Research Article

Transport of Ionic Species in Skin: Contribution of Pores to the Overall Skin Conductance

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Received March 30, 1993; accepted July 3, 1993

Two methods are reported that allow visualization of high conductance paths in skin at current densities typically used during clinical iontophoretic drug delivery (10–200 μA/cm²). In the first method, the counter-directional iontophoretic transport of Fe(CN)₆⁴⁻ and Fe³⁺ across skin results in the precipitation of colloidal prussian blue, Fe₄[Fe(CN)₆]₃, at sites of high iontophoretic flux. The appearance of localized deposits of Fe₄[Fe(CN)₆]₃ is recorded by video microscopy and used to document the activation of low-resistance paths. In the second method, the ionic flux of Fe(CN)₆⁴⁻ through pores is directly imaged by scanning electrochemical microscopy (SECM). Both methods demonstrate that the iontophoretic flux across skin is highly localized. Activation of low-resistance pores in hairless mouse skin is shown to occur during iontophoresis. The spatial density of current carrying pores increases from 0 to 100–600 pores/cm² during the first 30–60 min of iontophoresis. At longer times, the active pore density approaches a quasi-steady-state value that is proportional to the applied current density. The total conductance of the skin is proportional to the number of pores, consistent with a model of conduction in skin that is comprised of low-resistivity pores in parallel with a high-resistivity bulk phase. The contribution of pores to the total skin conductance during iontophoresis increases from an initial value of 0–5% to a quasi-steady-state value of 50–95%.

KEY WORDS: iontophoresis; electrotransport; hairless mouse; skin resistance; shunt pathways; scanning electrochemical microscopy.

INTRODUCTION

Knowledge of the electrical properties of skin is important in the area of electrofacilitated transcutaneous drug transport (iontophoresis). The skin has been modeled electrically as a parallel resistor and capacitor, in series with a second resistor (1–4). The value of the series resistance is low compared to that of the parallel resistor, such that the parallel resistor/capacitor element dominates the skin impedance at low frequencies. Removal of skin’s outer layer, the stratum corneum, has been shown effectively to eliminate the parallel resistance, leading to the conclusion that the dominant parallel resistive component is located exclusively in the stratum corneum (2). Thus, it is generally accepted that the stratum corneum is the major barrier to iontophoretic transport (5).

Homogeneous models of membrane permeability have proven unsatisfactory for describing iontophoretic transport across skin, and the importance of skin appendages (e.g., hair follicles, sweat ducts) as low-resistance shunt pathways through which ions traverse the skin has been the subject of debate for decades (6). It is generally agreed that skin appendages can provide low-resistivity channels across the otherwise highly resistive stratum corneum. We use the terms “pore” and “shunt” to denote a local, high-conductance pathway, without making presumptions about the physiological nature of such a structure. Experimental results, based on staining of pores during iontophoresis of charged dyes, as well as using potentiometric microelectrodes to measure electric field gradients, have shown that appendages are associated with regions of high current density (7–11). However, the small size and spatial density of appendages have prevented quantitative determination of the contribution of shunt paths to the overall conductance of skin (12–17).

During iontophoresis, the dc resistance of skin ($R_s$) is a function of time and the magnitude of the applied current (18). Typically, $R_s$ decreases rapidly during the initial ~10 min of iontophoresis and gradually levels off to a quasi-steady-state value. Because of this effect, the skin’s current/voltage response, while apparently ohmic at any given instant during iontophoresis, cannot be described by constant-valued electrical circuit components. While previous works have shown that the stratum corneum is chiefly responsible for skin’s high resistance and that the resistance of this layer changes in time, there is a need to address what changes...
occur within this layer which are responsible for the decrease in resistance.

We report on studies of iontophoresis through hairless mouse skin. The hairless mouse has been commonly used as a model for transcutaneous transport (19). Although mature hairless mice have few hairs (as the name suggests), the presence of several hundred hair follicles per square centimeter allows the use of this animal model to study whether such follicles behave as shunt pathways. Our experiments involved simultaneous measurements of the dc skin resistance and the number of shunt pathways during iontopheric and passive transport of ferrocyanide \( \text{Fe(CN)}_6^{4-} \) across hairless mouse skin. The current densities used in our transport studies (10–200 \( \mu \text{A/cm}^2 \)) are commonly used in iontophoretic drug delivery (20–24). Scanning electrochemical microscopy (SECM) and video microscopy are used to document the activation of shunt pathways during iontophoresis. By monitoring the number of pores present and the overall skin conductance over time, both the conductance of individual pores and the relative contribution of pores to the overall conductance of skin are quantitatively assessed. During iontophoresis, the resistance of skin is inversely correlated with both time and magnitude of current. It is shown that both of these phenomena can be attributed to an increased number of high-conductance shunt pathways across the stratum corneum.

MATERIALS AND METHODS

The experimental apparatus shown in Fig. 1 performs four distinct functions used in analyses of iontophoretic currents: (i) a constant current across skin samples is supplied by the galvanostat and two large-area Ag/AgCl electrodes; (ii) the electrical resistance of skin is measured by the four-point probe method using the two saturated calomel reference electrodes (SCE) as voltage-measuring electrodes; (iii) the deposition of Prussian blue dye on the skin surface is monitored during iontophoresis using a videocamera and telephoto lens; and (iv) ion transport rates through individual pores are measured quantitatively by a scanning electrochemical microscope. All experiments were made at ambient room temperature. Details of the instrumental components that perform each function are described below.

A disk-shaped area of skin separates the solutions in the donor (lower) and receptor (upper) compartments (Fig. 1) of a custom-built Teflon iontophoresis cell. A glass window is mounted at the top of the receptor compartment for optical imaging of skin by video microscopy. Ports in the diffusion cell provide connections to SCEs and Ag/AgCl current-driving electrodes. Glass frits are used to separate the Ag/AgCl electrodes from the main compartments of the cell. A detailed description of the cell construction and the specimen mounting procedure are given in Ref. 11.

Skin Samples. Skin was removed from the back and sides of freshly sacrificed hairless mice (male, age 7–12 weeks, Charles River, strain SKH-1). The loosely attached subcutaneous brown fat was removed by gentle rubbing with a damp gauze sponge. Skin samples were placed between layers of sterile saline-soaked gauze and stored in a refrigerator until use. Storage times ranged from 2 to 80 hr. Disk-shaped areas of skin (0.50 cm\(^2\)) were exposed to test solutions in the iontophoresis cell, with the epidermal side facing the upper (receptor) compartment.

**Scanning Electrochemical Microscopy (SECM).** A microelectrode, prepared by deposition of platinum at the end of an 8-\( \mu \text{m}-\)diameter carbon fiber, is used to measure the concentration of an electroactive species as it emerges from the skin (Fig. 1). The potential of the microelectrode is biased, using a potentiostat, at a constant value which is sufficiently positive (or negative) to oxidize (or reduce) the electroactive species at the mass transport-controlled rate. In the investigations reported below, which are concerned with imaging the local pathways of Fe(CN)\(_6\)^{4-} transport in skin, the microelectrode is poised at 0.4–0.5 V vs a saturated calomel electrode (SCE). At such a potential, the oxidation of Fe(CN)\(_6\)^{4-} at the Pt tip, Eq. (1), is diffusion limited.

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\text{Fe(CN)}_6^{4-} \rightarrow \text{Fe(CN)}_6^{3-} + e^- \quad E^0 = 0.185 \text{ vs SCE}
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Fig. 1. Schematic diagram of scanning electrochemical microscope (SECM) and iontophoresis cell.