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In vitro measurement of transepidermal water loss: a rapid alternative to tritiated water permeation for assessing skin barrier functions

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Abstract

Transepidermal water loss (TEWL), as measured with an evaporimeter, was used as a rapid assessment of the integrity of the barrier properties of skin as part of in vitro skin permeation studies. For a variety of physical and chemical treatments (i.e. solvent extraction, surfactants, mechanical abrasion and bases) TEWL correlated strongly with tritiated water permeation at short times. In contrast to the tedious process of measuring permeation of a finite dose of tritiated water, TEWL is a rapid, convenient measurement, and it provides a clear indication of the time dependence of barrier integrity. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

In the course of performing in vitro percutaneous absorption studies, it is important to ensure the integrity of the stratum corneum. In vitro assessment of the permeability of chemicals is often preceded by studying the rate of penetration of tritiated water into the test skin (Bronaugh et al., 1986; Scott et al., 1986). However, this technique has several limitations. For example, it results in hydrated stratum corneum possibly affecting the penetration of the compound under investigation. This method cannot be used if the test compound is tritium labeled. In addition, it is time-consuming and requires a license and facili-

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ties to handle radioactive materials. In practice, the repetitive measurement of tritiated water in laboratories is accomplished by determining the fraction of the dose applied absorbed at a fixed time. Selection of that fixed time is difficult without a priori knowledge of the permeability, and improper selection can lead to an erroneous assessment of the barrier properties of skin.

Measurement of transepidermal water loss (TEWL) with an evaporimeter is often used to evaluate the competency of the skin barrier in vivo (Leveque et al., 1979; Roskos et al., 1989) and provides a rapid and continuous measurement of the rate of water permeation. Investigators have also addressed the possibility of using this technique in vitro (Wilson et al., 1982; Moloney, 1988). In a study by Moloney, an evaporimeter was used to investigate the structural requirements of lipids, which are capable of altering the barrier functions and thus promoting skin permeability. Subtle changes in the skin barrier were observed with this device, suggesting TEWL's usefulness in detecting damaged skin. TEWL is also a relevant and sensitive indicator in vivo and in vitro for prediction of percutaneous absorption of other chemicals (Murahata et al., 1986; Lotte et al., 1987). It was thus reasonable to anticipate a close relationship between TEWL and the permeation of tritiated water through excised skin placed on a diffusion cell.

The present investigation evaluates the integrity of skin barrier functions by an evaporimeter before conducting percutaneous absorption studies in vitro. To evaluate TEWL as an indicator of skin damage, human cadaver skin slices were exposed to physical and chemical insults and were examined for TEWL. After altering the skin barrier, tritiated water flux measurements were obtained and correlated with the TEWL measurements.

2. Materials and methods

2.1. Materials

Norephedrine and imipramine hydrochloride were obtained from Aldrich, Milwaukee, WI. Naphazoline hydrochloride, antipyrine, and sodium lauryl sulfate (SLS, 99% pure) were purchased from Sigma Chemical, St. Louis, MO. Mecamylamine hydrochloride was donated by Merck, Sharp and Dohme, Rahway, NJ. For each drug, the free-base form was prepared by titration; the details may be found elsewhere (Berner et al., 1990; 1994). Antipyrine was used as received. Antipyrine, norephedrine, and sodium lauryl sulfate solutions were prepared in distilled water; the remaining bases were dissolved in ethanol to obtain the appropriate concentrations. Ether and methanol were procured from Fisher Scientific, PA. Chloroform was obtained from J.T. Baker, Phillipsburg, NJ. Tritiated water with specific activity of 1.0 mCi/g, was purchased from New England Nuclear, Boston, MA.

2.2. Methods

2.2.1. Skin preparation

Dermatomed human abdominal skin (0.25 mm, Dermatome Mod. B, Padgett, Kansas City, MO), from a 17-year-old Caucasian male, was obtained at autopsy. The skin samples were stored at -20°C until used. Two hours before the experiment, the frozen skin samples were thawed at room temperature. The samples were inspected against bright light for any damage. The epidermis was separated from the dermis by sandwiching the thin sections between two layers of aluminum foil and pressing tightly against a slide warmer for 45 s at 50°C. The epidermis was peeled off the dermis with dissection forceps (Romani et al., 1989).

