Normalization of Stratum Corneum Barrier Function and Transepidermal Water Loss *In Vivo*

Yogeshvar N. Kalia,[1–3] Ingo Alberti,[1,2] Nabila Sekkat,[1,2] Catherine Curdy,[1,2] Aarti Naik,[1,2] and Richard H. Guy[1,2]

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INTRODUCTION

The principal homeostatic function of human stratum corneum (SC) is to restrict the loss of water to the external environment. Although the SC forms only the outermost 10–20 μm of the epidermis, it fulfils this role remarkably well, despite the presence of a steep water concentration gradient across the membrane. Typical basal values of transepidermal water loss (TEWL) in adults with healthy skin are only of the order of 5–10 $g.m^{-2}h^{-1}$. Perturbation of the barrier, either by physical disruption, chemical attack or because of disease, can severely compromise the role of the SC. Therefore, it follows that measurement of TEWL can be an effective marker to report on the health and efficiency of SC barrier function *in vivo*.

Tape-stripping is a powerful, relatively noninvasive technique (1), that enables the position-dependent transport properties of molecules within the SC to be determined (2,3) and, when coupled with analytical methods of detection, provides data on the penetration of topically applied substances *in vivo* (4–7). In a previous paper we combined tape-stripping with TEWL measurements *in vivo* in man to show that, despite its heterogeneous structure, the SC functioned as a homogeneous barrier to water transport *in vivo,* with the diffusional resistance equally distributed throughout the membrane and not restricted to a particular tissue layer (2). A linearized form of Fick's 1st Law was used to estimate water diffusivity across the SC and the membrane thickness:

$$\frac{1}{TEWL_x} = \frac{H}{K\Delta C \cdot D} - \frac{x}{K\Delta C \cdot D} \qquad (1)$$

where $TEWL_x$ is the transepidermal water flux when x μm of SC has been removed by tape-stripping; K is the SC-viable tissue partition coefficient of water; D is the average apparent diffusivity of water in the SC of thickness, H (μm), and ΔC is the water concentration difference across the membrane (i.e., ~55M ~ 1 g cm^{-3}). The calculated parameter values for water diffusivity ($D = 3.8 \pm 1.3 \times 10^{-9}$ $cm^2.s^{-1}$) and SC membrane thickness ($H = 12.7 \pm 3.3$ μm) were in good agreement with previous *in vitro* measurements (8). Recently, this same approach has been used to identify site-dependent variations in these parameters (9).

As mentioned above, the absolute parameter values were quite reasonable and the variation about the mean was well within acceptable limits. However, it was apparent that removing the same amount of SC from different individuals did not result in the same increase in TEWL. For example, while removal of ~8 μm of SC thickness from one volunteer resulted in a ~10-fold increase over basal TEWL (indicating significant barrier disruption) stripping a similar amount from another subject resulted in only a ~2-fold increase over the initial, pre-stripping rate of water loss. It seemed reasonable to ask whether this variation was a function of inter-individual differences in the thickness of the intact membrane. Therefore, in this work we have analyzed TEWL data, recognizing these inter-individual differences, and we have normalized the results for each subject with respect to the corresponding intact SC thickness.

The results are directly relevant to methods proposed for the evaluation of topical drug bioavailability and bioequivalence. Since passage across the SC is the rate determining step for the transport of most topically applied therapeutic agents, the drug concentration within this membrane will be directly related to that in the epidermis and dermis which are the most frequent target sites. Therefore, the evaluation and comparison of the concentration profiles of different drug formulations in the SC over a period of time can be used, for example, to assess their bioequivalence or lack thereof. A recent FDA draft Guidance (10) recommends a protocol for assessing dermatopharmacokinetic equivalence in which ten tape-strips are removed to collect the "majority" of the drug in the SC (although more tape-strips can be used if necessary); the first tape-strip is to be discarded, and the nine remaining tape-strips are used to quantify the drug in the skin. However, as we show here, the number of tape-strips is a poor indicator of the actual amount of SC tissue removed. It conveys no information about the relative position with respect to the intact membrane, and fails therefore, to permit meaningful comparisons between individuals and, in turn, between different formulations applied to different skin sites on the same individual.

MATERIALS AND METHODS

Equipment

TEWL measurements were made using a Servo Med Evaporimeter EP1 (Servomed AB, Stockholm, Sweden) (11). A Mettler AT261 Deltarange (Mettler-Toledo GmbH, Greifensee, Switzerland) precision balance (0.01mg) was used to weigh the tape strips.

Subjects

Thirteen human volunteers (6 male, 7 female), aged from 24–39 years, participated in the study. All subjects were in good general health and had no history of dermatological disease. Informed consent was obtained from all participants. The study was approved by the *Commission d'Ethique, Département des Neurosciences cliniques et Dermatologie, Hôpitaux Universitaires de Genève.*

[1] Centre Interuniversitaire de Recherche et d'Enseignement, "Pharmapeptides," Campus Universitaire, Parc d'Affaires International, F-74166 Archamps, France.

[2] Faculté des Sciences—Section de Pharmacie, Laboratoire de Pharmacie Galénique, Université de Genève, CH-1211 Genève 4, Switzerland.

