Dermal exposure to chemicals — evaluation of skin barrier damage

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Abstract: Dermal exposure to xenobiotic compounds occurs on a daily basis in many humans, in intended as well as unintended ways. Serious skin problems are caused by household chemical products, mainly by strong anionic surfactants. The purpose of this study was to assess the suitability of two in vitro methods for evaluation of sodium lauryl sulphate (SLS) potential on skin barrier damage. Transdermal electrical conductivity (TEC) according to our design, and the method of in vitro skin permeability of indigotine as a chemical skin integrity marker were used. The TEC values across the skin membrane damaged with 5, 10 and 15 % aqueous SLS solutions for 1 h were 3.92, 5.79, and 7.29-fold higher respectively than the data of TEC across the intact skin membrane. The amounts of indigotine after 20 h permeation through the skin membrane damaged with 5, 10 and 15 % SLS were 2.48, 4.04, and 5.81-fold higher respectively than the measured amount of indigotine permeated through the intact skin. We consider that the measurement of TEC, especially, in combination with a suitable chemical marker can be simple, quick, safe and cost effective in vitro method for prediction the skin barrier damage not only by surfactants, but also for hazard and risk evaluation of other chemical compounds the human skin is exposed to.

Keywords: *in vitro* skin permeability, skin barrier damage, sodium lauryl sulphate, transdermal electrical conductivity

Abbreviations

DCS, diffusion cell system; ER, electrical resistance; IN, indigotine; OECD, Organization for Economic Co-operation and Development; PBS, phosphate buffered-saline; RF, receptor fluid; SD, standard deviation; SLS, sodium lauryl sulphate; TEC, transdermal electrical conductivity; TEWL, transepidermal water loss.

Introduction

As the largest organ of the body, human skin is continuously exposed to xenobiotics through everyday life products. In many cases, skin is a more significant route of exposure to chemicals than the lung or oral cavity. This is particularly true for non-volatile substances which are relatively toxic and which remain on skin surfaces for long periods of time (EU-OSHA, 2008; OSHA, 2012).

The human skin itself produces own defence system — the skin barrier, but its capacity is limited. Repeated and long-term dermal effects of xenobiotics can lead to protein denaturation, disorganization of the lipid layers of lamellae, reduction of intercellular cohesion between skin cells or removal of natural moisturizing factor(s). As a result of damage, there is a dry and itchy skin with susceptibility to various diseases, or completely damaged skin barrier, which is unable to fulfil its defensive function (De Paepe et al., 2002; Kartono and Maibach, 2006; Atrux-Tallau et al., 2010).

There are three major types of chemical-skin interactions from the aspect of health risks. Firstly, a chemical substance can be transported from the outer surface of the skin both into the skin and into the systemic circulation. This process of *dermal absorption* (WHO, 2006) can be divided into three steps: a) *penetration* — the entry of a substance into a particular layer or structure such as the entrance of a compound into the stratum corneum; b) *permeation* — the penetration through one layer into another, which is both functionally and structurally different from the first layer; c) *resorption* — the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment. Secondly, a chemical substance can induce local effects ranging from irritation through burns to permanent degradation of the skin barrier properties (skin corrosion). The Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UNECE, 2009) defines skin irritation as "the production of reversible damage to the skin following the application of a test substance" and defines skin corrosion as "the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance". Lastly, a chemical substance can evoke allergic skin reactions through complex immune system responses at both the point of contact and

at skin sites remote to the contact (Boeniger, 2003; Semple, 2004).

Dermal exposure to chemicals in everyday life can be intentional or unintentional. Intentional exposure may take place through the use of cosmetics, toiletries as well as topical drugs among which some chemicals are expected to remain on the outside or within the upper layers whereas other chemicals are intended for systemic absorption. But a variety of xenobiotics exposed to human skin for a long time and/or repeatedly and/or over a large area without the person noticing it. Such unintentional dermal exposure can occur to chemical released from clothing, footwear, bed linens, etc. Serious skin problems are caused by household chemical products, mainly strong anionic surfactants, such as sodium lauryl sulphate — an ingredient in detergents, dishwashing preparations and household cleaners, as well as in many cleaning cosmetic and toiletry products, which we use daily (Nielsen et al., 2007; NIOSH, 2011).

