Toxicology in Vitro 29 (2015) 176–181

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Development of an *in vitro* model for studying the penetration of chemicals through compromised skin

Diane J. Davies [a], Jon R. Heylings [a,*], Timothy J. McCarthy [b], Catherine M. Correa [b]

[a] Dermal Technology Laboratory Ltd, Keele, Staffordshire, UK
[b] Johnson & Johnson Consumer & Personal Care Products, Skillman, NJ, USA

ARTICLE INFO

Article history:
Received 4 June 2014
Accepted 23 September 2014
Available online 19 October 2014

Keywords:
Dermatomed pig skin
Electrical Resistance (ER)
Trans-Epidermal Water Loss (TEWL)
Tritiated Water Flux (TWF)
Tape stripping
In vitro percutaneous absorption

ABSTRACT

The conventional safety approach that includes dermal absorption of pharmaceutical or consumer products uses models that are based on intact skin. However, when products are intended for application to skin with a less effective barrier, such as in new-born infants, or in cases where the skin is mildly damaged or diseased, there are instances where absorption through compromised skin is also important. A tape stripping procedure was investigated using dermatomed pig skin to assess if an *in vitro* model could replicate the typical changes in barrier function observed in humans with compromised skin. The relationship between Trans-Epidermal Water Loss (TEWL), Electrical Resistance (ER) and Tritiated Water Flux (TWF), markers of skin barrier function in OECD 428 studies was investigated. There was a step-wise reduction in ER from normal (control) skin following 5, 10, 15 or 20 tape strips. This was mirrored by increases in both TWF and TEWL. An *in vitro* experimental protocol using 5 tape strips, ER and dermatomed pig skin provided a rapid, robust and reproducible approach equivalent to the 3–4-fold increases in TEWL observed clinically in compromised skin.

1. Introduction

Skin that has a compromised *stratum corneum* is likely to provide a less effective barrier to topically applied chemicals when compared with normal skin. For example, skin that is impaired due to irritation, sensitisation or more chronic skin disease, such as psoriasis, is likely to be a less effective barrier to the entry of chemicals into the systemic circulation via the dermal route (Goon et al., 2004; Kim et al., 2006; Stamatas et al., 2011). The measurement of dermal absorption of chemicals for consumer products intended for application to the skin is an important part of risk assessment. However, the *in vitro* animal and human models that assess the dermal penetration of topically applied products in Franz-type diffusion cells utilise intact skin (Franz, 1975; OECD, 2004a, 2004b; SCCS, 2010). Since there is no standardised model for evaluating skin penetration in conditions where the barrier properties of the *stratum corneum* are impaired, the use of additional safety factors to accommodate this is arbitrary, despite the fact that many products are targeted for use on skin that has impaired barrier properties. Therefore, a simple and robust *in vitro* technique would be useful for studying the dermal absorption of chemicals in compromised skin.

The purpose of this study was, therefore, to explore whether the tape stripping procedure used to assess the distribution of chemicals in the skin in regulatory protocols could be adapted, *in vitro,* to mimic damage to the *stratum corneum* barrier. Dermatomed pig skin[1] was used in these investigations since the morphological and permeability characteristics of the skin of this species are very similar to humans (Dick and Scott, 1992; Scott and Clowes, 1992) and pig skin is an accepted model for the skin penetration assessment of cosmetic ingredients (SCCS, 2010). One of the requirements of these regulatory studies that involve resected human or animal skin is to establish that the permeability characteristics of each skin sample is normal prior to the application of a test article to the skin surface. The commonly used skin integrity tests in OECD 428 *in vitro* dermal penetration studies using Franz diffusion cells include the measurement of Electrical Resistance (ER), Tritiated Water Flux (TWF) and Trans-Epidermal Water Loss (TEWL). Historically, the TWF approach was the most common barrier function test, but this

Abbreviations: ER, Electrical Resistance; TEWL, Trans-Epidermal Water Loss; TWF, Tritiated Water Flux; LSC, Liquid Scintillation Counting.

* Corresponding author at: Dermal Technology Laboratory Ltd, MedIC4, Keele University Science Park, Keele ST5 5NL, UK. Tel.: +44 1782 44 30 44.
E-mail address: j.heylings@dermaltechnology.com (J.R. Heylings).

[1] Animals were sacrificed for non-cosmetic purposes before the skin was harvested.

http://dx.doi.org/10.1016/j.tiv.2014.09.012
0887-2333/

has been largely replaced by the ER approach which is more practical, since the establishment of a steady state for water permeation takes several hours (Dugard et al., 1984; Lawrence, 1997). TEWL is also a useful method since it is non-invasive and the same instrument can be used for *in vitro* and *in vivo* barrier function assessment (Imhof et al., 2009). A major drawback for TEWL assessment is that it requires a period of stabilisation in a controlled temperature and humidity environment around the probe and is therefore not a practical option for studies involving large numbers of diffusion cells.

Values for "normal" or acceptable skin barrier properties for the three skin integrity parameters (ER, TWF and TEWL) have been published for six species, including human (Heylings et al., 2001; Davies et al., 2004). Of these methods, the ER approach has been shown to be the most practical and robust (Davies et al., 2004). However, different laboratories utilise different Databridge equipment to measure this resistance or impedance parameter and sometimes use different direct current and frequency settings. In addition, there are many different types of diffusion cells where the skin surface area and cell design also has an impact on the technique. Therefore, care has to be taken in the interpretation of values between laboratories (White et al., 2011). Ideally, investigators undertaking such work should link their own impedance/ER methodology to in-house TWF data for the same skin samples, in order to demonstrate the reliability of integrity data that is based on electrical properties of the skin membrane.

