Two-Layer Membrane Model for Iontophoretic Drug Transport through Excised Rat Skin

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Iontophoretic and passive transport of an ionized drug (sulfisoxazole) across excised rat skin was studied using a two-chamber cell with four electrodes under successive experimental conditions: without electrical current (stage-I) and with electrical current (stage-II). Two iontophoretic/diffusion models, i.e. a one-layer membrane model and a two-layer membrane model, in which a difference in the electrical potential gradient was taken into account between the stratum corneum and epidermis/dermis layer, were constructed to describe the non-steady-state drug permeation process during iontophoresis. The observed iontophoretic lag-time was two times greater than the calculated value based on the one-layer membrane model. According to the two-layer membrane model, the calculated iontophoretic lag-time agreed with the observed value. It was revealed by model adaptation to the observed data that the stratum corneum fraction of the electro-chemical potential difference across the whole skin caused by the iontophoresis was around 90%. This result was consistent with the observation that the direct current resistance of whole skin was seven times greater than that of stripped skin.

Keywords iontophoresis; sulfisoxazole; two-layer-membrane model; lag-time; FILT

Introduction

For a quantitative expression of the iontophoretic drug permeation process across the skin, most authors have attempted to apply the Nernst-Planck flux equation, assuming that the skin is a uniform membrane which is under the influence of a uniform electric field. In our previous report on iontophoretic flux enhancement of sulfisoxazole (SIX) across excised rat skin, it was demonstrated that the steady-state flux of SIX during iontophoresis approximately correlated to the potential difference across the skin (E) using Goldman’s equation of a uniform single layer membrane.

From the viewpoint of electrical property and permeability, however, the skin is considered to be at least a two-layer membrane, i.e. the least conductive and permeable stratum corneum layer and epidermis/dermis layer.

The purpose of the present study was to verify the applicability of the two-layer membrane model for quantitatively expressing the permeation of SIX (pK_a = 1.55, pK_a = 5.10), used as a model ionized drug, across excised rat skin during iontophoresis. To characterize the transient process between two steady states of SIX transport (stage-I and stage-II), iontophoretic lag-time introduced in the previous study was utilized (Fig. 1).

Theoretical

Membrane Model for SIX Transport

The four types of membrane model employed in the present study are listed in Table I. For the respective models, the Laplace transform of the cumulative drug amount which permeated the skin (Q) are expressed as follow. Definitions of the symbols used in all the equations are listed in Table II.

\[
\hat{Q}_{1-x} = \frac{DKC_0}{s^2 \sinh(\lambda L)}
\]

\[
\lambda = \frac{s}{D}
\]

Fig. 1. Definition of Iontophoretic Lag-Time

\(t_0\), diffusional lag-time; \(t_1(0)\); \(t_1 - t_0\), iontophoretic lag-time; \(t_1(v)\).

\[
\tilde{Q}_{1-x} = \frac{DKC_0}{s^2 L} + \frac{D^2 KC_0}{s^3 L} \left[ \frac{v}{2} + \frac{\mu [\cosh(\mu L) - e^{\mu L}]}{\sinh(\mu L)} \right]
\]

\[
\nu = \frac{zFE}{RTL} + \frac{V_s}{D}, \quad \mu = \sqrt{\frac{\nu^2}{2}} + \frac{s}{D}
\]

\[
\tilde{Q}_{2-x} = \frac{C_0}{s^3} \left[ \frac{\cosh(\lambda_2 L_2) \sinh(\lambda_1 L_1) + \cosh(\lambda_1 L_1) \sinh(\lambda_2 L_2)}{D_1 K_1 \lambda_1} + \frac{D_2 K_2 \lambda_2}{D_2 K_2 \lambda_2} \right]
\]

\[
\lambda_j = \sqrt{\frac{s}{D_j}} \quad (j = 1, 2)
\]

\[
\tilde{Q}_{2-x} = \frac{C_{inf}}{s^2} + \frac{D_{2} C_{inf}}{s^3} \left[ A_2 R_1 e^{\xi_1 \lambda_1} + B_2 R_2 e^{\xi_2 \lambda_2} \right]
\]

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### Table I. Model Definitions

