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SHORT COMMUNICATION

Qualitative and quantitative comparison of heat separated epidermis and dermatomed skin in percutaneous absorption studies

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Abstract Transepidermal water loss (TEWL), mainly regulated by the stratum corneum, was quantitatively correlated to percutaneous absorption of compounds in human and suggested for the ex vivo assessment of skin integrity. The present study investigated qualitatively and quantitatively the relevance of 100-μm heat separated epidermis (HSE) in percutaneous absorption studies as compared to 500-μm dermatomed skin by dual complementary approaches. Percutaneous absorption of caffeine delivered from aqueous solution through dermatomed skin or HSE specimens (n = 9) was measured using vertical static diffusion cells coupled with an unventilated evaporimeter enabling the assessment of TEWL and skin integrity for 21 h. Permeation of caffeine exhibited different finite dose-like profiles ranged according to the thickness of skin specimens (cumulative dose absorbed up to 21 h: 11.5 ± 11.5 μg/cm^2 and 29.4 ± 36.2 μg/cm^2 through dermatomed skin and HSE, respectively). Normalized TEWL and caffeine fluxes were similar through dermatomed skin and HSE suggesting that the intrinsic permeability properties of both models were undifferentiated over time. Interestingly, a significant relationship was shown between TEWL and caffeine fluxes, suggesting the usefulness of TEWL measurement as an element in the estimation of percutaneous drug absorption. In conclusion, the present showed that percutaneous absorption through HSE was qualitatively and quantitatively similar to dermatomed skin when TEWL as endogenous standard and skin thickness were considered in permeability data comparisons.

Keywords Heat separated epidermis · Dermatomed skin · Caffeine · Transepidermal water loss · Skin permeability

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The Organisation for Economic Co-operation and Development (OECD) guidelines specify the use of split skin (e.g., dermatomed to about 0.5 mm) or the full thickness skin (not more than 1 mm thick) in percutaneous absorption studies [7]. However, the inter- and intra-variability of full skin thickness from human donors and skin animal species might compromise the comparison of skin absorption parameters (e.g., percentage of dose absorbed, apparent flux), whereas the use of dermatomed skin requires specialized staff [15, 16]. Therefore, taking into account its relative constant thickness, reconstructed epidermis was proposed for the determination of percutaneous uptake [13]. However, the permeation of caffeine and testosterone (i.e., reference compounds recommended by OECD in percutaneous penetration studies) through different reconstructed epidermis models was found higher than that determined through human epidermal sheet and full thickness porcine skin questioning the validity of such models in percutaneous absorption studies [13].

The present study examined the relevance of heat separated epidermis (HSE) in percutaneous absorption studies by using complementary procedures including caffeine permeation and transepidermal water loss (TEWL) measurements, as recommended by the OECD in test guidelines, in which TEWL allocates the evaluation of stratum corneum thickness [8], skin barrier function in chemical testing [7, 9] as an alternative to tritiated water permeation [5] and skin electrical resistance assay [7]. The permeation of caffeine as exogenous compound was analysed concomitantly to the water transport (i.e., TEWL) taken as an endogenous standard of dermatomed skin and HSE permeability [3].

