Evaluation of Skin Barrier Function Using Direct Current I: Effects of Conductivity, Voltage, Distance between Electrodes and Electrode Area

Makoto KANEBAKO,*a Toshio INAGI,a and KOZO TAKAYAMA,b

*a Fuji Research Laboratories, Pharmaceutical Division, Kowa Company, LTD.; 332–1 Ohnoshinden, Fuji, Shizuoka 417–8650, Japan: and b Department of Pharmaceutics, Hoshi University; 2–4–41 Ebara, Shinagawa, Tokyo 142–8501, Japan. Received April 12, 2002; accepted August 28, 2002

The objective of this study was to evaluate the reduction in skin barrier function caused by direct current iontophoresis by measuring resistance in the short term. The experiments were carried out using rat abdominal skin in vivo. The resistance was measured every 125 ms and analyzed using a two-compartment model consisting of surface and skin resistance. Moreover, the initial value and the rate constant of each resistance were calculated with the non-linear approximation program. The proposed method could evaluate the reduction in barrier function from the initial value and the rate constant of surface resistance with high sensitivity and accuracy. Using this proposed method, the effects of the conductivity of an adhesive pad, voltage, the distance between electrodes and the area of electrode were examined. The increase in conductivity of the adhesive pad decreased the initial value since the rate constant increased. The reduction in barrier function depends on voltage. Although the barrier function decreased up until an electrode distance of 1 cm, it increased beyond 1 cm. These phenomena contributed to the current pass portion in the skin because the resistance was in the order of the stratum corneum, epidermis and dermis. The initial value decreased with increasing electrode area. However, the rate constant was little affected since the current density of a topical electrode adjacent to the other electrode was high.

Key words iontophoresis; skin barrier function; resistance; two-compartment model; initial value; rate constant

Iontophoresis is a method to transport ionic material by electric potential. Numerous investigations have been performed on transdermal pharmaceutical preparations since Veratti advocated their use back in the 18th century.1—6) We focused on iontophoresis to enhance transdermal delivery. Modified equations for the amount of drug delivered7) and the mechanism of enhanced transdermal delivery8) by iontophoresis were proposed using indomethacin as a model. Additionally, it was shown that the amount of drug that migrates by iontophoresis was the sum of that delivered by enhanced passive diffusion and by current application. Moreover, it was concluded that an analysis of the reduction in barrier function would be necessary to clarify the amount of drug transported by enhanced passive diffusion.7)

As regards the reduction in barrier function on direct current application, several studies have been published. Kasting and Bowman examined the voltage-current properties and the ability of sodium ion to cross excised human skin in order to evaluate the efficiency of transdermal delivery by iontophoresis.9) Pikal and Shah compared the resistance of excised hairless mouse skin to pulsed current and continuous current application.9) Dinh et al. measured the change in excised human skin resistance with time. They showed that the upper limit of resistance was the normal state and the lower limit the steady-state by current application.10) Inada et al. evaluated barrier function in terms of the reduction and recovery of excised human skin resistance under constant voltage. They showed that voltage made the largest contribution to the decrease in skin resistance, and the recovery time depended on the voltage and the application time.12)

It is known that skin resistance following the application of direct current changes remarkably in a matter of microseconds and gradually in seconds. In the studies mentioned above, skin resistance was examined over seconds/hours, while very short-term changes were not referred to. Moreover, these investigations used excised skin and diffusion cells. Thus, it is difficult to comment on the effectiveness of external pharmaceutical preparations.

In this study, in vivo rat skin resistance was measured in the order of milliseconds taking into consideration the electrical properties of the skin. The resistance was divided into surface and skin resistance. The initial value and the rate constant of each resistance were calculated with a non-linear approximation program using a two-compartment model. Using this proposed method, the effects of the conductivity of an adhesive pad, voltage, the distance between electrodes and the area of the electrode were evaluated.