In our investigation we have explored the usefulness of Electrical Resistance (ER), Tritiated Water Flux (TWF) and Trans-Epidermal Water Loss (TEWL), for predicting the degree of skin damage achieved through sequential tape stripping of the skin surface. We aimed to establish how the permeability properties of skin changes with varying degrees of skin stripping using dermatomed pig skin in our glass static diffusion cells.

2. Materials and methods

Skin was obtained from suckling pigs (aged 6–8 weeks) of the British White strain that were sacrificed for non-cosmetic purposes before the skin was harvested. Pig skin is a predictive model for human skin penetration as it has very similar morphology and permeability properties to human skin (Dick and Scott, 1992) and it is permitted in regulatory studies to assess the skin penetration of cosmetic ingredients (SCCS, 2010).

2.1. Preparation of dermatomed skin membranes

Samples of whole skin were excised from the trunk area. Excess hair was removed and strips of skin membranes (approximately 6 cm diameter) were cut at a thickness of 200–500 μm using an electric dermatome. Each membrane was given an identifying number and stored frozen, at −20 °C, on aluminium foil, until required for use. The dermatomed skin membranes were used within 6 months of preparation.

2.2. In vitro static diffusion cell equipment

Details of the approach used in these investigations are similar to those described in the OECD guidance document No. 28 (OECD, 2004a). Discs of dermatomed skin membranes approximately 3.3 cm diameter were mounted dermal side down in Franz-type static diffusion cells with an exposed area of 2.54 cm^2 (Dugard et al., 1984; Scott and Clowes, 1992). The receptor chambers were filled with a recorded volume of physiological saline and placed on a magnetic stirrer plate in a water bath maintained at 32 ± 1 °C. Diffusion cells containing membranes intended for TEWL measurement were placed in an incubator maintained at the same temperature.

2.3. Measurement of skin barrier function

Three measures of skin barrier function (ER, TEWL and TWF) were utilised in this study using methods and previously established cut-off values for the rejection of abnormal samples (Davies et al., 2004; Heylings et al., 2001; Imhof et al., 2009).

For ER, this was measured using a PRISM Electronics AIM6401 LCR data bridge connected to two stainless steel electrodes using a setting of 100 kHz and ER was expressed as kΩ for the exposed skin surface area (2.54 cm^2). Further details on the equipment used can be found in our previous publication (Davies et al., 2004). The diffusion chambers were allowed to equilibrate in a water bath at 32 °C for approximately 30 min. One electrode was inserted into the saline in the receptor chamber underneath the skin via the side arm and the other electrode immersed in 2 ml of saline in the donor chamber above the skin. When the resistance across the skin sample had stabilised, the ER reading was recorded.

TWF was determined by firstly allowing the membranes to equilibrate in a water bath at 32 °C for approximately 30 min after which a 2 ml aliquot of tritiated water (T_2O), containing a known amount of radioactivity, was applied to the surface of the membranes. Contact between the T_2O and the skin membrane was deemed to be the start of the experiment (time zero). Samples of the receptor fluid were taken 3, 4, 5 and 6 h after application and analysed for radioactivity content by LSC. The receptor fluid removed was not replaced. However, the receptor fluid and skin membranes were in good contact throughout the T_2O permeability measurement. A permeability coefficient (K_p) was calculated by dividing the steady state absorption rate by the radioactivity concentration of the T_2O applied to the membranes.

TEWL was measured by firstly placing the diffusion cells containing skin membranes in a humidity (40–60%) and temperature-controlled incubator at 32 °C. The cells were allowed to equilibrate for at least 30 min before taking a measurement using a calibrated, ServoMed EP-2 Evaporimeter (ServoMed, Varberg, Sweden) by placing the probe directly on to the dry skin surface. Once the TEWL value had stabilised the reading was recorded.

2.4. Experimental design

Part of the pre-selection criteria of the membranes was a conventional ER skin integrity test which was used to identify any damaged pig skin samples. Any skin sample, in our static diffusion cells, that did not meet the cut-off value of 3 kΩ was discarded and not used in these investigations. The criteria for barrier damage in dermatomed pig skin was as described previously (Davies et al., 2004). Normal skin samples from five different animals were then randomly assigned to groups to be left unstripped (control membranes) or to groups to be subjected to tape stripping 5, 10, 15 or 20 times, in order to remove different proportions of the *stratum corneum*. Further skin samples from the same animals that had passed the initial ER skin integrity test were also randomly assigned to groups to be subjected to individual tape stripping (5 tape strips then individual strips up to a maximum of 14 strips). The tape stripping method followed the standard approach described in the OECD 428 test guideline (Trebilcock et al., 1994), using 22 mm diameter Cuderm D-Squame stripping discs (CuDerm Corporation, Dallas, USA) which were applied to the dry skin surface at a constant pressure of 225 g/cm^2 for five seconds using a purpose-built applicator. The three measures of skin barrier function (ER, TEWL and TWF) were recorded before the tape stripping procedure. The three values were recorded again after removal of the specified number of tape strips of the *stratum corneum* and finally for a third time after 24 h following the tape stripping procedure. Initial and 24 h measurements were also performed for the unstripped control membranes. For comparative purposes,