Skin Penetration Flux and Lag-Time of Steroids Across Hydrated and Dehydrated Human Skin in Vitro

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To study the effect of hydration on skin absorption, we investigated penetration across human skin of twelve model chemicals having steroid structure but different molecular weight and compared the steady-state penetration rate (J) and lag-time (t) across hydration intact skin (J d and t d) with that across dehydrated intact skin (J h and t h). Stratum corneum (SC) thickness of hydrated (52 μm) is 3.3 times that of dehydrated skin (16 μm). Transepidermal water loss (TEWL) of hydrated (7.6±2.1 g/m²/h) is twice that of dehydrated skin (3.4±1.6 g/m²/h, p<0.05) which are similar to in vivo values, suggesting the SC barrier function was recovered. The ratio of J d/J h ranged between 0.7 and 3.6 (average of 1.9). On the other hand, the ratio of t d/t h was almost constant (average of 0.8). Ratios of J d/J h and t d/t h were independent of MW and K ow. In percutaneous absorption experiments in vitro, skin was preserved in culture medium until use and SC might swell during that time. Therefore, we consider the possibility that J and t varied between hydrated and dehydrated skin. We confirmed the difference of J and t between hydrated and dehydrated skin in vitro and now need to define these results under in vivo condition.

Key words hydrated skin; dehydrated skin; penetration flux; lag-time; in vitro experiment; transepidermal water loss (TEWL)

Skin has a outermost thin layer, stratum corneum (SC), and underlayed viable epidermis and dermis. Because the SC is highly lipophilic, dry and a relatively effective percutaneous barrier, skin penetration is influenced by physicochemical properties of compounds. Hydrophobic compounds penetrate more easier than hydrophilics and lower than high molecular weight. 1 Relationships between penetration flux and physicochemical properties include: lipophilicity, 2 melting point, 3 molecular weight, 4 and pH of skin and vehicle. 5 Water content of SC is 30 to 50% (w/w) of SC dry weight in vivo 6 and is varied when occluded by a water impermeable membrane and stored into water (e.g. phosphate buffered saline) to be used to skin transplantation and percutaneous absorption experiments in vitro. Increasing SC hydration alters barrier function, hence often increasing percutaneous absorption in vitro. 7 Bucks and Maibach, reporting the effect of occlusion on percutaneous absorption in human in vivo, discussed that the skin occlusion caused SC hydration did not necessarily increase percutaneous absorption of hydrophilic compounds. 8 Thus, the SC hydration is an important factor to reveal the in vivo/in vitro correlation of percutaneous absorption.

This study investigated a procedure to prepare dehydrated skin from hydrated skin stored in culture medium for some days and the effect of skin hydration on skin penetration of twelve steroids as model chemicals because they had a similar basic structure and different molecular weight.

MATERIALS AND METHODS

Materials We selected twelve steroids having a different molecular weight. Physicochemical properties of these chemicals were summarized in Table 1. Polyethylene glycol 400 (PEG400) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Methanol (HPLC grade), acetonitrile (HPLC grade), and distilled water (HPLC grade) were purchased from Fisher Chemicals (Fair Lawn, NJ, U.S.A.).

Skin Preparation Human cadaver skin samples (leg or back) were obtained from 39 Caucasian males between the age of 21 and 81 years old (average age with S.D., 53.9±15.6 years) at the Northern California Transplant Bank (Oakland, CA, U.S.A.). The skin samples were kept in MEM Eagle’s medium with Earle’s BSS (MEM medium) at 4 °C. SC of skin samples was hydrated because of preservation in MEM medium more than 2 d and we set the limitation of skin use on 5 d to avoid skin deterioration. We prepared three types of samples: stripped skin, hydrated intact skin and dehydrated intact skin. Stripped skin was obtained by SC stripping thirty times using adhesive tape (#1527-1, 3M Health Care). Hydrated intact skin was immediately used of storage in MEM medium. Dehydrated intact skin was prepared as follows: hydrated intact skin was placed on a 20 ml sample