<table>
<thead>
<tr>
<th>Model</th>
<th>Partial differential equations</th>
<th>Initial and boundary conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-d</td>
<td>( \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} )</td>
<td>( [t=0] C = 0 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=0] C = KC_0 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=L] C = 0 )</td>
</tr>
<tr>
<td>1-i</td>
<td>( \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - Dv \frac{\partial C}{\partial x} )</td>
<td>( [t=0] C = KC_0 \left[ 1 - \frac{x}{L} \right] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=0] C = KC_0 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=L] C = 0 )</td>
</tr>
<tr>
<td>2-d</td>
<td>( \frac{\partial C_1}{\partial t} = D_1 \frac{\partial^2 C_1}{\partial x^2} )</td>
<td>( [t=0] C_1 = 0 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=-L_1] C_1 = K_1 C_0 )</td>
</tr>
<tr>
<td></td>
<td>( \frac{\partial C_2}{\partial t} = D_2 \frac{\partial^2 C_2}{\partial x^2} )</td>
<td>( [x=0] K_2 C_1 = K_2 C_2 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=L_2] C_2 = 0 )</td>
</tr>
<tr>
<td>2-i</td>
<td>( \frac{\partial C_1}{\partial t} = D_1 \frac{\partial^2 C_1}{\partial x^2} - D_1 v_1 \frac{\partial C_1}{\partial x} )</td>
<td>( [t=0] C_1 = K_1 C_0 \left[ \frac{L_2 - x}{D_1 K_2} \right] )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=-L_1] C_1 = K_1 C_0 )</td>
</tr>
<tr>
<td></td>
<td>( \frac{\partial C_2}{\partial t} = D_2 \frac{\partial^2 C_2}{\partial x^2} - D_2 v_2 \frac{\partial C_2}{\partial x} )</td>
<td>( [x=0] K_2 C_1 = K_2 C_2 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( [x=L_2] C_2 = 0 )</td>
</tr>
</tbody>
</table>

### Materials and Methods

**Chemicals** Sulfoxazole (JP XII, Yamanouchi Pharm. Co., Tokyo) was obtained commercially. Trypsin (Purified Pancreas, type IX, Sigma Chemical, St. Louis) was purchased commercially and was used without further purification. Other reagents used for buffers, i.e., disodium phosphate, citric acid and sodium chloride, were of analytical grade.

**Diffusion Cell** Diffusion cells with four electrodes, described in the previous report,\(^{20}\) were used in all the transport studies. A 5.6 cm\(^2\) area of excised skin was exposed to the donor and acceptor compartments of each diffusion cell. The reservoirs (15.6 ml each) were stirred mechanically by external electric motors (Oriental Motor Co., Ltd., Tokyo). Diffusion cells were immersed in a water bath maintained at 32 ± 0.5°C to simulate the condition in which a transdermal therapeutic system is used. A set of electrodes consisted of an anode, a cathode and two reference electrodes. The anode and cathode were each positioned 4 cm from the side of the skin; the cathode was placed on the epidermal side and the anode on the dermal side. The reference electrodes were attached carefully to the skin so as not to damage its surface. Constant electric potential was supplied by a programmable constant electric potential source (Seto Electric Co., Toyama). The pH of the reservoirs was monitored by a pH meter (Horiba F-7 type, Kyoto) during iontophoresis and was maintained within 7.4 ± 0.1.

**Skin Preparation** Male albino rats (Wistar strain, Shizuoka Laboratory Animal Service Center, Hamamatsu) weighing 250—300 g were used. Three different skin samples, i.e., whole skin, stripped skin and isolated stratum corneum, were used. Stripped skin was prepared according to the method of Washitake et al.\(^{21}\) using cellophane adhesive tape. Isolated stratum
TABLE II. Symbols and Definitions

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$</td>
<td>Drug concentration in the donor compartment, $\mu$g/ml</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion coefficient in the membrane, cm$^2$/s</td>
</tr>
<tr>
<td>$E$</td>
<td>Electric potential difference across the membrane, V</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday’s constant</td>
</tr>
<tr>
<td>$J(0)$</td>
<td>Steady-state permeation rate of drug through the skin in stage-I (diffusional), $\mu$g/cm$^2$</td>
</tr>
<tr>
<td>$J(v)$</td>
<td>Steady-state permeation rate of drug through the skin in stage-II (iontophoretic), $\mu$g/cm$^2$</td>
</tr>
<tr>
<td>$K$</td>
<td>Partition coefficient of drug to the membrane</td>
</tr>
<tr>
<td>$L$</td>
<td>Thickness of the membrane, cm</td>
</tr>
<tr>
<td>$Q$</td>
<td>Cumulative amount of drug permeated, $\mu$g/cm$^2$</td>
</tr>
<tr>
<td>$R$</td>
<td>Gas constant</td>
</tr>
<tr>
<td>$s$</td>
<td>Variable of Laplace transform</td>
</tr>
<tr>
<td>$T$</td>
<td>Absolute temperature</td>
</tr>
<tr>
<td>$t_c(0)$</td>
<td>Diffusional lag-time, h</td>
</tr>
<tr>
<td>$t_i(v)$</td>
<td>Iontophoretic lag-time, h</td>
</tr>
<tr>
<td>$V_x$</td>
<td>Volume flow parameter, cm$^3$/s</td>
</tr>
<tr>
<td>$z$</td>
<td>Ionic valence</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Iontophoretic factor, including effects of voltage drop and volume flow, cm$^{-1}$</td>
</tr>
</tbody>
</table>