2.2.2. Skin permeation and TEWL measurements

Circular pieces of skin were cut (using a scalpel) and mounted between the donor and receptor compartments of flow-through glass diffusion cells (Gummer et al., 1987) (Laboratory Glass Apparatus, Berkeley, CA). The exposed skin area was 1.0 cm^2. The receptor fluid was 0.01% (w/v) gentamicin sulfate in physiological saline (Berner et al., 1990) and was perfused through the chamber with a multichannel peristaltic pump at a rate of 1.5 ml/h and stirred by a Teflon-coated magnetic bar at 150 rpm. The chamber temperature

was regulated by a thermostat to maintain the skin surface temperature at 32°C.

To assess the effect of various physical and chemical insults on skin barrier functions, water loss through the skin was measured with an unventilated evaporimeter (Model EP-1, Servomed AB, Stockholm, Vallingby, Sweden) (Nilsson, 1977; Wilson et al., 1982; Pinnagoda et al., 1990). TEWL measurements were made by placing the collared probe on top of the tube of the upper half of the permeation cell (Fig. 1) and leaving there until a constant value was established (≈ 2 min). An unstirred air column of 2.5 cm above the skin provided little resistance to water permeation.

All measurements were performed in a single ventilated room having an ambient temperature of 23 ± 2°C and relative humidity of 50–70%. Intact skin at 32°C is equivalent to ≈ 50 cm of column of air, which was calculated based on transepidermal water loss and the diffusion coefficient and vapour pressure of water in air at that temperature (Weast, 1969).

2.2.3. Tritiated water permeation

Two hundred microliters of tritiated water (1 μCi) was applied to the epidermal skin surface of the donor compartment, which was subsequently covered with Parafilm® (American National, Greenwich, CT). For the next 20 h perfusate samples were collected every 4 h on a fraction collector and assayed for radioactivity with a liquid scintillation counter (Packard Instruments, Tri-Carb 4640, Packard Instrument, Downers Grove, IL). All experiments were performed in quadruplicate.

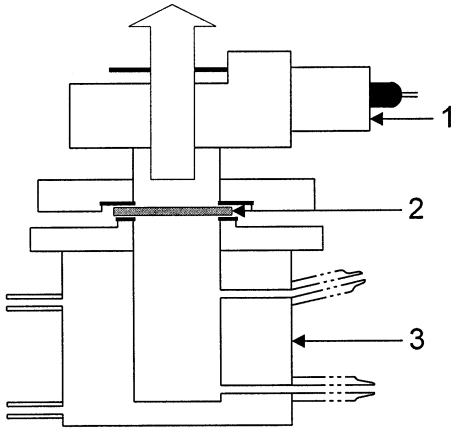

Fig. 1. Schematic diagram of the diffusion cell coupled to an evaporimeter: (1) evaporimeter probe; (2) skin slice; (3) diffusion cell.

In all cases, positive and negative controls were used. The positive control consisted of an uncovered cell filled with water; the negative control was a cell covered with an impermeable Teflon membrane.

2.2.4. Skin treatments

Five categories of experimentally damaged skin models were investigated.

2.2.4.1. Chloroform–methanol treatment. For delipidization, 0.5 ml of a mixture of chloroform and methanol (2:1% v/v) was applied to the epidermal surface for 1.5, 3, 6, 12 and 24 h under occlusion with Parafilm. At the end of the pre-treatment episode, the solvent was aspirated and the skin gently blotted dry with a tissue paper.

2.2.4.2. Surfactant-induced irritation. Sodium lauryl sulfate solution (SLS, 0.2 ml, 2% w/v) was applied under occlusion to the epidermal sides of the skin slices for 3, 10, 30 and 60 min. After treatment the surfactant solution was removed and the skin was gently washed three times with 0.5 ml of distilled water and finally dried using a tissue paper. Various concentrations of SLS (0.2 ml of 0.125, 0.5, 2, and 3% w/v) were also applied for 24 h to select a concentration that could completely damage the water barrier functions.

2.2.4.3. Mechanical damage. Mechanical insult to the skin slices was induced by either abrading it with sandpaper or a sharp needle, or by stripping it with adhesive tape. Medium grade sandpaper was gently stroked ten times across the skin in the same direction to alter the barrier functions. For tape stripping, the skin surface was cellophane-tape stripped (Scotch™ 600, 3M, St. Paul, MN) 10 times to remove most of the stratum corneum. A hypodermic needle was lightly drawn over a 1 cm^2 area of skin eight times in the form of a grid.

2.2.4.4. Treatment with basic compounds. Bases were selected that provided a wide range of basicity and induced skin damage in vitro (Nangia et al., 1993) . Epidermal surfaces of mounted skin slices were exposed to either (1) 0.2 ml of either an aqueous solution (5% w/v) of norephedrine or antipyrine; or (2) ethanolic solutions of 2.5% w/v imipramine, naphazoline, or mecamylamine, then the cell tops were covered with Parafilm. To observe the effect of the solvent on the skin, samples were also treated with 0.2 ml of water and ethanol as control pretreatments. After 24 h of exposure, excess solution was drained, the samples were rinsed with distilled water (0.5 ml) three times, and the skin was dried by gentle blotting with a tissue paper.