Fresh dermatomed skin specimens (~500 μm in thickness) were prepared from human donors (three males, 68 ± 18 years) collected by the Northern California Transplant Bank (Oakland, CA, USA), then stored in media at 4°C and finally used within 12–48 h. Epidermis was separated from dermatomed skin specimens (2 cm × 6 cm) by heating to 55°C for 30 s in phosphate buffer saline solution. HSE was carefully removed from underlying tissue by using forceps, then transferred to petri dishes filled with phosphate buffer saline solution until use within 1 h. Both dermatomed skin and HSE (2 cm × 2 cm) were maintained in static vertical glass diffusion cell (surface area: 0.78 cm^2) kept at 37°C insuring a skin surface temperature of 32°C. The surface of the skin was opened to ambient air (24 ± 2°C under relative humidity of 30 ± 10%). The receptor compartment was filled with 3.5 ml of phosphate buffer saline solution continuously stirred with a magnetic stirrer. Therefore, dermatomed skin and HSE specimens were allowed to equilibrate for 2.5 h, then caffeine (Sigma, St Louis, MO, USA) aqueous solution (4 mg/ml) was deposited onto the surface (caffeine dose: 100 μg; caffeine dose per area unit: 128 μg/cm^2). The receptor fluids were withdrawn from the receptor compartments at regular intervals of time (0–21 h). The volume of fluid withdrawn was replaced by 800 μl of fresh receptor fluid. The top of the donor compartment was uncapped in order to avoid occlusion. Concomitantly, TEWL was measured using an unventilated evaporimeter (Vapometer Delfin SWL3 type, Delfin Technologies Ltd., Finland) for 21 h [11, 14]. Each permeation sample, filtered through 0.45 μm filter was analysed by high performance liquid chromatography (HPLC, Hewlett Packard serie 1100) system. Detection was performed at λ 272 nm. Caffeine assays were performed by using Eclipse® XDB C18 5 μm (4.6 × 150 mm) column (Agilent, Santa Clara, CA, USA). The mobile phase was a mixture (30/70, v/v) of methanol and 25 mM phosphate buffer solution (pH 5.2). Sample volume injected was 10 μl. The retention time of caffeine was 3.86 ± 0.03 min. For concentrations between 0.5 and 500 μg/ml, the chromatograms were linear with a factor correlation of 0.999 ($P < 0.001$).

Taking into account the maximum of caffeine solubility in water (i.e., ~22 mg/ml), the ex vivo study was performed under sink conditions in the receptor fluid reservoir (i.e., maximal theoretical concentration of caffeine in receptor solution, defined as the initial dose per receptor volume, ~30 μg/ml). Therefore, rates of percutaneous absorption were calculated as followed [1]:

$$J_t = \frac{1}{A} \cdot \frac{dQ}{dt} = \frac{1}{A} \cdot \frac{[Q_t - Q_0]}{(t - 0)} \quad (1)$$

where Q_t and Q_0 ($Q_0 = 0$) were respectively the cumulative amount of caffeine assayed in receptor fluid at time t and $t = 0$, A was the skin surface area, and J_t was the apparent flux of caffeine through skin specimens at time t. In order to compare the extent of permeation through different skin specimens (i.e., dermatomed skin versus HSE), the rates of percutaneous absorption were linearised as followed:

$$\text{Log}\ (J_t - J_{21\,\text{h}}) = \text{Log}\ (\Delta J) = -k \cdot t \quad (2)$$

where $J_{21\ \text{h}}$ was the flux calculated at 21 h and $t < 21$ h.

Similarly, the variations of TEWL through skin specimens as a function of time were plotted as followed:

$$\text{Log}\ (\text{TEWL}_t - \text{TEWL}_{21\text{h}}) = \text{Log}\ (\Delta\text{TEWL}) = -k' \cdot t \quad (3)$$

where $\text{TEWL}_{21\ \text{h}}$ was TEWL measured at 21 h $t < 21$ h.

The relationships between the logarithm of caffeine and TEWL flux variations versus time exposure were tested using regression analysis. The chosen level of significance was $P < 0.05$.

