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Effects of iontophoresis on the electrical properties of human skin in vivo

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Abstract

Preliminary measurements have been made of the effect of iontophoresis on the electrical properties of human skin in vivo. Skin impedance was measured as a function of frequency and as a function of applied direct (i.e., iontophoretic) current. The resistance of the skin was then determined from the accumulated impedance data in the normal way. After the electrode chambers were attached to the forearm, and the pretreatment resistance had stabilized, direct current was applied and the % change from baseline resistance was determined. At the end of current passage, the recovery of resistance was monitored for up to 4 h. Current application involved three current densities applied for three different times (combined in such a way that three consistent amounts of total charge were delivered): 10 $\mu A/cm^2$ for 10, 20 and 50 min; 50 $\mu A/cm^2$ for 2, 4 and 10 min; and 100 $\mu A/cm^2$ for 1, 2 and 5 min. Current application caused skin resistance to drop rapidly (at all currents, most of the change occurs within 10 seconds of beginning the current flow). At all current levels, the decrease in skin resistance leveled off at a value which was dependent upon current density, but somewhat independent of time of current application: 10 $\mu A/cm^2$ –approx. 45% of pretreatment value; 50 $\mu A/cm^2$ –approx. 20% of pretreatment value; and 100 $\mu A/cm^2$ – approx. 10% of pretreatment value. The time required for recovery of skin resistance increased with (a) increasing time of current application (at constant current density), and (b) increasing current density. It is concluded that measurements of skin impedance (and derived values of skin resistance) in vivo, in man, can therefore provide direct electrical evaluation of the effects of iontophoresis on the tissue. Such measurements, we believe, are of vital importance with respect to the long-term application of iontophoresis as a method of drug delivery.

Keywords: Iontophoresis; Skin impedance; Skin resistance; Transdermal drug delivery; Percutaneous absorption

Iontophoresis has the potential to deliver charged and uncharged drug molecules across the skin at an enhanced rate in a controllable manner (Burnette, 1989). To optimize iontophoretic drug delivery, a detailed understanding of the electrical properties of the skin, and the manner in which they are altered by current passage, is essential. Most importantly, information is required on the effects of the electrical potential gradient on human skin in vivo, and the ability of the tissue's electrical properties to recover following a period of iontophoresis.

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Electrically, the skin may be represented as a parallel combination of a resistance (R) and a capacitance (C) (Yamamoto and Yamamoto, 1976). The former is associated with current-conducting pathways through the membrane, the latter being believed to originate in the lipids of the stratum corneum (Yamamoto and Yamamoto, 1976; Burnette and Ongpipattanakul, 1987; Kasting and Bowman, 1990; Cullander, 1992; Oh et al., 1993). In an earlier study (Oh et al., 1993), we measured the impedance of hairless mouse skin in vitro as a function of frequency to determine how the R and C of the membrane were affected by the magnitude of applied current. As the magnitude of the applied current increased, there was a decrease in R, with little change in C. Changes in R following alterations in current were rapid and irreversible, suggesting that current-induced changes in the nature of the ion-conducting pathways had occurred. In this work, we have further studied the dependence of R, as a function of both time and level of current applied, in human skin in vivo. We also have used impedance spectroscopy (DeNuzzio and Berner, 1990; Kontturi et al., 1993; Kontturi and Murtomaki, 1994) to study the recovery of the skin's electrical properties after current application.

The measurement cells were small polypropylene cylinders, of area 0.95 cm^2, holding a volume of approx. 1.5 ml. The cell covers had inlets for six electrodes: two for the alternating current (a.c.) signal, two for direct current (d.c.) supply, and two for a.c. sensing. The cells were adhered to the volar surface of the forearm, and the electrodes carefully positioned to avoid any inter-electrode contact. The a.c. sensing electrodes were placed as close as possible to the skin surface. All electrodes were Ag/AgCl, prepared electrochemically. A short length (3 mm) of Ag wire (99.9%, Aldrich, Milwaukee, WI) (1 mm diameter) was lightly sanded with emery paper, washed in acetone, and then cleaned in 1 M HCl for 20 min at 50°C. After rinsing with distilled water, the Ag was anodically plated with AgCl (using a Pt cathode) by immersion in 0.5 M KCl and application of a 0.1 mA current. Signal electrodes and d.c. supplying electrodes were plated for 5 h, sensing electrodes for 20 min. NaCl