2.2.5. Data analysis

Differences in the means of TEWL values and fraction of tritiated water absorbed were analyzed using the nonparametric Student's *t*-test. The linear correlation between TEWL and the fraction of tritiated water absorbed was examined for each treatment.

3. Results

The mean value of TEWL for untreated intact excised skin was 5.6 ± 1.8 g/m^2 per hour, which agrees well with the values reported in the literature (Pinnagoda et al., 1990). Treatment with water resulted in hydrated skin and showed a higher TEWL i.e. 26.5 ± 4.1 g/m^2 per hour when measured initially and then declined sharply to the baseline value in 1 h. As an upper bound to evaluate skin damage, a skin diffusion cell containing water, but not skin, was used as the positive control. The TEWL value observed with this 'water cup' assembly was 27.5 ± 5.7 g/m^2 per hour. TEWL values were measured up to 7 h after treatment, and the fraction of the dose of absorbed tritiated water was measured over the course of 20 h. Values of TEWL, not statistically different from zero, were observed with an impermeable Teflon membrane.

Exposure to the chloroform–methanol mixture resulted in rapid and irreversible damage to the barrier properties. The extent of damage increased with the contact time (Fig. 2). An exposure of 3 min had a pronounced effect and resulted in a TEWL of 22.1 ± 8.0 g/m^2 per hour, which was approximately five times higher than the control; this value slightly decreased when measured after 24 h. Damage to the water barrier of the excised skin was most effective when the skins were exposed for 10 min with a TEWL value of 29.9 ± 8.0 g/m^2 per hour. This value was similar to that of an open 'water cup' assembly, suggesting that 10 min of exposure to solvent is adequate to destroy completely the water barrier of the skin. Treatment for 60 min did not exhibit further damage since the TEWL value remained similar to that of 30 min (28.6 ± 3.2 g/m^2 per hour). Chloroform–methanol treatment also resulted in an increased fraction of tritiated water absorbed in 4 h; these results were consistent with the trend for TEWL. In Fig. 2, the correlation between the effects of chloroform–methanol treatment on TEWL and the fraction of tritiated water absorbed was found to be excellent ($r^2 = 0.96$).

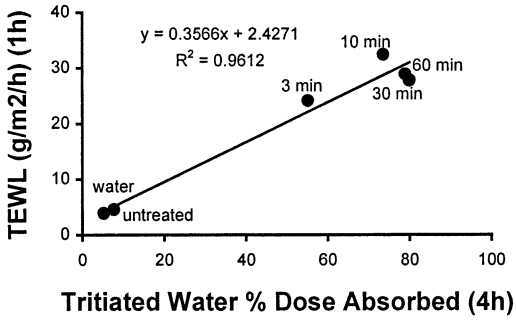

Fig. 2. A linear correlation between TEWL (measured after 1 h) and the fraction of the dose of tritiated water absorbed (in 4 h) after chloroform–methanol treatment applied to excised human skin.

The concentration-dependent increases in TEWL and tritiated water absorbed by SLS treatment are shown in Fig. 3. A good correlation between the two was observed ($r^2 = 0.98$). The non-linearity at high concentrations may relate to the lack of relationship between the fraction of tritiated water absorbed and water permeability for large fractions absorbed, i.e. it measures only the dose applied (see Eq. (4)). Treatment with

SLS resulted in a significant increase in TEWL at all concentrations. A concentration below the critical micelle concentration (CMC) (i.e. 0.125% w/v) caused the least damage to the skin and raised the TEWL of intact skin from 5.2 ± 1.0 g/m^2 to 8.3 ± 4.5 g/m^2 per hour. However, the extent of damage observed with concentrations higher than the CMC was more severe. A linear dependence of TEWL on surfactant concentration between 0.125 and 2% w/v was observed. No further increase in TEWL was evident at 3% w/v suggesting that 2% w/v SLS solution caused maximum possible damage to the skin.

At a fixed concentration (2% w/v) of SLS, the time dependence of the effect of SLS on the barrier function was characterized. The extent of damage, as reflected by either TEWL or the fraction of tritiated water absorbed, increased linearly with contact time (Fig. 4) ($r^2 = 0.82$ and $r^2 = 0.93$ for TEWL and tritiated water measurements, respectively).