Table 1. Physicochemical Properties of Model Chemicals

<table>
<thead>
<tr>
<th>M.W.</th>
<th>logK ow</th>
<th>Cs in 40% PEG400 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESE</td>
<td>270.36</td>
<td>2.76 a</td>
</tr>
<tr>
<td>ESL</td>
<td>272.37</td>
<td>2.69 a</td>
</tr>
<tr>
<td>AND</td>
<td>286.40</td>
<td>2.75 a</td>
</tr>
<tr>
<td>TES</td>
<td>288.41</td>
<td>3.31 a</td>
</tr>
<tr>
<td>ETE</td>
<td>296.39</td>
<td>4.06±0.10</td>
</tr>
<tr>
<td>PRO</td>
<td>314.45</td>
<td>3.84±0.05</td>
</tr>
<tr>
<td>COC</td>
<td>346.45</td>
<td>2.00±0.01</td>
</tr>
<tr>
<td>PNS</td>
<td>358.44</td>
<td>1.46 a</td>
</tr>
<tr>
<td>PNL</td>
<td>360.44</td>
<td>1.49±0.04</td>
</tr>
<tr>
<td>COR</td>
<td>360.46</td>
<td>1.47 a</td>
</tr>
<tr>
<td>HYC</td>
<td>362.47</td>
<td>1.53 a</td>
</tr>
<tr>
<td>BET</td>
<td>392.45</td>
<td>2.02±0.03</td>
</tr>
</tbody>
</table>

a) LogK ow are cited data of Hansch and Leo, 1979. b) Saturated concentrations Cs are measured at 37 °C. ESE, estrone; ESL, 17β-estradiol; AND, androstenedione; TES, testosterone; ETE, ethinyl estradiol; PRO, progesterone; COC, corticosterone; PNS, pindinone; PNL, pindinosone; COR, cortisone; HYC, hydrocortisone; and BET, betamethasone.

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vial filled with MEM medium, that is, dermis side kept in contact with MEM medium and desiccated air, respectively. This sample keeps at 4 °C for 72 h.

### Transepidermal Water Loss Measurements in Vitro

To ensure the restoration from hydrated to dehydrated skin, transepidermal water loss (TEWL) was measured using an evaporimeter (Tewameter TM210, Courage & Khazaka, Germany) at 0, 24, 48, 72, and 96 h after skin preparation started in desiccator. Because the dehydrated skins were prepared at 4 °C, skin samples were left at room temperature for 30 min and then TEWL was measured. Measurements were performed at 23 ± 2 °C room temperature and relative humidity of 57 ± 3%.

**In Vitro Skin Penetration Experiments**  Skin samples were mounted between the two half cells of in vitro side-by-side permeation system (the effective volume is 5 ml and the maximum difference (Δ)) and lag-time (t), x-axis of J vs. time plot, for hydrated skin (Jh and th) vs. dehydrated skin (Jd and td) is shown in Table 3. The ratio of Jh/Jd ranged between 0.7 and 3.7 (average value of 1.8). On the other hand, the ratio of th/td is almost constant (average 0.8). Liner regression of Jh/td and th/td against MW and log Kow did not yield a good relationship (r²=0.001—0.024) (Figs. 3, 4).

### Effect of Hydration on Skin Penetration

Physicochemical properties of model chemicals are summarized in Table 1. Octanol/water partition coefficients (log Kow) of ESE, ESL, AND, TES, PNS, COR, and HYC are referred from Hansch and Leo.10) The ratio of steady-state penetration flux (J) and lag-time (t), x-axis of J on cumulative amount of penetrated vs. time plot, for hydrated skin (Jh and th) to dehydrated skin (Jd and td) is shown in Table 3. The ratio of Jh/Jd ranged between 0.7 and 3.7 (average value of 1.8). On the other hand, the ratio of th/td is almost constant (average 0.8).

#### RESULTS

**Effect of Hydration on Skin Thickness**  Skin thickness are 1.147±0.256 mm (stripped skin, n=77), 1.163±0.286 mm (dehydrated skin, n=67), and 1.199±0.319 mm (hydrated skin, n=79), respectively. SC thickness of hydrated skin (52 μm) are 3.3 times that of dehydrated skin (16 μm).