Subscripts:
- 1: stratum corneum layer
- 2: epidermis/dermis layer
- d: One-layer-diffusion
- i: One-layer-iontophoresis
- t: Two-layer-diffusion
- T: Two-layer-iontophoresis

The corneum was prepared according to Knutson et al., incubating the excised skin in 0.5% trypsin solution for 4 h at 37°C. The thickness of the whole/stripped skin was measured using a micrometer, before and after the transport studies. The thickness of the stratum corneum and total skin was measured using a micrometer.

Determinant of Lag-Time The values of diffusional and iontophoretic lag-time were evaluated graphically from the time course of the cumulative amount of drug which permeated the skin over time. 

Results Observed Data The mean values of the cumulative amount of SIC which permeated the subcutaneous skin with and without electric current exposure (Table III, exper. 1-I and 1-II), and the mean values of the cumulative amount of SIC which permeated the stripped skin (exper. 2) are shown in Figs. 2 and 3, respectively.

Adaptation of One-Layer Membrane Model Assuming that the skin is a homogeneous membrane and the electric potential gradient is constant throughout the skin, the adaptability of the one-layer membrane model (model 1-d for stage-I and model 1-i for stage-II) to the observed data shown in Fig. 2 was examined.

Table III. Experimental Conditions and Estimated Parameters

<table>
<thead>
<tr>
<th>Exper.</th>
<th>Skin</th>
<th>Potential across skin (V)</th>
<th>Known parameters</th>
<th>Estimated parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-I$^a$</td>
<td>Intact</td>
<td>0</td>
<td>$C_0$, $L$, $J(0)$, $D$, $K$</td>
<td></td>
</tr>
<tr>
<td>1-II$^a$</td>
<td>Intact</td>
<td>0.3</td>
<td>$J(v)$, $t_i(v)$, $\nu$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Stripped</td>
<td>0</td>
<td>$C_0$, $L_2$, $D_2$, $K_2$</td>
<td></td>
</tr>
<tr>
<td>1-I</td>
<td>Intact</td>
<td>0</td>
<td>$C_0$, $L_1$, $D_1$, $K_1$</td>
<td></td>
</tr>
<tr>
<td>1-II</td>
<td>Intact</td>
<td>0.3</td>
<td>$C_0$, $L_1$, $D_1$, $K_1$, $v_1$, $v_2$</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Cumulative Amount of SIX Which Permeated Excised Rat Whole Skin in Stage-I and Stage-II

(a) stage-I, (b) stage-II. The potential difference across the skin was regulated automatically to 0.3 V in stage-II. The net amount of SIX which permeated the skin with electric current is plotted. The dotted square point and solid curve represent the theoretical value calculated with the parameters of Table V.

a) Exper. 1-I and 1-II were successive experiments. b) $\nu$ value was estimated from flux enhancement ratio using Goldman’s equation, $J(v)/(J(0)) = vL/(1 - \exp(-vL))$, since the model adaptation failed.
parameters of the epidermis/dermis layer ($D_2$ and $K_2$) were estimated. Then, with the $D_2$ and $K_2$ values fixed, model 2-d was adapted to the observed data of exper. 1-I, and the parameters of the stratum corneum ($D_1$ and $K_1$) were estimated. Finally, iontophoretic parameters ($v_1$ and $v_2$) were estimated by the adaptation of model 2-i to the observed data of exper. 1-II, with all the other parameters fixed. Parameters estimated by the least squares adaptation of the two-layer membrane model are shown in Table V. The estimations of the two-layer membrane model using the obtained parameters are shown in Fig. 2 with solid curves. The iontophoretic lag-time $t_L(v)$ based on the two-layer membrane model was calculated with the procedure shown in the Appendix using the parameter values of Table V. The calculated lag-time (iontophoretic) was 0.3438 h, comparable with the observed lag-time of 0.326 ± 0.118 h.