Of the three acute physical injuries created, abrasion with sandpaper inflicted the greatest damage while a needle had little effect on the increase in TEWL and tritiated water permeation (Fig. 5). For these acute injuries, TEWL correlated well with results for water permeation ($r^2 = 0.89$; Fig. 5).

Table 1 shows the TEWL values after exposure to five basic permeants. Application of the antipyrine solution ($pK_a = 1.4$, non-irritant control)

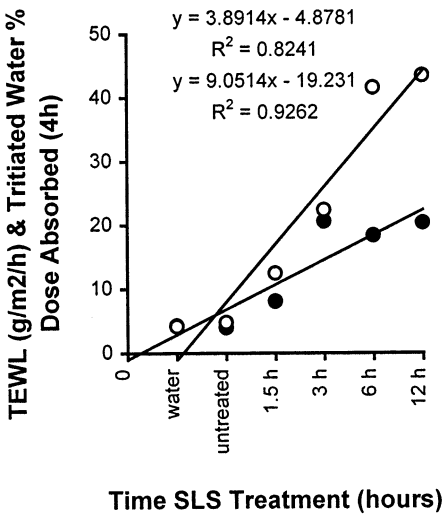

Fig. 4. A linear correlation between TEWL (measured after 1 h) and the fraction of the dose of tritiated water absorbed (in 4 h) after different durations of SLS treatment applied to excised human skin. ●, TEWL; ○, tritiated water absorbed.

for 24 h did not cause any damage to the skin, and the TEWL was similar to that for water-treated skin. All the remaining four basic compounds, norephedrine ($pK_a = 9$), imipramine ($pK_a = 9.5$), naphazoline ($pK_a = 10.9$), and mecamylamine ($pK_a = 11.2$) resulted in an increase in TEWL and the fraction of tritiated water absorbed with the maximum effect seen with mecamylamine, followed by naphazoline, imipramine, norephedrine and antipyrine. The

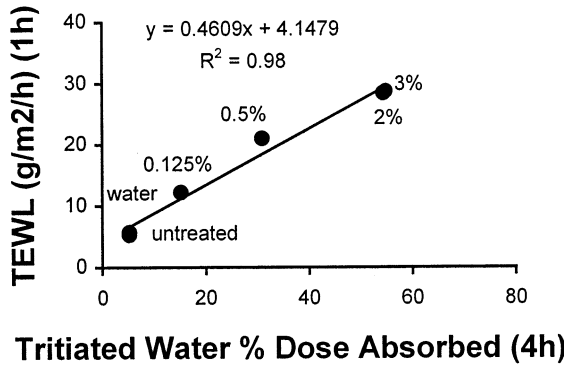

Fig. 3. A linear correlation between TEWL (measured after 1 h) and the fraction of the dose of tritiated water absorbed (in 4 h) at various concentrations of SLS (after 4 h) applied to excised human skin.

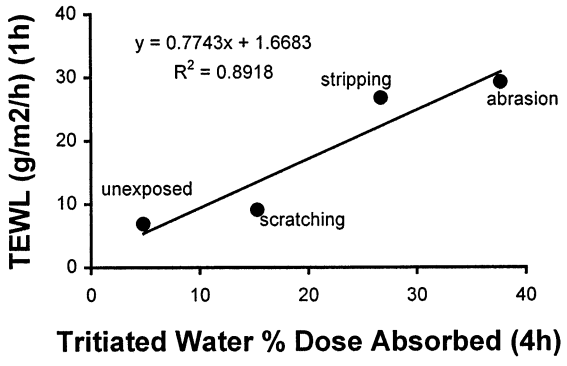

Fig. 5. A linear correlation between TEWL (measured after 1 h) and the fraction of the dose of tritiated water absorbed (in 4 h) after various physical injuries at 4 h applied to excised human skin.

Table 1
Irritation induced by basic compounds with increasing pKa values

Compound	pKa	TEWL values[a](g/m^2 per hour)	Dose ^3H_2O(% absorbed in 20 h)
Antipyrine	1.4	4.8 ± 0.8	30.0
Norephedrine	9.0	5.2 ± 1.6	45.2
Imipramine	9.5	9.8 ± 0.6	55.0
Naphazoline	10.9	13.8 ± 4.1	90.5
Mecamylamine	11.2	28.4 ± 6.2	88.7
Water	—	4.6 ± 1.3	28.2
Ethanol	—	7.7 ± 1.2	43.6

Antipyrine and norephedrine solutions were prepared in distilled water. Imipramine, naphazoline and mecamylamine solutions were prepared in ethanol.
[a] TEWL values were measured in vitro after 1 h using the evaporimeter.