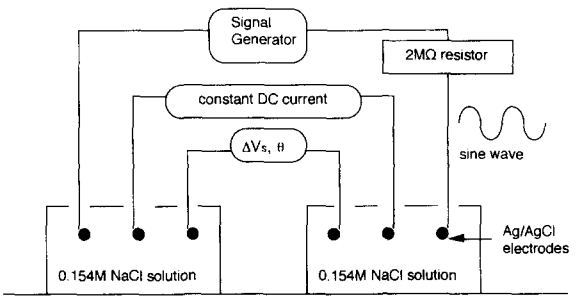

Fig. 1. Schematic diagram of the experimental setup used to determine the impedance of the skin in vivo. The signal generator supplies a sine wave of amplitude 1 V (peak-to-peak). The maximum current that flows across the skin is ± 0.5 μA, due to the 2 MΩ resistor in series with the signal generator. Constant d.c. current was supplied using a constant voltage source in series with a 4 MΩ resistor. The voltage drop (ΔV_S) and phase shift (θ) across the skin were measured using a lock-in amplifier. All electrodes used were Ag/AgCl.

solution was prepared with distilled water from a Milli-Q UF Plus water purification system (Millipore, Bedford, MA). Solutions were degassed under vacuum with sonication before use.

The change in resistance, in response to passage of an iontophoretic current, was followed by measuring impedance (Z) and phase shift (θ), using a lock-in amplifier (Stanford Research Systems SR530, Sunnyvale, CA) at a single frequency (see Fig. 1). Four, normal healthy volunteers, with no history of skin disease, participated in the study. All experiments were carried out at a relative humidity of 55–65%, and temperature 22–24°C. The cells were positioned about 3 cm apart and were filled with 0.154 M NaCl solution. After complete hydration of the skin (approx. 2 h), a direct current was applied, and the change in impedance simultaneously measured. Data were automatically recorded by the lock-in amplifier every second for the first few minutes; subsequently, measurements were collected manually at predetermined time intervals. The d.c. densities studied were 10, 50, 100 and 500 $\mu A/cm^2$. The durations of d.c. application were 10, 20 and 50 min for 10 $\mu A/cm^2$ (experimental series A); 2, 4 and 10 min for 50 $\mu A/cm^2$ (experimental

series B); 1, 2 and 5 min for 100 $\mu A/cm^2$ (experimental series C); and 3.3 and 5 min for 500 $\mu A/cm^2$ (experimental series D). Thus, series A, B and C represent three different sets of current profiles, each of which delivers successively 'doses' of 100, 200 and 500 μA min. At the end of d.c. application, the recovery of skin impedance was measured continuously for between 1 and 4 h (depending on the magnitude and duration of d.c. application). For the first 3 min post-iontophoresis, data were recorded every second; subsequently, measurements were collected manually at predetermined time intervals.

Skin impedance was determined from the potential drop (ΔV_S), and the shift in phase (θ), across the skin as measured by the sensing electrodes. The electrical circuit used in the experiments included a 2 MΩ resistor in series with the skin. At an applied voltage of 1 V (peak-to-peak), a sinusoidal current was applied via a signal generator (Hewlett Packard 8116A, Mountain View, CA) to one of the signal electrodes (the other was grounded). Since skin impedance was routinely 150–400 kΩ, the current could not be assumed to be determined solely by the 2 MΩ resistor in series and that constant current was therefore flowing. However, because ΔV_S is known, the potential drop across the 2 MΩ resistor (ΔV_R) is simply $\{1\ V - \Delta V_S\}$. Hence, from the ratio ($\Delta V_S/\Delta V_R$), and the impedance of the resistor (2 MΩ), the impedance of the skin can be calculated. Knowing the impedance (Z) of the skin, its resistance (R) was determined from $Z/\cos\theta$, assuming that the skin can be represented by a parallel RC circuit (7). The contributions of the electrolyte solutions to the impedance values measured were ignored in this calculation because their combined magnitude is typically $< 1\%$ of the impedance of skin. A constant d.c. current source was provided by a 4 MΩ resistor connected in series with a constant voltage source (Model APH1000M, Kepco, Inc., Flushing, NY). Due to the high input impedance (10 MΩ) of the signal generator, together with the 2 MΩ resistor connected in series, direct current from the d.c. source flows only through the skin, not through the signal generator. Similarly, due to the much higher impedance (relative to that of the skin) of the 4 MΩ resistor in series with the constant voltage source than that of the skin, alternating current from the signal generator flows only through the skin.

Initial skin resistances were typically 150–400 kΩ cm^2, and decreased 20–40% during the 2 h (pretreatment) hydration period. This initial degree of variability was also manifest in the responses to iontophoretic current (although the relative responses were similar between subjects) – consequently, for the purposes of clarity, the data presented are those from a single individual. Fig. 2a, Fig. 3a and Fig. 4a show the changes in skin resistance during current application in experimental series A, B and C, respectively. Fig. 2b, 3b and 4b show the recoveries of skin resistance following current application in the corresponding experimental series A, B and C, respectively. Current application caused skin resistance to drop rapidly (at all currents, most of the change occurred within 10 s of beginning the current flow). At all current levels, the decrease in skin resistance leveled off at a value which was dependent upon current density, but somewhat independent of time of current application (Fig. 2a, 3a and 4a): 10 $\mu A/cm^2$ – approx. 45% of pretreatment value; 50 $\mu A/cm^2$ –approx. 20% of pretreatment value; 100 $\mu A/cm^2$ –approx. 10% of pretreatment value. At an applied current density of 500 $\mu A/cm^2$, for only a few minutes, skin resistance dropped, within a few seconds, to essentially that of the electrolyte contained in the electrode chambers. At this current density, skin resistance had recovered to only 35% of its pretreatment value 4 h after termination of current flow (data not shown).