The profiles of percutaneous absorption of caffeine through dermatomed skin and HSE are in Fig. 1. The convex profiles of the caffeine permeation, exhibiting a linear increase then a plateau phase, suggested finite dose (10 and ~23% of the initial dose absorbed through dermatomed skin and HSE, respectively up to 21 h) and receptor sink conditions [10]. However, taking into account that caffeine exposure to the skin was experienced with a small donor volume (25 μl), the finite dose conditions should be re-considered as finite vehicle undergoing a dramatic decrease of caffeine fluxes whatever the nature of skin specimens. This interpretation was reinforced by the visual inspection of skin surface after 21 h showing detectable caffeine residues. Although no statistical differences were shown, the extent of caffeine permeation through skin specimens was ranged according to the thickness of the membrane (dermatomed skin: 11.5 ± 11.5 μg/cm^2; HSE: 29.4 ± 36.2 μg/cm^2 after 21-h skin exposure) confirming the relationship between the pathlength of diffusion and the total amount permeated. In consequence, the use of HSE

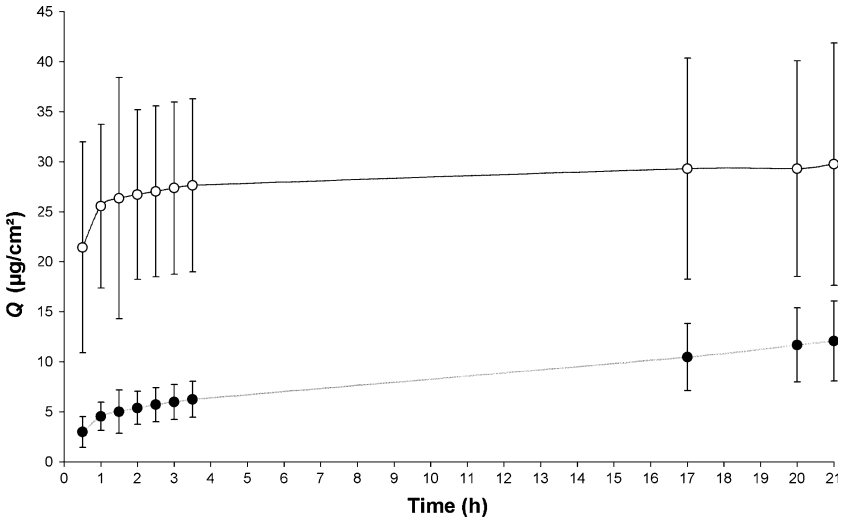

Fig. 1 Cumulative amounts of caffeine absorbed (*Q*) through dermatomed skin (*filled circle*) and heat separated epidermis (*HSE*) specimens (*open circle*) as a function of time. Each data is the mean ± standard error of the mean of 9 experimental determinations. The initial dose applied was 128 µg cm^{-2}. In respective to membrane thickness, higher permeation of caffeine through HSE was shown as compared to dermatomed skin