rank for the bases was in accordance with their pK_a values; i.e. TEWL increases with higher pK_a values. Mecamylamine, with the highest pK_a of 11.2, perturbed the skin completely and the resultant TEWL (28.4 ± 6.2 g/m^2 per hour) was comparable to that of an open 'water cup' assembly. The maximum percentage of tritiated water absorbed through cadaver skin also ranked the permeants in a manner similar to that of TEWL, and the correlation between TEWL and water permeation was found to be reasonably good ($r^2 = 0.71$). Deviations occurred for large fractions of water absorbed, because in that regime this measurement no longer reflects water permeation.

4. Discussion

Rapid and direct TEWL measurements with a small probe are extremely convenient for routine analysis. Under controlled environmental conditions, an elevated TEWL is predictive of altered barrier properties and has been used to verify the integrity of excised human cadaver skin before it is used to study permeation of different compounds (Abrams et al., 1993).

In this study we have examined the relationship between TEWL and skin barrier functions that have been damaged to varying extents by different techniques. For all treatment effects studied, the excellent correlations between TEWL and tritiated water absorbed at short times, indicate the usefulness of TEWL as a measure of the integrity of skin barrier functions. The rapid and continuous nature of the TEWL measurement allows studies that yield more detailed information about the changes in barrier properties with time.

Measurement of TEWL by an evaporimeter is a continuous measurement of water permeation through skin under gradient conditions. In contrast, tritiated water permeation is a cumulative measurement over time. With increasing time, finite dose tritiated water permeation methods reflect the dose absorbed and not the permeability of the tissue. Assuming a negligible time lag for water permeation (10–15 min), from mass balance we find that

$$\frac{dC}{dt} = -APC/V \tag{1}$$

where C is the concentration of tritiated water in the donor solution, t is the time, A is the surface area, V is the volume of tritiated water applied and is assumed to be constant, and P is the permeability of skin. By integrating, the fraction absorbed may be obtained as

$$\text{Fraction absorbed} = 1 - \exp\left[-(A/V)\int_0^t dt\, P\right] \tag{2}$$

For constant P at short times ($APt/V \ll 1$)

$$\text{Fraction absorbed} = APt/V \tag{3}$$

and the tritiated water permeation reflects the permeability of skin. However, in general, even for constant P

$$\text{Fraction absorbed} = 1 - \exp(-APt/V) \quad (4)$$

At long times, particularly, the fraction absorbed of tritiated water equals unity because it does not depend on the permeability. In contrast, TEWL is a measure of the skin permeability at all times. For severely damaged skin, tritiated water permeation did not vary linearly with TEWL because the fraction absorbed was nearly unity.

As shown in Figs. 2–4, an excellent correlation between the dose of tritiated water absorbed and TEWL was demonstrated for barrier damage by delipidization or SLS treatments. TEWL also permitted detailed studies of non-steady-state phenomenon.

Investigations (Bronaugh et al., 1986; Scott et al., 1986) revealed an increase in water permeability of rat skin that was subjected to both tape stripping and sandpaper abrasion, and indicated that the former technique caused more damage to the barrier than the latter one. Our results are in contradiction to these findings with higher TEWL values recorded after sandpaper abrasion of cadaver skin. A plausible explanation for this difference may be that a different species and a different tape were used our study. The stratum corneum of human skin (10–20 μm) is thicker and more coherent than that of the rat (Bartek et al., 1972).

Previous studies have suggested that the dissociation constant (pK_a) of a permeant is predictive of skin irritation (Nangia et al., 1990). Using 14 basic permeants with diverse physicochemical characteristics, the degree of irritation was found to increase as the pK_a of the conjugate acid of the penetrant increased above eight (Nangia et al., 1996). It was also observed that these bases, in addition to nonspecific effects, i.e. erythema and edema, damage the skin by altering: (1) biomatter osmolality; (2) physiological pH of the viable epidermis and dermal vasculature; and (3) barrier functions (Nangia et al., 1990; 1993).

A survey of literature indicates that barrier function of skin has been evaluated using several non-invasive techniques, such as measurement of transcutaneous carbon dioxide loss, skin impedance, skin blood flow, and cutaneous water content measurements. The importance of using TEWL as an integrity indicator has been acknowledged more often due to the recent reports that have validated the use of an evaporimeter to measure cutaneous irritation. Using an evaporimeter is a simple and rapid method of screening the integrity of the barrier function of skin in vitro and thus can be used as an alternative to the tritiated water permeation.

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