THEORETICAL

Calculation of Resistance Using a Skin Equivalent Circuit

The experimental set-up for in vivo transdermal delivery and the equivalent circuit by iontophoresis is shown in Figs. 1a and b, respectively.11—15) The anodal pad resistance, Ra, at a constant thickness and drug concentration is determined as follows:

\[
\frac{1}{R_a} = \frac{1}{R_1} + \frac{1}{R_2} + \ldots + \frac{1}{R_n} = \frac{n}{R_e}
\]  

where \(n\) is the number of electrodes and \(Ra\) is given by

\[
R_a = \frac{Ra'}{n'}
\]

where \(R_1\) is an anode pad resistance per unit area and \(n\) is anode pad area. Similarly, the cathodal pad resistance, Rc, is given by

\[
R_c = \frac{Ra'}{n'}
\]

where \(R_2\) is cathode pad resistance per unit area and \(n'\) is cathode pad area. From Eqs. 2 and 3, it is clear that the resistance decreases with pad area. The surface and skin resis-
tance are defined as parallel circuits consisting of resistance and capacitance. The anodal surface resistance, $R_{sa}$, is shown as

$$R_{sa} = \frac{R_3}{j\omega C_1 + 1} \tag{4}$$

where $R_3$ is resistance, $j$ is imaginary unit, $\omega$ is angular frequency and $C_1$ is capacitance. In the case of direct current application, Eq. 4 is similar to Eq. 5 since the participation of the capacitor can be neglected. The reason is that the capacitor does not pass direct current.

$$R_{sa} = R_3 \tag{5}$$

In the same way, the skin resistance, $R_{SK}$, and the cathodal surface resistance, $R_{sc}$, are represented by Eqs. 6 and 7, respectively.

$$R_{SK} = R_4 \tag{6}$$

$$R_{sc} = R_5 \tag{7}$$

$R_4$ is the skin resistance and $R_5$ is the cathode surface resistance. The total resistance, $R_t$, is shown as follows:

$$R_t = \frac{R_1}{n} + R_2 + R_4 + R_5 + \frac{R_6}{n} \tag{8}$$

Finally, Eq. 8 is similar to Eq. 9 since $R_1$, $R_4$, and $R_5$ are about 100—1000 times greater than $R_2$ and $R_5$.

$$R_t = R_1 + R_4 + R_5 \tag{9}$$

**Analysis Using the Two-Compartment Model** Equa- tion 10 shows that the total resistance, $R_t$, is the sum of the surface resistance, $R_{SUS}$, and skin resistance, $R_{SK}$.

$$R_t = R_1 + R_4 + R_5 = (R_1 + R_3) + R_4 = R_{SUS} + R_{SK} \tag{10}$$

Under the constant voltage condition, the change in the mol number of electrons is applied to a two-compartment model, which consists of surface (SUC), skin (SKC) and source compartments (SOC). As shown in Fig. 2, electrons migrate to the SUC. After a reversible reaction between SUC and SKC, they eliminate to the SOC. The differential equations for the numbers of electrons in each compartment are as follows:

$$\frac{dE}{dt} = -k_{12}E - k_{21}E_{SK} \tag{11}$$

$$\frac{dE_{SK}}{dt} = k_{12}E - k_{21}E_{SK} \tag{12}$$

$$\frac{dE_s}{dt} = k_1E_s \tag{13}$$

where $E$ is the mol number of electrons in SUC, $E_{SK}$ is that in SKC and $E_s$ is that in SOC. In addition, $k_{12}$, $k_{21}$ and $k_1$ represent the rate constant from SUC to SKC, from SKC to SUC and from SUC to SOC, respectively. Solving Eqs. 11, 12 and 13 according to the usual method yields

$$E = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \tag{14}$$

$$C_1 = \frac{D(k_{12} - \lambda_1)}{\lambda_1 - \lambda_2} \tag{15}$$

$$C_2 = \frac{D(k_{21} - \lambda_2)}{\lambda_1 - \lambda_2} \tag{16}$$

where $D$ is the mol number of electrons that migrate to SOC. Moreover, $\lambda_1$ and $\lambda_2$ are the real solutions of Eqs. 11, 12 and 13. On the other hand, the mol number of electrons, $E$, is derived from Faraday’s law:

