. The buffer solution is 0.1 *M* NaCl, 0.05 *M* glucose, 0.01 *M* Tris buffer at pH 8.6. The pulse forms are redrawn from the oscilloscope trace: A, buffer solution alone at 0.31 mA (0.1 mA/cm^2); B, hairless house skin in buffer solution at 0.31 mA (0.1 mA/cm^2); C, hairless mouse skin in buffer solution at 3.1 mA (1.0 mA/cm^2).

with a 7-μsec time constant. At 50 kHz, we find that the current is essentially constant over the 16-μsec "on" portion of the pulse and reverses in sign during the "off" portion of the pulse, remaining constant from >16 to 20 μsec (data not shown). The experiments reported by Okabe and co-workers (1) were *in vivo* experiments on human subjects and were performed using electrodes potentially susceptible to polarization effects, differences which might be responsible for the discrepancy in time constants. Note that, using the usual circuit model for the electrical properties of skin (3), the time constant is approximately equal to the product of the capacitance in the stratum corneum and the resistance in the viable skin. Thus, differences in capacitance and/or resistance between skin from different species would be expected to produce different time constants.

The effect of current density on the time dependence of resistance in experiments using continuous current is shown in Fig. 2. Error bars denoting the standard error are given for

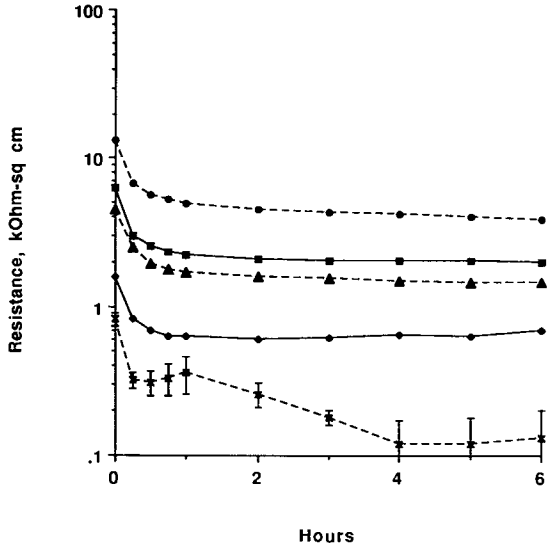

Fig. 2. Time dependence of the DC resistance of hydrated hairless mouse skin at 37°C as a function of current density: continuous DC current. (●) 0.1 mA/cm^2; (■) 0.2 mA/cm^2; (▲) 0.3 mA/cm^2; (♦) 1.0 mA/cm^2; (x) 3.0 mA/cm^2.

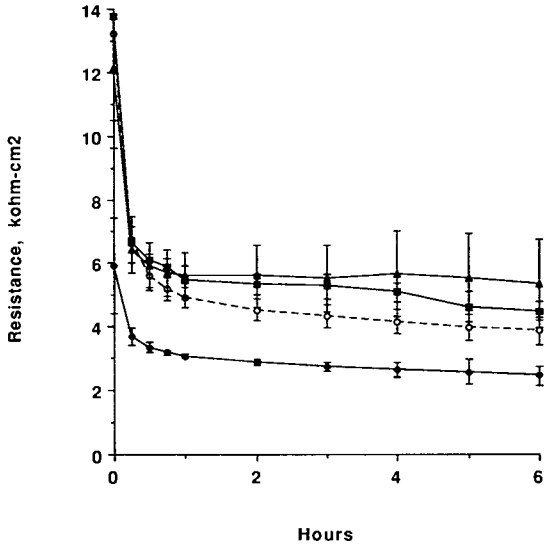

Fig. 3. Time dependence of the DC resistance of hydrated hairless mouse skin at 37°C as a function of pulse frequency: duty cycle of 4:1 on:off and current density of 0.1 mA/cm^2. (o) Continuous DC; (♦) 2 kHz; (■) 10 kHz; (▲) 50 kHz.

the data at 3 mA/cm^2, as here the standard error is significantly larger than the size of the plotting symbol. Consistent with earlier observations (7,8), the resistance decreases significantly over the first hour of iontophoresis, and at all time points, the resistance is much lower at the higher current densities.