[3] To whom correspondence should be addressed. (e-mail: kalia@pharma1.cur-archamps.fr)

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Experimental Procedure

After recording an initial TEWL measurement, a mask consisting of a 5 × 5 cm section of Scotch No. 845 Book Tape (gift from 3M Medica, Borken, Germany) with a central aperture of 3.4 cm in diameter was fixed onto a predetermined site on the ventral forearm surface (this method ensured that the area of SC removed by each tape-strip was constant). A strip of adhesive tape was placed on the forearm surface and pressed firmly on to the skin. Subsequently, the strip was removed in a single continuous motion, in an attempt to remove a relatively uniform layer of stratum corneum. An "alternating" stripping method was used: i.e., the first strip was removed in the direction wrist-to-elbow, the second in the opposite sense, and so on. This method was adopted since it was found to provide more uniform removal of the SC using fewer tape-strips. TEWL measurements were made after every second tape-strip. Typically, between 20 and 30 tape-strips were removed until the TEWL reached >70 $g.m^{-2}.h^{-1}$, or became constant. The tape-strips were weighed and the cumulative amount and thickness of SC removed (x) was calculated, assuming a density of 1 g cm^{-3} and uniform coverage of SC on the tape-strip (2). The density of skin has been previously determined and reported to be in the range of 0.8–1.3 $g.cm^{-3}$ (12). The skin was not pretreated in any way before the measurements; each subject refrained from applying any cosmetic formulations (moisturizers etc.) on the day of the experiment.

RESULTS AND DISCUSSION

The initial (pre-stripping) values of TEWL ($TEWL_0$) were all in the "normal" range (13) and corresponded to a mean (± S.D., n = 20) permeability coefficient ($K_p = TEWL_0/\Delta C$, where ΔC = 1 g/ml) of 1.8 (± 0.3) × 10^{-7} cm s^{-1}, a value in good agreement with the literature (8,14). Figure 1 shows a conventional representation of the increase in TEWL as a function of the number of tape-strips removed. The data are the pooled results from 20 experiments (n = 225 points). However, Figure 1 conveys neither information about the quantity of SC removed during the tape-stripping process nor of the percentage reduction in barrier function achieved after a certain number of tape-strips. Certainly, the data in Figure 1 reveal important inter-individual differences but how these may be related to differences in SC thickness is not apparent.

When the same TEWL data are replotted as a function of the SC thickness removed (x/μm) by the serial tape-stripping procedure (Figure 2), a different and more revealing picture is obtained. Note that x was determined in this case (and as previously described (2)) from the cumulative mass of SC removed during the tape-stripping process, a method which has been recently validated colorimetrically (15). The significant scattering of points in Figure 2 reflects the inter-individual differences in intact SC membrane thickness and their effect on TEWL as different amounts of the membrane are removed in different subjects by a similar number of tape-strips. The data in Figure 2 were subsequently transformed, for each subject, and fitted to Equation (1). In each case, a linear dependence of $TEWL_x$ upon x was found (for n = 20, mean r^2 = 0.94). Intercepts on the x-axis equalled the values of H (i.e., the SC thickness) for each volunteer; it was found that (for n = 20) H = 10.9 ± 3.5 μm.

It is clear from Figure 2 that, for x ≤ 4 μm, the TEWL data are essentially superimposable and lie within the limits of ~5 ≤ TEWL ≤ ~20 $g.m^{-2}.h^{-2}$. TEWL only begins to show appreciable variation between subjects when 4 ≤ x ≤ 8 μm; at x = 8 μm, TEWL ranged from ~10 ≤ TEWL ≤ ~65 $g.m^{-2}.h^{-1}$. The reason for this 6-fold variation in TEWL lies in the inter-individual differences of SC thickness. That is, removing 6 μm of SC from an individual whose total SC thickness is 8 μm has a much more profound effect on barrier function, and hence TEWL, than removing the same amount of tissue from someone with a SC twice as thick.

To normalize the data, therefore, it was decided to divide the cumulative SC removed (x) for each individual by the corresponding total SC thickness (H). Figure 3a shows the same pooled TEWL results presented in Figures 1 and 2 now plotted against the corresponding values of (x/H). Remarkably, *all* data now conform to a single functional dependence (the empirical fit shown in Figure 3a is a biexponential function with r^2 = 0.90); that is, Figure 3a demonstrates clearly that, once the intrinsic inter-individual differences in the thickness of the intact SC are taken into account by normalizing the SC thickness removed (x) with respect to H, the same degree of barrier disruption induces the same increase in TEWL in each individual. Replotting the water loss data against (1-x/H) enables TEWL to be expressed as a function of % barrier efficiency (Figure 3b). The data demonstrate categorically the effect of deteriorating barrier efficiency on TEWL. Remarkably, in general, TEWL increases dramatically only when about 75% of the SC has been removed.