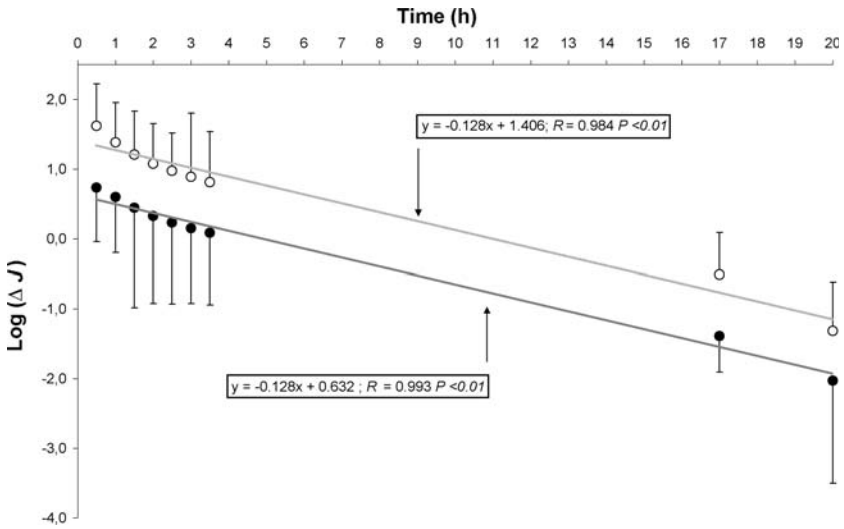

Fig. 2 Logarithmic variation of caffeine flux (Log (Δ*J*)) through dermatomed skin (*filled circle*) and HSE specimens (*open circle*) as a function of time (Eq. 2, see text for details). Caffeine fluxes at 21 h ($J_{21\ h}$) through dermatomed skin and HSE specimens were respectively 0.58 ± 0.57 µg cm^{-2} h^{-1} and 1.42 ± 1.73 µg cm^{-2} h^{-1}. Each data is the mean ± standard deviation of nine experimental determinations. Logarithmic variations of caffeine fluxes were comparable in both HSE and dermatomed skin suggesting a similar behaviour of both skin models in finite donor volume conditions

instead of dermatomed skin in percutaneous absorption studies enhanced quantitatively the extent of permeation data in relation to the tissue thickness.

Thus, the question arises whether the qualitative characteristic of permeation differed between the dermatomed skin and HSE. Figures 2 and 3 reported the logarithmic variation of caffeine and water fluxes through dermatomed skin and HSE as a function of time, respectively. Clearly, the normalized flux-time courses of exogenous (caffeine) and endogenous (water) compounds were comparable through dermatomed skin and HSE suggesting similar permeability properties in both skin models.

From conventional understanding, the main barrier to water permeability is primarily located in the stratum corneum which should be thus identical in dermatomed skin and HSE. However, lower TEWL was found through dermatomed skin as compared to HSE. Such discrepancies might be explained by the water-binding proteins (e.g., collagen) and free amino-acids in the dermis which restricted subsequent water transfer to superficial epidermis. Therefore, higher water transport through HSE was assumed by enhanced availability of unbound water for epidermal matrix.

Interestingly, the water fluxes through both membranes presented a dramatic decrease during skin permeation studies suggesting that the skin barrier function of both skin models varied likewise over time. The reduction of water fluxes during the skin exposure might be explained

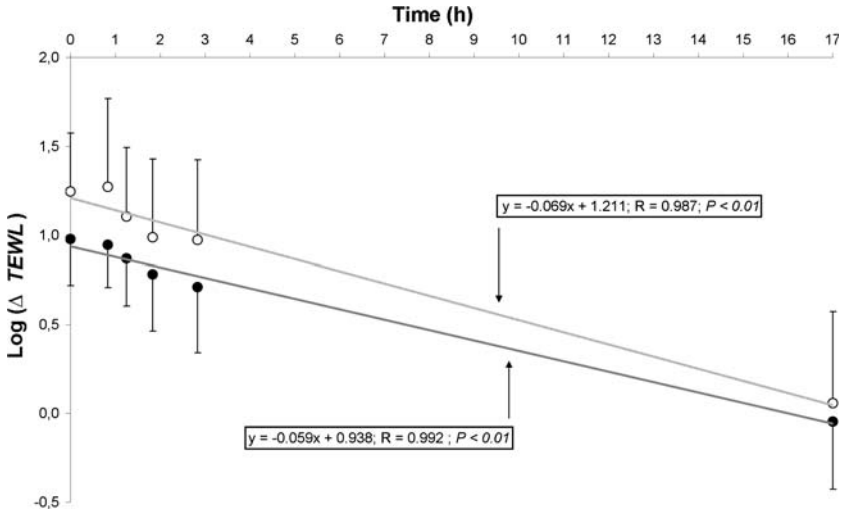

Fig. 3 Logarithmic variation of transepidermal water loss (Log (ΔTEWL)) through dermatomed skin (*filled circle*) and HSE specimens (*open circle*) as a function of time (Eq. 3, see text for details). TEWL at 21 h ($TEWL_{21\ h}$) through dermatomed skin and HSE specimens were respectively 5.5 ± 6.7 g m^{-2} h^{-1} and 4.0 ± 3.0 g m^{-2} h^{-1}. Each data is the mean ± standard deviation of nine experimental determinations. Logarithmic variations of TEWL were comparable in both HSE and dermatomed skin suggesting a similar behaviour of both skin models barrier function in finite donor volume conditions