Corresponding data were obtained using pulsed DC under the following conditions: 2 kHz with an 80% duty cycle at current densities of 0.1, 0.2, 0.3, and 1.0 mA/cm^2; 2 kHz with a 50% duty cycle at 0.1 and 0.2 mA/cm^2; 10 kHz with an 80% duty cycle at 0.1 mA/cm^2; and 50 kHz with an 80% duty cycle at 0.1 mA/cm^2. With the single exception of pulsed DC at 2 kHz, 80% duty cycle, and 0.1 mA/cm^2, the resistance-versus-time curves for pulsed DC either superimpose on the corresponding continuous current curve (2 kHz, 80% duty, 1.0 mA/cm^2) or lie slightly above the corresponding continuous DC curves. A selection of these data is shown in Fig. 3 where the effect of frequency at an 80% duty cycle on the resistance–time curve at 0.1 mA/cm^2 is displayed. Although the 50-kHz curve does lie well above the continuous current curve, the unusually large variability in the 50-kHz data compromises any interpretation of these differences (ANOVA; $P \approx 0.3$ to 0.4 for data from 0.25 to 6 hr). However, the resistance–time curve measured at 2 kHz is significantly lower than the continuous current curve (ANOVA; $P \approx 0.05$ for data up to 1 hr). The ratio of the "continuous" resistance to the "2-kHz" resistance varies from ≈2 near time zero to ≈1.4 after several hours.

Figure 4 compares ^{14}C-glucose flux measured using pulsed current (2 kHz, 80% duty) with corresponding data obtained with continuous DC current in an earlier study (9). Flux is expressed in terms of equivalent volume of donor solution transported in units of μl hr^{-1} cm^{-2}, which is numerically equivalent to the molar flux of glucose in nmol hr^{-1} cm^{-2} from a donor solution of 1 m*M* glucose. The experimental protocol (9) consisted of a 3-hr passive transport period (Passive 1), anodic [(+)IONTO] or cathodic [(−)IONTO] iontophoresis at 2 mA, and passive transport for 18 hr (Passive 2). The stratum corneum side of the cell was always the donor side. Since, as a good first approximation, Passive 2 flux does not depend on the current polarity during the iontophoresis phase (9), Passive 2 flux data in Fig. 4 represent means of the passive data obtained following anodic and cathodic iontophoresis. The continuous current experiments were replicated six (anodic) or five (cathodic) times, while the pulsed studies were run only in duplicate. Error bars represent standard errors. It is clear that solution flux is *not* larger for the pulsed DC mode of current delivery. In fact, both anodic flux and Passive 2 flux are

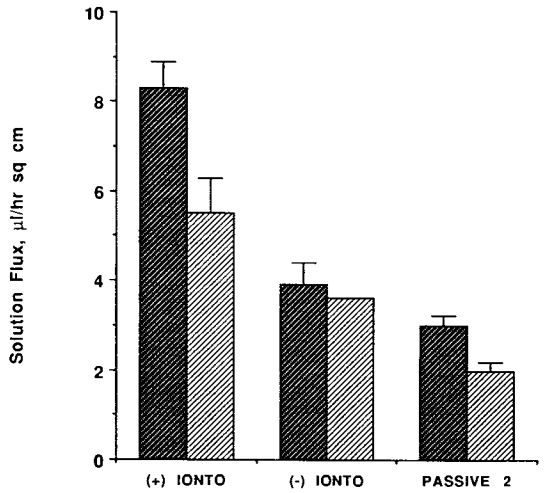

Fig. 4. Comparison of ^{14}C-glucose transport in hydrated hairless mouse skin at 37°C for pulsed and continuous DC current. Iontophoresis is at 2 mA (3.1 mA/cm^2). Dark shading, continuous DC; light shading, pulsed DC (2 kHz at a 4:1 on:off duty cycle); (+) IONTO, anodic iontophoresis; (−) IONTO, cathodic iontophoresis; PASSIVE 2, passive delivery (zero current) after the iontophoresis period.

possibly *lower* for the pulsed DC experiments (SAS/GLM, $P = 0.07$).

DISCUSSION

As argued earlier, if an "impact energy" is generated in the pulsed current mode and if this impact energy can increase drug transport, one would expect greater fluid flow and enhanced ^{14}C-glucose flux during pulsed anodic delivery. However, the data (Fig. 4) clearly do not support this prediction. Rather, glucose fluxes observed with pulsed current (2 kHz, 80% duty) are systematically slightly less than the corresponding fluxes determined with continuous DC. However, using the ratio of anodix flux to Passive 2 flux as a rough measure of flux enhancement as a result of iontophoresis (9,10), one finds that the enhancement ratio is identical (2.8) for pulsed and continuous current modes. It is possible that the increase in skin permeability from skin damage is less for pulsed current flow than for continuous current at these high current densities, thereby giving both lower "intrinsic permeability" (9) and lower "Passive 2" transport for the pulsed case. Since "intrinsic permeability" cancels in the enhancement ratio, the iontophoretic flux enhancement ratio is not altered. Our pulse generator did not supply sufficient voltage to generate a current density of 3 mA/cm^2 for the large cell used for resistance measurements (3.1-cm^2 skin area), so a comparison of resistance between continuous and pulsed current modes could not be made at 3 mA/cm^2. However, regardless of the detailed interpretation of the lower fluxes achieved during pulsed DC current flow, it is clear that anodic pulsed flux, or pulsed flux enhancement ratio, is *not* increased over that achieved with continuous current. Thus, the data (Fig. 4) do not support the "impact energy" concept as applied to iontophoresis.

