Impact of Humidity on *In Vitro* Human Skin Permeation Experiments for Predicting *In Vivo* Permeability

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ABSTRACT: *In vitro* skin permeation studies have been commonly conducted to predict *in vivo* permeability for the development of transdermal therapeutic systems (TTSs). We clarified the impact of humidity on *in vitro* human skin permeation of two TTSs having different breathability and then elucidated the predictability of *in vivo* permeability based on *in vitro* experimental data. Nicotinell® TTS® 20 and Frandol® tape 40mg were used as model TTSs in this study. The *in vitro* human skin permeation experiments were conducted under humidity levels similar to those used in clinical trials (approximately 50%) as well as under higher humidity levels (approximately 95%). The skin permeability values of drugs at 95% humidity were higher than those at 50% humidity. The time profiles of the human plasma concentrations after TTS application fitted well with the clinical data when predicted based on the *in vitro* permeation parameters at 50% humidity. On the other hand, those profiles predicted based on the parameters at 95% humidity were overestimated. The impact of humidity was higher for the more breathable TTS; Frandol® tape 40mg. These results show that *in vitro* human skin permeation experiments should be investigated under realistic clinical humidity levels especially for breathable TTSs. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:4223–4231, 2015
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INTRODUCTION

Today, numerous drugs including estradiol, clonidine, fentanyl, nicotine, testosterone, and tulobuterol are widely marketed as transdermal therapeutic systems (TTSs). TTSs have many advantages including reductions in the first-pass effect and gastrointestinal incompatibility, the ability to maintain a constant therapeutic drug level, and increased patient compliance. These advantages have, in turn, increased the popularity of these systems. *In vitro* permeation methods using human or animal skin have been commonly used to estimate *in vivo* permeability during the development of TTSs. The use of human skin for *in vitro* skin permeation experiments was recommended in a guidance note published by the Organization for Economic Cooperation and Development (OECD)[1] because of concerns over animal welfare. And there is a long history of *in vitro*/*in vivo* correlation analyses using *in vitro* human skin permeation experiments. In 1975, Franz reported that *in vitro* human skin permeability of 12 organic compounds approximately reflected the relationship of *in vivo* human skin permeability of these compounds.[2] After this report was published, the relationship of *in vitro* and *in vivo* human skin permeability have been elucidated.[3–5]

Franz-type diffusion cells have been widely used for *in vitro* permeation experiments.[6–8] A vertical Franz-type diffusion cell has also been used for evaluating TTSs, and the diffusion cell is maintained at a constant temperature using a recirculation device or incubator. The results of permeation experiments are likely affected by the laboratory environment when a recirculation device is used. The impact of temperature on *in vitro* hairless mouse skin permeation experiments has been evaluated by Tominaga and Tojo.[9] On the other hand, Chang and Riviere[10] reported that *in vitro* pig skin permeability of parathion increased when the *in vitro* skin permeation experiments was conducted under high humidity condition (over 70%).

The hydration of the stratum corneum is known to increase as the humidity level increases.[11] Consequently, the hydration of the stratum corneum of skin samples used for *in vitro* skin permeation experiments is likely to increase if the experiment is performed under a high humidity, thereby influencing the *in vitro* skin permeability. In this situation, the condition of the stratum corneum is thought to differ from that under clinical conditions, and the predicted *in vivo* skin permeability based on such experiments would likely not be accurate. Indeed, a humidity level of 30%–70% is recommended in the OECD guideline of *in vitro* skin permeation experiments.[12] From these results, it is quite likely that conducting *in vitro* skin permeation experiments under appropriate conditions is one of the most important points to predict *in vivo* skin permeability from *in vitro* skin permeability. However, the humidity impact on the predictability of plasma concentration–time profiles is actually still unclear. Additionally, the magnitude of the discrepancy in the prediction among TTSs with different breathability is also poorly understood.

The objective of this study was to clarify the impact of humidity on *in vitro* human skin permeation of two TTSs having different breathability and then elucidate the predictability of

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in vivo permeability based on *in vitro* experimental data. In the present study, the *in vitro* human skin permeability was compared under a clinical humidity level (approximately 50%) and under excess high humidity level (approximately 95%). We selected 95% humidity as excess high humidity level because it exceeds the recommended humidity in the OECD guideline[12] and it is maximum condition that we can conduct *in vitro* skin permeation experiments. Two different TTSs for water-soluble drugs (nicotine, log $K_{o/w}$: 1.20; and isosorbide dinitrate [ISDN], log $K_{o/w}$: 1.31) were selected, as water-soluble drugs were easily affected by the hydration of the stratum corneum.[13] Moreover, the selected two TTSs had different breathability. Finally, we predicted the human plasma concentration–time profiles of nicotine and ISDN after TTS application based on *in vitro* human skin permeation parameters to clarify the impact of humidity on the predictability of such profiles.