TABLE IV. Comparison of Calculated and Observed Iontophoretic Lag-Time

<table>
<thead>
<tr>
<th>Enhancement ratio</th>
<th>Iontophoretic factor, $v$ (cm$^{-1}$)</th>
<th>$t_L(v)$ Calculated (h)</th>
<th>$t_L(v)$ Observed (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J(v)/J(0)$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.67 ± 2.65</td>
<td>121 ± 46</td>
<td>0.143 ± 0.075</td>
<td>0.326 ± 0.118</td>
</tr>
</tbody>
</table>

a) Each value represents the mean value of 5 experiments ± S.D. b) Difference between calculated and observed $t_L(v)$ is significant ($p < 0.01$).

TABLE V. Estimated and Other Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion coefficient (10$^{-7}$ cm$^2$/s)</td>
<td></td>
</tr>
<tr>
<td>Stratum corneum $D_1$</td>
<td>0.394 ± 0.282</td>
</tr>
<tr>
<td>Epidermis/dermis $D_2$</td>
<td>4.97 ± 0.70</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td></td>
</tr>
<tr>
<td>Stratum corneum $K_1$</td>
<td>0.00523 ± 0.00377</td>
</tr>
<tr>
<td>Epidermis/dermis $K_2$</td>
<td>0.878 ± 0.141</td>
</tr>
<tr>
<td>Iontophoretic factor (cm$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>Stratum corneum $v_1$</td>
<td>2761 ± 23</td>
</tr>
<tr>
<td>Epidermis/dermis $v_2$</td>
<td>10.05 ± 0.80</td>
</tr>
<tr>
<td>Membrane thickness (cm)</td>
<td></td>
</tr>
<tr>
<td>Stratum corneum $L_1$</td>
<td>0.002 ± 0.000</td>
</tr>
<tr>
<td>Epidermis/dermis $L_2$</td>
<td>0.042 ± 0.004</td>
</tr>
</tbody>
</table>

The diffusivity in the epidermis/dermis layer is 10 times greater than that in the stratum corneum layer. In addition, the partition coefficient of the epidermis/dermis layer is 200 times greater than that of the stratum corneum layer. As shown in Table VI, the observed direct-current resistance of the whole skin was 7 times greater than that of the stripped skin, indicating that 90% of the whole-skin electric potential drop occurred in the stratum corneum layer during iontophoresis.

Estimation of the trans-stratum-corneum-layer and trans-epidermis/dermis-layer potential difference was attempted by the parameter values of Table V, using the volume flow parameters reported in the previous report. Results are shown in Table VII. Although the exact volume flow parameter is not known, it is clear that the effect of the volume flow to the iontophoretic permeation of SIX was small and that 90% of the whole-skin potential difference occurred in the stratum corneum layer, supporting the results of Table VI. These facts confirm again that the stratum corneum is the main barrier for the passive permeation of SIX and that the flux enhancement caused by the electric potential gradient occurs mainly in the stratum corneum.

Discussion

In the studies of passive drug permeation through the skin, it has frequently been assumed that the skin is a homogeneous membrane. This is because the homogeneous membrane model is simple and easy to handle. In the studies of drug permeation during iontophoresis, too, the skin has been assumed to be a homogeneous single layer. For the calculation of a flux enhancement factor by iontophoresis, Srinivasan et al. used Goldman’s equation, which is based on the assumption that the skin is a homogeneous membrane and the electric potential gradient is constant through the skin. In the present study, however, the iontophoretic factor...
v, which was estimated from the flux enhancement ratio assuming a homogeneous membrane (one-layer membrane model), was unable to properly simulate the observed iontophoretic lag-time (Table IV).

In actuality, the electric resistance of the stratum corneum is much larger than that of epidermis/dermis layer, and the homogeneous assumption is not free from argument. Tojo et al.8) asserted that the skin should be treated as at least a two-layer membrane, consisting of the stratum corneum and a viable epidermis/dermis layer, to elucidate the concentration profiles through the skin under passive diffusion, because some drugs are metabolized only in the viable skin and, in addition, the adsorption and enzymatic processes occurring in the skin are quite different in stratum corneum and in viable skin. Assuming the two-layer membrane model of the present study, it was revealed that the theoretical lag-time (iontophoretic, 0.3438 h) calculated by the model parameters of Table V was close to the observed value (0.326 ± 0.118 h).