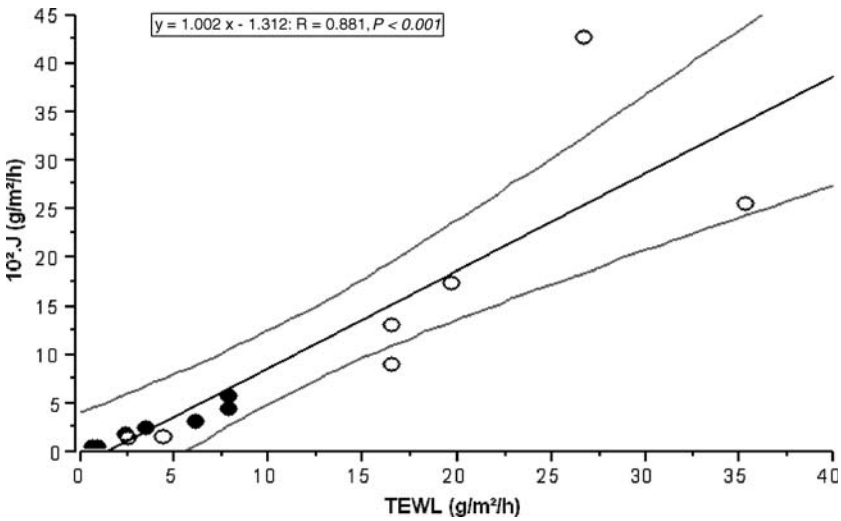

Fig. 4 Ex vivo relationship between TEWL and apparent caffeine flux through dermatomed skin (*filled circle*) and HSE specimens (*open circle*). Each data is the mean of seven experimental determinations (standard deviations are omitted for clarity reason). Caffeine permeation through HSE and dermatomed skin was strongly correlated to TEWL used as an endogeneous standard for the assessment of skin barrier properties

by the progressive dryness of the skin in spite of receptor fluid beneath the innermost parts of the membranes. During HSE preparation, the extraction of free amino-acids and/or the rearrangement of stratum corneum lipids, leading to a decrease of water holding capacity, might explain the characteristic brittleness of HSE observed at the end of the exposure [2]. Therefore, the decrease of caffeine fluxes (Fig. 2) might be explained by the finite volume of vehicle used and the concomitant change of skin barrier properties.

The suitability of TEWL measurements was investigated in vitro as a barrier integrity test for intact and punctured HSE [6]. The relevance of TEWL was questioned since no difference was detected through intact and punctured HSE as revealed by using an inert membrane (i.e., Teflon®), and because the transdermal flux of flufenamic acid was clearly affected by the mechanical damage. However, TEWL was only measured immediately before skin permeation study. As a result, punctual TEWL measurement (i.e., before topical treatment) and course-time permeation data seemed difficult to compare. For these reasons, in our study, both TEWL and permeation data were compared parallely all over the experiment to detect potential skin properties modifications. As observed, TEWL values decreased dramatically along with the time suggesting that both water and model drug fluxes should be considered concomitantly for more appropriate data analysis in transdermal studies. Therefore, as depicted in Fig. 4, our study supported previous works emphasising

the usefulness of TEWL to correlate and predict the percutaneous absorption of various drugs [4, 12].

In conclusion, the present study emphasized the feasibility of HSE use in percutaneous absorption studies. Permeation data of caffeine ponderated by TEWL measurements used as endogenous standard of permeability, showed no significant difference between dermatomed skin and HSE. Consequently, the data analysis in percutaneous absorption studies must considerer the apparent flux of exogenous compound comparatively to water flux measured as endogenous standard of skin integrity.