Appreciable increases in drug transport relative to continuous DC have been reported using pulsed DC frequencies in the range of 1 to 40 kHz and duty cycles ranging from 80 to 10% (2–5) with current densities of the order of ≈0.16–0.33 mA/cm^2 (2–4). If one attributes these increases to a lowered skin resistance with pulsed current, our studies should have shown much lower resistance during pulsed current flow than during continuous current flow under most, if not all, of the pulse conditions studied. We observe a lower resistance during pulsed DC *only* under one set of conditions (2 kHz, 80% duty, and 0.1 mA/cm^2), and even in this case, the reduction in resistance was rather modest compared to the greatly enhanced solute flux previously reported as resulting from pulsed current (2–5). The data usually cited (6) to justify the concept of reduced resistance during pulsed current flow were obtained using skin not previously exposed to direct current flow. Moreover, the experimental procedure utilizes alternating current (AC) at extremely low levels (≈1 μA/cm^2) to measure resistance. Under such conditions, both human skin (6) and nude mouse skin (7) show a significant reduction of resistance as the frequency is increased. However, iontophoresis experiments are not carried out with AC at the microamp level. Rather, DC or pulsed DC with a time-averaged current density in the range 0.1–0.5 mA/cm^2 are usually employed. As the data presented in this report demonstrate, even a brief current flow at these levels generally destroys the frequency effect, and the observations normally cited (6) to rationalize the "pulsed current effect" are not necessarily relevant to either drug transport or resistance during an actual iontophoresis experiment.

While our data do not support the concept of greatly lowered resistance under pulsed DC as a general concept, we did observe (Fig. 3) a statistically significant (ANOVA, $P = 0.05$) reduction of resistance relative to continuous current under one set of pulse conditions (2 kHz, 80% duty, 0.1 mA/cm^2). Of course, it is possible that the statistical conclusion of significance simply represents the 1 in 20 chance that the statistical conclusion is wrong. This interpretation appears most consistent with the recent study by Bagniefski and Burnette, which concludes that transport of Na^+ is independent of the frequency of a square-wave current pulse (11). However, it should be noted their raw data and associated uncertainty (11) are compatible with a modest increase in Na^+ flux at a frequency of 1 kHz.

Alternately, the lower resistance measured under this particular set of pulse conditions (2 kHz, 80% duty, 0.1 mA/cm^2) could be real. It should be noted that these conditions represent a combination of circumstances where the lowest pulse current density studied is combined with a frequency which allows nearly complete discharge of the capacitor portion of the circuit (see Fig. 1). At a time-average current density of 0.1 mA/cm^2, an 80% duty cycle requires a steady-state current density (i.e., current during the "on" phase of the pulse) of 0.125 mA/cm^2, but with a 50% duty cycle, the corresponding current density is 0.2 mA/cm^2. Furthermore, at a frequency of 2 kHz, the polarization has time to dissipate (i.e., the capacitor discharges) before the next pulse begins, but at 10 kHz, little depolarization occurs during the "off" portion of the pulse. Therefore, it seems reasonable to postulate that the frequency dependence of resistance observed during AC measurements with very low current densities also occurs during pulsed DC iontophoresis as long as (a) the current density during the on phase of the pulse is very low, i.e., ≤0.125 mA/cm^2 for HMS *in vitro*, and (b) the capacitor equivalent of the stratum corneum has time to discharge before the next voltage pulse is applied, i.e., ≈100 μsec for the off phase of the pulse with hairless mouse skin *in vitro*. These requirements for lowered resistance, and enhanced drug transport, should apply to *in vitro* and *in vivo* experiments with skin from other species, but the current density limits and frequency limits may well be different. Specifically, as noted earlier, the time constant for capacitor discharge seems much shorter for human skin *in vivo* than we find for hairless mouse skin *in vitro*, so the frequency limit for human skin would be significantly higher than 2 kHz. Thus, it is possible that the reported flux enhancement using pulsed DC has its origin in a lowered resistance, although the conditions for flux enhancement are far more complex than simply "pulsed current vs continuous current" with the optimum pulsed conditions being specific to the animal species being studied. Moreover, the modest resistance reduction observed using pulsed DC suggests that the corresponding solute flux enhancement would also be modest. These tentative conclusions, while plausible, require support from equivalent experiments conducted on skin samples other than excised hairless mouse skin.

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