The time required for recovery of skin resistance increased with (a) increasing time of current application (at constant current density), and (b) increasing current density (see Fig. 2b, 3b and 4b). This point is illustrated in Table 1, which presents the time required for 80% recovery of skin resistance post-iontophoresis as a function of (i) applied current density, and (ii) total current 'dose'. It is clear from the values in Table 1 that, for an equivalent current dose, the short application of high current is more perturbing than a longer application of lower current density. It is

worth noting that the decreases in skin resistance observed in these in vivo experiments occurred much faster than those which have been reported in vitro. In experiments involving the application of a fixed voltage across excised human epidermal membrane, for example, it has been found that 250 mV for up to 1 h causes no change in resistance (Inada et al., 1994). At 750 mV, and above, however, progressively larger decreases in resistance were seen (with concomitantly poorer abilities for recovery). The decreases, though,

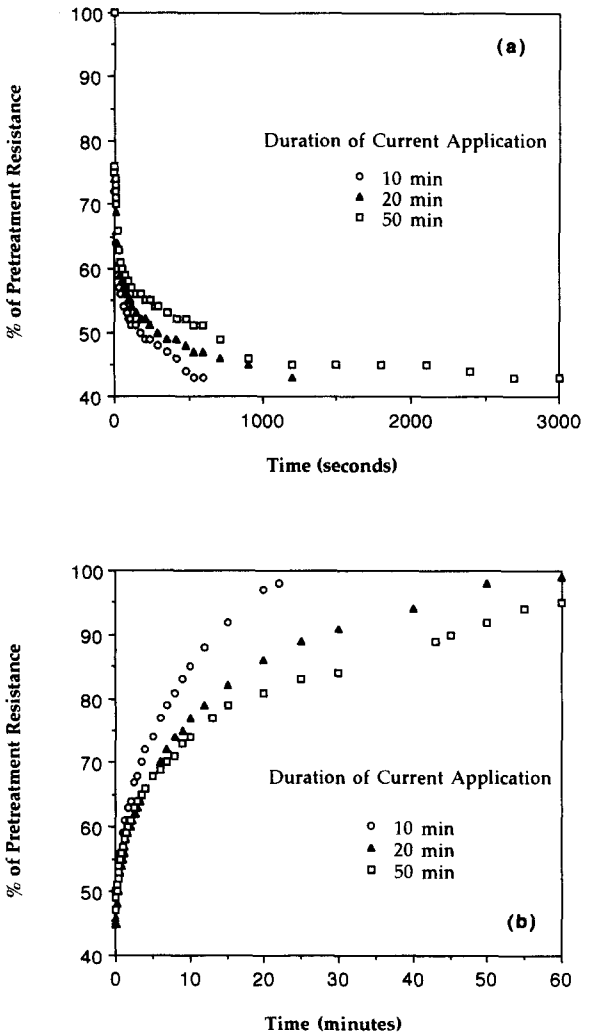

Fig. 2. (a) Change of skin resistance as a function of time during the application of 10 $\mu A/cm^2$ for 10, 20 and 50 min. (b) Subsequent recovery of skin resistance after current application.