$$E = \frac{I}{F} \tag{17}$$

where $I$ is current (A), and $F$ is Faraday's constant (96487 C/mol). The change of current with time is obtained by combining Eqs. 14 and 17:

$$I = FE = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \tag{18}$$

$$C_1 = FC_1 \tag{19}$$

$$C_2 = FC_2 \tag{20}$$

According to Ohm’s law, total resistance in Fig. 1b is represented as the sum of the exponential function of the surface compartment (i.e., surface resistance) and the skin compartment (i.e., skin resistance) under constant voltage.

$$R^{-1} = \frac{1}{V} \left( \frac{1}{V} \left( C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \right) \right) \tag{21}$$
MATERIALS AND METHODS

Materials and Animals  N-Vinyl acetamide sodium acrylate copolymer (PNVA) was purchased from Showa Denko K. K. (Tokyo, Japan). PNVA was a white powder with molecular weights of about 2300000 (number-average molecular weight; Mn), 2600000 (weight-average molecular weight; Mw) and 3000000 (z-average molecular weight; Mz), respectively. All other commercially available chemicals of analytical grade were obtained from dealers. Silver and silver chloride electrodes (Ionview) were bought from ADVANCE Co., Ltd. (Tokyo, Japan). Male Wistar rats (8 weeks old, 200 g) were purchased from Charles River Japan, Inc. (Tokyo, Japan).

Preparation of the Adhesive Pad  Sodium chloride (0.05, 0.1, 0.2 g), tartaric acid (0.2 g) and carrageenan (0.5 g) were dissolved in purified water (50 g) at 50 °C (water phase). Meanwhile, anhydrous aluminum hydroxide gel (0.4 g), hydroxypropylcellulose (3 g) and PNVA (10 g) were suspended in macrogol 400 (30 g) at room temperature (oil phase). After the water phase had cooled to room temperature, the oil phase was added. Total weight was adjusted to 100 g by adding purified water. The mixture was shaken using a mixer (HIVISIMIX, Tokushukika Kogyo Co., Ltd., Osaka, Japan) at a combination of 40 rpm (revolution) and 80 rpm (rotation) for 10 min. The mixture was rolled 750 μm thick between the polyethylene liners, the surface of which was processed with silicone, using a roller machine (Ikeda Machine Industry Co., Ltd., Osaka, Japan). After the rolling, the liner on one side was peeled away. The silver and silver chloride electrodes were then installed on the mixture and the mixture cut circularly (1—3 cm²) with electrode and liner as shown in Fig. 1a. Thus, the adhesive pads were prepared.

Measurement of Resistance in Vivo  The rats were anesthetized with an intraperitoneal injection of pentobarbital (0.05 mg/g) and their abdominal hair was clipped. Two adhesive pads were applied to the abdominal skin with distance of 0.5—3 cm. The Ag (anode) and AgCl (cathode) electrodes were connected to a DC voltage current generator (Type 7651, Yokogawa Electric Co., Ltd., Tokyo, Japan) as shown in Fig. 1a. The experiments were carried out at a constant voltage. The voltage and current were measured with an oscillographic recorder (Type OR-100, Yokogawa Electric Co., Ltd., Tokyo, Japan) at intervals of 125 ms for 10 s. The data were transformed to Microsoft Excell 97 for windows using Handy OR Application (Yokogawa Electric Co., Ltd., Tokyo, Japan). Each resistance at intervals of 125 ms was calculated according to Ohm’s law using Microsoft Excell 97 for windows. The above mentioned time-resistance data were fitted to Eq. 21 using the non-linear approximation program, MULTI. Furthermore, the initial value and the rate constant of the surface and skin resistance were calculated.