References

1. Collier S, Bronaugh RL (1991) Expression of absorption data. In: Bronaugh RL, Maibach HI (eds) In vitro percutaneous absorption: principles, fundamentals and applications. CRC Press Inc., Boca Raton, pp 157–164
2. Imokawa G, Kuno H, Kawai M (1991) Stratum corneum lipids serve as a bound-water modulator. J Invest Dermatol 96(6): 845–851
3. Levin J, Maibach HI (2005) The correlation between transepidermal water loss and percutaneous absorption: an overview. J Control Release 103(2):291–299
4. Nangia A, Berner B, Maibach HI (1999) Transepidermal water loss measurements for assessing skin barrier functions during in vitro percutaneous absorption studies. In: Bronaugh RL, Maibach HI (eds) Percutaneous absorption: drugs–cosmetics–mechanisms–methodology. Marcel Dekker, Inc., New York, pp 587–594
5. Nangia A, Patil S, Berner B, Boman A, Maibach HI (1998) In vitro measurement of transepidermal water loss: a rapid alternative to tritiated water permeation for assessing skin barrier functions. Int J Pharm 170(1):33–40
6. Netzlaff F, Kostka KH, Lehr CM, Schaefer UF (2006) TEWL measurements as a routine method for evaluating the integrity of epidermis sheets in static Franz type diffusion cells in vitro. Limitations shown by transport data testing. Eur J Pharm Biopharm 63(1):44–50
7. OECD (2004) OECD Guidance document for the conduct of skin absorption studies. OECD series on testing and assessment, Paris, pp 31
8. Pirot F, Berardesca E, Kalia YN, Singh M, Maibach HI, Guy RH (1998) Stratum corneum thickness and apparent water diffusivity: facile and noninvasive quantitation in vivo. Pharm Res 15(3):492–494
9. Pirot F, Kalia YN, Stinchcomb AL, Keating G, Bunge A, Guy RH (1997) Characterization of the permeability barrier of human skin in vivo. Proc Natl Acad Sci USA 94(4):1562–1567
10. Roberts M, Anissimov Y, Gonsalvez R (1999) Mathematical models in percutaneous absorption. In: Bronaugh RL, Maibach HI (eds) Percutaneous absorption: drugs–cosmetics–mechanisms–methodology. Marcel Dekker Inc., New York, pp 3–55
11. Rogiers V, Houben E, De Paepe K (2005) Transepidermal water loss measurements in dermato-cosmetic sciences. Bioengineering of the skin. CRC press, Boca Raton, pp 63–76
12. Rougier A, Lotte C, Maibach HI (1999) In vivo relationship between percutaneous absorption and transepidermal water loss. In: Bronaugh RL, Maibach HI (eds) Percutaneous absorption: drugs–cosmetics–mechanisms–methodology. Marcel Dekker, Inc., New York, pp 117–132
13. Schreiber S, Mahmoud A, Vuia A, Rubbelke MK, Schmidt E, Schaller M, Kandarova H, Haberland A, Schafer UF, Bock U, Korting HC, Liebsch M, Schafer-Korting M (2005) Reconstructed epidermis versus human and animal skin in skin absorption studies. Toxicol In Vitro 19(6):813–822
14. Shah JH, Zhai H., Maibach HI (2005) Comparative evaporimetry in humans. J Am Acad Dermatol Suppl Am Acad Dermatol Poster Abstr 52(3 Supplement 1):P98
15. van de Sandt JJM, van Burgsteden JA, Cage S, Carmichael PL, Dick I, Kenyon S, Korinth G, Larese F, Limasset JC, Maas WJM (2004) In vitro predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. Regul Toxicol Pharmacol 39(3):271–281
16. Wilkinson SC, Maas WJ, Nielsen JB, Greaves LC, van de Sandt JJ, Williams FM (2006) Interactions of skin thickness and physicochemical properties of test compounds in percutaneous penetration studies. Int Arch Occup Environ Health 79(5):405–413