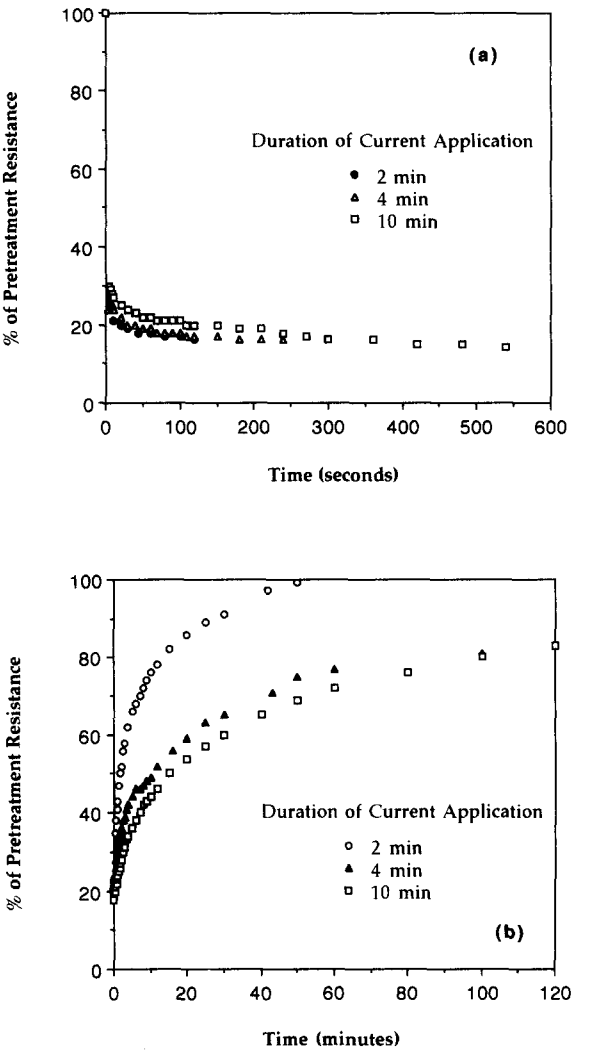

Fig. 3. (a) Change of skin resistance as a function of time during the application of 50 $\mu A/cm^2$ for 2, 4 and 10 min. (b) Subsequent recovery of skin resistance after current application.

were quantitatively smaller than those measured in vivo, and the kinetics of change were significantly slower. Precisely why such differences exist between in vivo and in vitro situations is not known. An obvious possibility is that removal of the skin alters (perhaps, even destroys) some of the current-conducting pathways through the membrane (e.g., occlusion of sweat glands in vitro as suggested by Burnette and Ongpipattanakul (1987)).

In conclusion, measurements of skin impedance (and derived values of skin resistance) in vivo, in man, can provide direct electrical evaluation of the effects of iontophoresis on the tissue. Such measurements are clearly of vital importance with respect to the long-term application of iontophoresis as a method of drug delivery (Cullander and Guy, 1992; Sage and Riviere, 1992). Several key questions now emerge: For example, are these electrical changes indicative of physical alterations in barrier function? Of biological changes in the epidermis (Ledger, 1992)? Over what time course? Can the changes be modulated by alterations in the pattern of current delivery, or the electrolyte composition applied?

Table 1
Time (in min) required for 80% recovery of skin resistance post-iontophoresis as a function of (i) applied current density, and (ii) total current 'dose'

Current density (μA/cm^2)	Total current dose (μA min cm^{-2})		
	100	200	500
10	8	12	20
50	15	90	100
100	30	100	210

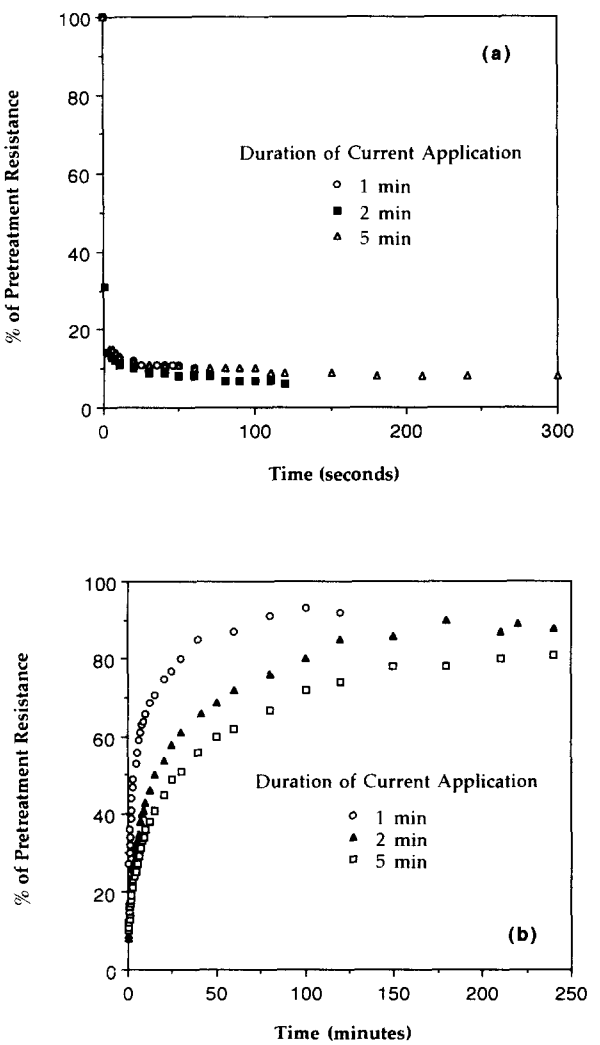

Fig. 4. (a) Change of skin resistance as a function of time during the application of 100 μA/cm^2 for 1, 2 and 5 min. (b) Subsequent recovery of skin resistance after current application.

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