MATERIAL AND METHODS

Materials

Nicotine, ISDN, Nicotinell®TTS® 20 (Nicotinell), and Frandol®tape 40mg (Frandol) were purchased from Sigma–Aldrich Company (St. Louis, Missouri), Toronto Research Chemicals Inc. (North York, Ontario, Canada), Novartis Pharma K.K. (Tokyo, Japan), and Astellas Pharma Inc. (Tokyo, Japan), respectively. Other chemicals and solvents were of reagent grade and were obtained commercially.

Silicone membrane (500 μm thick) was purchased from Sanshin Enterprise Company, Ltd. (Tokyo, Japan).

Preparation of Human Skin Membranes

Frozen abdominal (six donors) and back (three donors) skin tissues excised from male Caucasians (50.1 ± 13.1 years old) were used in this study. The skins were purchased from Human & Animal Bridging Research Organization (Chiba, Japan). The human skins were used in accordance with the "World Medical Association Declaration of Helsinki" and with the permission of the Ethics Committee of Taisho (approved numbers: A11-04, A13-04). The frozen skins were stored at −80°C until use.

For the *in vitro* human skin permeation experiments using Frandol, human abdominal skin was used. Human back skin was used for the *in vitro* human skin permeation experiments using Nicotinell and the measurement of the transepidermal water loss (TEWL) of human skin. Dermatomed and dermatome-stripped skin membranes were prepared according to our previously reported methodology.[14] Briefly, excised skins were thawed in a 5% CO_2 incubator (humidity: 95%) at 32°C for 20 min and then dermatomed to a thickness of approximately 500 μm using an electric dermatome (Model B; Padgett Instruments, Inc., Kansas, Missouri). Dermatome-stripped skin membranes were prepared from the dermatomed skin membranes by tape stripping 20 consecutive times to remove the stratum corneum completely. The skin membranes were placed on a paper towel soaked in saline and stored in a sealed container at 4°C overnight. The skin membranes were then placed in a 5% CO_2 incubator (humidity 95%) at 32°C for 20 min before the start of the skin permeation experiment.

The thickness of the dermatomed skin membranes was measured using a dial thickness gauge (Model H; Osaki MFG, Company, Ltd., Tokyo, Japan). The exact thicknesses of the dermatomed skin membranes were 520 ± 18 μm (Nicotinell, 50% humidity), 592 ± 41 μm (Nicotinell, 95% humidity), 484 ± 18 μm (Frandol, 50% humidity), and 566 ± 57 μm (Frandol, 95% humidity). The stratum corneum thickness of the dermatomed skin membrane was determined microscopically using microtomed sections after hematoxylin-eosin staining. The exact thicknesses of the stratum corneum tissues were 13.3 ± 4.9 μm (Nicotinell, 50% and 95% humidity), 15.7 ± 0.5 μm (Frandol, 50% humidity), and 15.2 ± 0.5 μm (Frandol, 95% humidity). These exact thicknesses were almost the same as previously reported stratum corneum thicknesses.[15,16]

In Vitro Permeation Experiments Using Human Skin or Silicone Membranes

A Franz-type diffusion cell (internal diameter: 15 mm) was used for the *in vitro* permeation experiments. The receptor compartment was filled with 8.5 mL of distilled water. Each TTS was applied to the skin or silicone membrane, and the diffusion cell was maintained at 32°C in a 5% CO_2 incubator. The humidity level in the CO_2 incubator was adjusted to the clinical trial level (50%; average humidity in Japan) or 95%. In the case of Nicotinell, the clinical humidity level was estimated based on the weather report for the day of the experiment. The receptor compartment was mixed throughout the experiment using a Teflon stirrer driven by a 150 rpm constant speed motor. At designated times, 1.00 mL sample aliquots were taken from the receptor compartment and the same volume of distilled water warmed to 32°C was added to the receptor compartment.

Measurement of TEWL from Dermatomed Skin or Silicone membranes

The receptor compartment of the Franz-type diffusion cell was filled with 32°C distilled water and the human back skin was mounted on the cell. Approximately 10 min thereafter, the TEWL from the surface of the skin membrane was measured using a TEWAMETER TM300 (CK Electronic, GmbH, Cologne, Germany). Then, each TTS was applied to the skin and the TEWL from the surface of the TTS was measured.

The TEWL from the silicone membrane was measured same as the skin membrane. The silicone membrane was mounted on the cell filled with 32°C distilled water. Approximately 10 min thereafter, the TEWL from the surface of the silicone membrane was measured.

Determination of Nicotine and ISDN

Each 50 μL sample from the *in vitro* skin permeation experiment was mixed with 200 μL of acetonitrile/methanol (9:1, v/v) solution containing an internal standard. The mixed solution was then centrifuged (4°C, 3600*g*, 10 min), and the supernatant (nicotine 0.3 μL, ISDN 8 μL) was subjected to liquid chromatography–tandem mass spectrometry (LC–MS/MS). The LC–MS/MS system was constructed using an LC-30AD HPLC system (Shimadzu, Kyoto, Japan) and a Triple quad 5500 tandem mass spectrometer (AB SCIEX, Foster City, California). The LC–MS/MS conditions for nicotine and ISDN were as follows.

1. Nicotine

Thiamidine-d_3 was used as the internal standard for the measurement of the nicotine level. Chromatographic

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