RESULTS AND DISCUSSION

It is known that skin resistance drops remarkably in the short term and gradually long term when a direct current is applied. In previous studies,7—8) we reported that a reduction in barrier function enhanced the migration of drug by passive diffusion under iontophoretic conditions. In the present study, this phenomenon was represented by the sum of two exponential functions, and a new quantitative method to evaluate the barrier function was established.

Establishment of a Method for Evaluating the Effect of the Conductivity of an Adhesive Pad  The barrier function of skin is affected by various factors including hydration,16) pH,17) chemical materials,17) the concentration and charge of electrolyte,18) temperature,18) aging,19) sweat,20) dermatosis21) and electric factors. To establish the new method, experiments on the change in the conductivity of the adhesive pad at 6 V, a distance between electrodes of 1 cm and an electrode area of 7 cm² were performed.

The total resistance (Rt), which is the sum of the surface (R_SU) and skin resistance (R_SK), when sodium chloride (0.1%) was contained in the adhesive pad decreased remarkably in less than 1 s and gradually thereafter (Fig. 3). The initial value and rate constant of 6.37±0.028 s⁻¹. R_SU and R_SK were calculated using the non-linear approximation program.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Initial value (kΩ) R_SU</th>
<th>Rate constant (s⁻¹) R_SU</th>
<th>Initial value (kΩ) R_SK</th>
<th>Rate constant (s⁻¹) R_SK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.643±0.032</td>
<td>2.837±0.032</td>
<td>1.129±0.028</td>
<td>0.01±0.002</td>
</tr>
<tr>
<td>0.10</td>
<td>0.599±0.074</td>
<td>3.432±0.154</td>
<td>0.964±0.018</td>
<td>0.015±0.003</td>
</tr>
<tr>
<td>0.20</td>
<td>0.537±0.009</td>
<td>6.795±1.275</td>
<td>0.682±0.091</td>
<td>0.021±0.004</td>
</tr>
</tbody>
</table>

Fig. 3. The Plot of the Total Resistance, Rt, the Surface Resistance, R_SU, and the Skin Resistance, R_SK, versus Time with the Adhesive Pad Containing 0.1% Sodium Chloride (Voltage 6 V; Distance between Electrodes 1 cm, Electrode area 7 cm²).

These results were analyzed using the proposed method, and then the initial value and the rate constant of R_SU and R_SK were calculated (Table 1). With respect to R_SK, although the initial value (V_SU) decreased, the rate constant (k_SU) increased with the concentration. On the other hand for R_SK,
the initial value ($V_{SK}$) decreased, but the rate constant ($k_{21}$ in Fig. 2) changed little with the concentration. The reduction of $V_{SU}$ and $V_{SK}$, and the increase of $k_{12}$ contributed to the enhancement of conductivity (i.e., increase of sodium chloride concentration) in the skin. The reason that the $k_{21}$ changed little was explained by the 2-compartment model (Fig. 2). Thus, $k_{21}$ became small when $k_{12}$ was very large since SUC and SKC were a reversible reaction. These findings showed that the skin barrier function was appreciable by both $V_{SU}$ and $k_{12}$. Previous methods$^{9–12}$ used steady-state resistance, which corresponds to $Rt$ at seconds/hours. The method proposed here obtains the two parameters mentioned above in a short term. Thus, it could evaluate the reduction in skin barrier function since it changed remarkably in the short term.

**Evaluation of the Effect of Voltage** Inada et al. reported that voltage is most dependent upon the reduction of $Rt$, i.e., the barrier function.$^{12}$ To clarify the effect of voltage on the barrier function, experiments were performed at a distance between electrodes of 1 cm, an electrode area of 7 cm$^2$ and a concentration of sodium chloride of 0.2% in the adhesive pad.