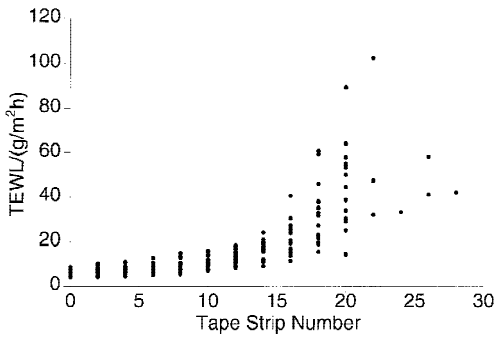

Fig. 1. Pooled data from 20 experiments (225 data points) showing the variation of transepidermal water loss (TEWL) as a function of tape-strip number.

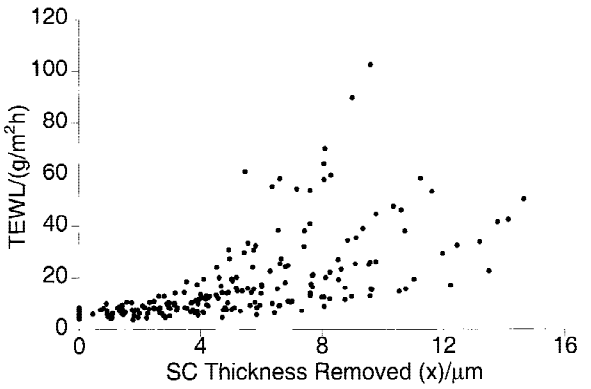

Fig. 2. Pooled data from 20 experiments (225 data points) showing the variation of transepidermal water loss (TEWL) as a function of position within the stratum corneum (SC) as quantified by the amount of SC thickness removed (x) by serial tape-stripping.

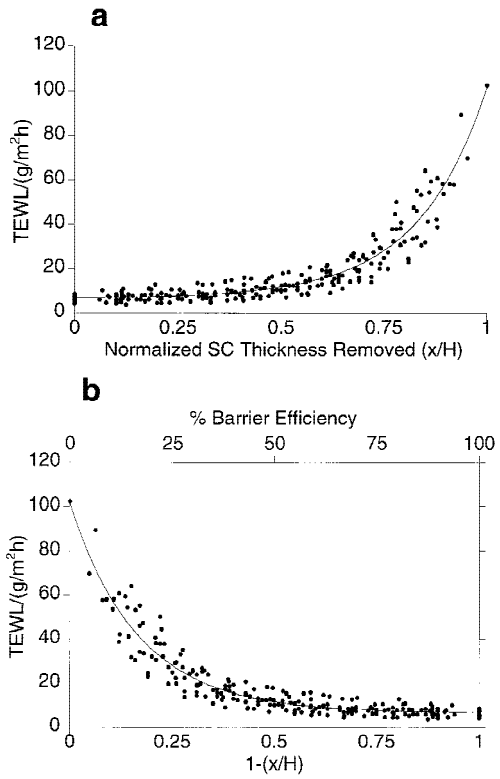

Fig. 3. Pooled data from 20 experiments (225 data points) showing: (a) the variation of TEWL as a function of normalized position within the SC. The SC thickness removed (x) values (shown in Figure 1) have been divided by the corresponding intact membrane thickness for each individual. The empirical fit to the data is the biexponential equation, TEWL = $0.215 \cdot \exp(6.07\ x/H) + 6.34 \cdot \exp(0.251\ x/H)$ ($r^2 = 0.90$); (b) the variation of TEWL as a function of barrier efficiency (%), defined as $100 \cdot (1\text{-}x/H)$, where x/H is the normalized SC thickness removed.

Hence, although the absolute thickness of intact SC on the ventral forearm may vary from 5 up to 20 μm in healthy adults, the *relative* barrier function of the membrane, as a function of position, is independent of the initial thickness (i.e., removal of the same percentage of the SC results in an equivalent degree of barrier disruption).

This finding is consistent with the fact that SC barrier function in healthy adults is relatively constant despite a rather wide range in the thickness of this barrier. It follows that individuals with thin SC "pack" more barrier function into each micron of tissue. Whether this is achieved by subtle changes in lipid composition or in membrane architecture (e.g., increased tortuosity) is a question to be addressed in future work.

The results presented here also have a bearing on the proposed use of tape-stripping methodology for the assessment of topical drug bioavailability (the so-called dermatopharmacokinetic approach described by the U.S. Food & Drug Administration). It is clear from the results presented here that, in order to compare objectively the uptake of drug into the SC from two formulations, one must minimally (a) represent the amount of compound in the SC *per unit mass of tissue* removed by stripping, and ideally (b) normalize the quantity of drug present in the membrane by the percentage of the total thickness sampled in the analytical procedure. In other words, a fixed number of tape-strips neither removes the same amount of SC in different individuals, nor the same relative percentage of the total barrier function.

In conclusion, it has been shown that, despite wide differences in SC thickness, the population of healthy human beings presents a very consistent barrier to TEWL. The relative efficiency of this barrier is a constant: removing a certain fraction of the SC thickness from a thick skin has the same impact on TEWL as removing the same fraction of a thin membrane. How this efficiency comes about is presently unknown. The implications of this observation, however, are of clear importance and relevance to the anticipated use of tape-stripping "dermatopharmacokinetic" studies for the assessment of topical drug availability.

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