The estimated potential difference across the whole skin \((E_1 + E_2)zF/RT\) was 6.03–6.36 (Table VII), whereas theoretical predictions are: \(E_1 + E_2 = 3.0 V, z = 1\) eq/mol \(F = 23.06 \times 10^3\) cal/V·eq, \(R = 1.987\) cal/mol·deg, \(T = 305\) deg and \((E_1 + E_2)zF/RT = 11.41\). The discrepancy is not big, a factor of 1.9.

According to Srinivasan et al.,1) the estimated enhancement factors for tetraethylammonium (TEA), citrate and butyrate are, in general, lower than the theoretical predictions, but within a factor of 2. The agreement appears to be much better for the TEA-ion (cation) than for the citrate or butyrate ion (anion). Since the model drug used in the present study, SIX, is an anion in the physiological environment, the discrepancy among the observed and the predicted potential difference within a factor of 2 is acceptable.

The low value of the volume flow, \(V_s = -5.22 \times 10^{-7}\) cm/s (Table VII) gives a \(V_s/D_1\) value of \(-13.2\) cm\(^{-1}\) and a \(V_s/D_2\) value of \(-1.05\) cm\(^{-1}\). In the iontophoretic factors of 2761 cm\(^{-1}\) (\(v_1\)) and 10.05 cm\(^{-1}\) (\(v_2\)), the contributions of the volume flow are 0.4% and 10.4%, respectively. For the high value of the volume flow, the contributions of the volume flow are 2.8% in \(v_1\) and 61.3% in \(v_2\). It was confirmed again that the effect of the applied voltage drop is greater than the water transport contribution, which is a secondary effect, especially in the stratum corneum layer.

Through the investigation of iontophoretic lag time in the present study, it was indicated that the difference in the electric potential gradient and diffusivity between the stratum corneum and epidermis/dermis layers affect the time course of the cumulative drug amount which permeates the skin. Consequently, it is concluded that the application of a two-layer membrane model, such as the one presented in this study, is necessary.

Appendix

Evaluation of Lag-Time Mathematical Procedure The Laplace transform of the cumulative drug amount which permeated the skin \((Q)\) is the form of Eq. 1A, where the denominator is assumed as Eq. 2A.

\[
Q = \frac{c_0}{s^2} \left[ \frac{f(s)}{g(s)} \right] \tag{1A}
\]

\[
g(s) = (s + a_1)(s + a_2)\cdots(s + a_n) \tag{2A}
\]

According to Crank,29) Eq. 1A is expanded as Eq. 3A, which is inversely transformed to Eq. 4A.

\[
Q = c_0 \left[ \frac{1}{s^2} \frac{f(s)}{g(s)}_{s=0} \right] + \frac{d}{ds} \left[ \frac{f(s)}{g(s)}_{s=0} \right] + \sum_{i=1}^{n} \frac{f(a_i)}{a_i^2} e^{-a_i s} \tag{3A}
\]

\[
Q = c_0 \left[ \frac{f(s)}{g(s)}_{s=0} \right] + \frac{d}{ds} \left[ \frac{f(s)}{g(s)}_{s=0} \right] + \sum_{i=1}^{n} \frac{f(a_i)}{a_i^2} e^{-a_i s} \tag{4A}
\]

Since the third term of Eq. 4A becomes negligible because of its large \(t\) value, the steady-state drug permeation is expressed by Eq. 5A and the lag-time is given by Eq. 6A.

\[
Q = c_0 \left[ \frac{f(s)}{g(s)}_{s=0} \right] + \left[ \frac{d}{ds} \left[ \frac{f(s)}{g(s)}_{s=0} \right] \right] \tag{5A}
\]

\[
\text{lag-time} = \left[ \frac{d}{ds} \left[ \frac{f(s)}{g(s)}_{s=0} \right] \right] \tag{6A}
\]

One-Layer Membrane Model Applying the above procedure to Eq. 2 in the text, Eq. 7A is obtained as presented in the previous report.41)

\[
t_l(v) = \frac{L}{D v} \left[ \frac{1 + e^{-v t_l}}{1 - e^{-v t_l}} \right] \left[ \frac{1 - e^{-v t_l}}{v L} \right] \tag{7A}
\]

Two-Layer Membrane Model After the expansion of Eq. 4 in the text, the above procedure is applied to the resulting equation and the lag-time is evaluated numerically.

References