Although $V_{SU}$ decreased with voltage, $k_{12}$ increased with one as shown in Fig. 4. These findings showed that the reduction of the barrier function depended on voltage. The analytic results of our proposed method were compared with those of previous methods.$^{9–12}$ $V_{SU}$ and the $Rt$ were plotted as a function of voltage (Fig. 5). The values of $Rt$ at 10 s were used because they were almost in equilibrium at that time. $V_{SU}$ had a negative linear relationship to voltage (●; $Y = -0.237x + 2.126, r^2 = 0.982$) in addition to $Rt$ (○; $Y = -0.189x + 1.716, r^2 = 0.993$). From the slope, standard error and correlation coefficient, it was recognized that $V_{SU}$ was almost same as $Rt$. These results suggested that the proposed method had accuracy and sensitivity similar to previous methods. In addition, it was shown that our method was useful since it obtained two parameters in the short term.

**Evaluation of Effect of Distance between Electrodes** Usually, iontophoretic pharmaceutical preparations consist of a donor containing drug and a receptor containing an electrolyte with an insulation distance. The distance affects the reduction in barrier function. The effect of the distance between electrodes was evaluated at 6 V, an electrode area of 7 cm$^2$ and a sodium chloride concentration of 0.2% in the adhesive pad.

Up until a distance of 1 cm, although $V_{SU}$ decreased, $k_{12}$ increased. Beyond 1 cm, however, although $V_{SU}$ increased, $k_{12}$ decreased (Fig. 6). These results showed that the skin barrier function changed with the distance between electrodes. Furthermore, a distance of 1 cm was most suitable across rat abdominal skin for the reduction in barrier function.

Taking into consideration the electrical properties of skin, the resistance is greatest in the stratum corneum, followed by the epidermis and then the dermis. In addition, current is supposed to pass through an area of low resistance, which is near to the skin surface. When the distance is short, the resistance increases because the current mainly passes through the stratum corneum. With the increase in distance, the resistance decreases since the current passes through the dermis and epidermis. Thus, the distance to minimize resistance depends on the thickness of the stratum corneum. This distance is important to the enhancement of the migration of a drug by iontophoresis.

**Evaluation of the Effect of Electrode Area** As shown in Eqs. 2 and 3, the resistance changes with electrode area. The effect of electrode area on barrier function was evaluated using the proposed method. Experiments were carried out at 6 V, a distance between electrodes of 1 cm and a sodium chloride concentration of 0.2% in the adhesive pad.

Although $V_{SU}$ decreased with electrode area, $k_{12}$ was almost constant as shown in Fig. 7. These findings suggested that $k_{12}$ little changed under same voltage and distance between electrodes because the current density of a topical electrode adjacent to the other electrode was high. Therefore,
to reduce the barrier function effectively, it is useful to increase the distance of the adjacent electrode. Further investigation of electrode shape is needed to prepare iontophoretic pharmaceutical preparations.

CONCLUSION

The reduction in skin barrier function caused by a direct current was evaluated using our proposed method, which measures short-term resistance. The resistance was taken as the sum of the surface \( R_{SU} \) and skin resistance \( R_{SK} \). Moreover, the initial value and rate constant of each resistance were calculated. The initial value \( V_{SU} \) and the rate constant \( k_{12} \) of \( R_{SU} \) could be used to evaluate the loss of barrier function using an adhesive pad containing sodium chloride. The barrier function decreased with voltage. In addition, the proposed method showed the accuracy and sensitivity similar to previous methods. The barrier function was affected by the distance between electrodes, which depends on the current pass portion in the skin. In addition, although the electrode area decreased \( V_{SU} \), \( k_{12} \) was little affected. These results suggest that current density was localized in the electrode portion adjacent to the other electrode. Using the proposed method, it was shown that an evaluation of the reduction in barrier function caused by direct current is possible in the short term with high sensitivity and accuracy. It is important to clarify the reduction in barrier function in detail. This is because the amount of drug that migrates by iontophoresis enhances due to the reduction in barrier function. In addition, the amount of drug delivered may be evaluated quantitatively. Further investigation of current application conditions (i.e., pulse) and electrode shape is needed to prepare iontophoretic drug delivery systems.

REFERENCES