**TEWL Measurement**  Time course of TEWL under the condition of dehydrated skin preparation shows in Fig. 2. TEWL of hydrated skin (0 h) is 7.6 ± 2.3 g/m²/h. The values after 24 h become a almost constant (average 3.7 g/m²/h) and differ from hydrated skin, having a statistically significant difference (p<0.05).

**Effect of Hydration on Skin Permeability**  Permeants have different molecular weight, molecular structure, lipophilicity (solubility in skin and donor compartment), melting point, and ionization degree. These physicochemical properties affect skin permeability.1–4) Skin ab-
The effect of hydration and sorption is also influenced by skin condition; water contents (hydration) of SC\(^1\) and skin surface pH.\(^5\) Researchers have discussed the influence of physicochemical properties of drugs on percutaneous absorption \textit{in vitro} and \textit{in vivo}\(^3\) and reported the effect of hydration and \textit{in vitro} experimental conditions (pH and composition of vehicle). We selected twelve model chemicals having a similar steroidal structure but a different molecular weight (270.4 to 392.5) and log \(K_{ow}\) (1.46 to 4.06) to investigate the effect of hydration on skin penetration flux \(J\) and lag-time \(t\) under the same experimental conditions \textit{in vitro}.

TEWL, a water permeability of skin, has a correlation with percutaneous absorption flux.\(^{12}\) Thus, integrity of SC barrier function was investigated by a measurement of TEWL. Water overlaid on the SC of hydrated skin was evaporated and the water vapor lasted for 10 min at least.\(^{13}\) The skin samples were left at room temperature more than 30 min for TEWL was accurately measured in this study. TEWL of hydrated skin (7.6±2.1 g/m²/h) is obviously larger than that of dehydrated skin (24 h; 4.39±1.74, 48 h; 3.4±1.6 g/m²/h, and 96 h; 3.9±1.9 g/m²/h, Fig. 2) which are almost same values with back \(K_{ow}\) data.\(^{14}\) Thus, SC barrier is recovered by the procedure of dehydrated skin preparation for 24 h and over.

Hydration of SC increases \(J\) and slightly decreases \(t\) through the skin (Table 3). The ratio of \(J_h/J_d\) and \(t_H/t_d\) had no relation to MW, a range between 270.4 to 392.5, and log \(K_{ow}\) from 1.5 to 4.1 (Figs. 3, 4). This result may be caused by a narrow range of MW and log \(K_{ow}\). Thus, we will eventually
confirm a relation between a wide range of physicochemical properties and skin penetration parameters.

The SC is composed of dehydrated-flat cells (hydrophilic domains) in hydrophobic lipid domains. Intercellular lipids are arranged in a dense and orderly bilayer and influence skin barrier function. Most lipophilic permeants penetrate across these lipid domains. One mechanism of penetration enhancers is to disrupt the arranged lipid bilayers and enhance skin permeability. The dried and flat corneocytes in SC absorb water. SC thickness of hydrated skin was 3.3 times thicker than that of dehydrated skin in this study. The swollen SC increases its weight to 300 to 400 times and water are mainly absorbed within the corneocytes resulting in disrupted lipid bilayers. This is a reason why SC barrier function decreased with increasing contact time with water. On the other hand, the SC barrier function caused by lipid bilayers might return to its original state (Fig. 2) because we prepared the dehydrated skin from the hydrated skin under a mild condition (at 4 °C and for 72 h). We used 40% PEG400 solution to maintain sink conditions. This solution rapidly decreased skin water content, mainly viable epidermis and dermis, from 68% (wt/wt) to 24% (wt/wt) for a few hours. This rapid change of the water content in the skin may keep disordering lipid bilayers in hydrated skin.

Skin samples obtained from a skin bank for research were generally preserved in culture medium until use for in vitro penetration experiments. Therefore, we consider the possibility that J was overestimated and t was underestimated using hydrated skin. We confirmed the in vitro difference of J and t between hydrated and dehydrated skin and will define these results under in vivo conditions.

REFERENCES