Materials and methods

Chemicals

Sodium lauryl sulphate (SLS; $C_{12}H_{25}NaO_4S$, CAS No. 151-21-3, Mw 288.38 g.moL^{-1}, purity ≥ 98 %) was obtained from Sigma-Aldrich (Seelze, Germany). Disodium 3,3'-dioxo-[delta 2,2'-biindoline]-5,5'-disulfonate, Indigotine (IN; FD&C Blue No. 2, CI Food Blue 1, $C_{16}H_8N_2Na_2O_8S_2$, CAS No, 860-22-0, Mw 466.36 g.moL^{-1}, purity 85 % of active colorant on a weight) was purchased from BASF (Ludwigshafen, Germany). All other reagent-grade chemicals were obtained from Mikrochem, Pezinok, Slovak Republic. The receptor fluid (RF) was phosphate buffered-saline (PBS; pH 7.4). The stability of indigotine as a marker under the used experimental condition was confirmed previously (unpublished results).

Skin membranes

As were confirmed by the Organisation for Economic Co-operation and Development (OECD, 2004a, 2010), just as by the World Health Organization (WHO, 2006) and by the European Commission (EC, 2008) the skin membrane of porcine ear is a good alternative for human skin because of a similar anatomy, physiology and chemistry.

The ears of six months old pigs (*Sus scrofa domestica*, Large White) excised from the carcass prior to the steam cleaning process (a local abattoir, Senec, Slovak Republic) were used in these studies as *in vitro* skin model. After immediate transport to our laboratory, the full-thickness skin membrane (*epidermis* plus *dermis*) was obtained in accordance to the OECD 428 method (OECD, 2004a). Membranes with no visible imperfections were wrapped in aluminium foil and kept frozen at –20 °C until required for used, up to 3 months. For experiments, skin membranes were thawed at room temperature and the thickness value was evaluated by micrometer (Digital micrometer SKW 1/0.001, Helios Messtechnik, Niedernhall, Germany). Skin thickness varied between 0.8 and 1.1 mm.

Diffusion cell equipment

The in vitro experiments were performed according to the OECD Test Guideline 428 (OECD, 2004a) using the vertical unjacketed glass static diffusion cells Franz-type (diffusion area of 2.00 cm^2/cell). The system consists of two half-cells where the upper cell compartment represents the donor chamber and the lower the receptor chamber. The skin membrane was mounted between the chambers with the stratum corneum uppermost and held in place by a clamp. The receptor chamber was filled with 5.5 mL of the RF and constantly stirred using a teflon-coated magnetic bar. Fourteen diffusion cells were placed at time on a magnetic stirrer plate (Variomag 15, Thermo Scientific, Karlsruhe, Germany) and semi-submerged in a water bath (Julabo Labortechnik, Seelbach, Germany) resulting in the skin membrane surface temperature of 32 ± 1 °C (the in vivo skin temperature).

Assessment of skin membrane integrity

Two electrical resistance (ER) methods were a base for the method used in our experiment: first method by Davies (Davies et al., 2004) recommended for skin integrity assessment in in vitro dermal absorption studies of chemicals and second method by OECD 430 (OECD, 2004b) for skin corrosion assessment of chemicals. In this study, the skin membrane integrity or skin damage was determined before and after each exposure to SLS by measurement of transdermal electrical conductivity (TEC) according to our design (Hojerová et al., unpublished results). An original conductivity probe connected to a simple FE30 electrical conductivity meter (Mettler-Toledo, Zürich, Switzerland, a resolution 0.1 µS/cm to 199.9 mS/cm and a limit of error ± 0.5 %) was adjusted by two platinum electrodes, one long and one short. After filling the donor and receptor chambers with RF of 0.6 mL and 5.5 mL respectively, the diffusion cell was kept at 32 ± 1 °C temperature for 30 min. The long electrode was inserted into the receptor chamber through the sampling outlet to touch bottom. The short electrode was placed into the donor chambers close to the membrane, but without touching it. The distance between the end of electrodes was always the same

(here 4 cm). Following a 30 s equilibration period, TEC readings were measured on three different locations of membrane and the mean TEC4 was determined. TEC1 value was calculated using the following expression:

$$TEC_1 = TEC_4 \times 4 \ [\mu S/cm] \quad (1)$$

where TEC_4 [μS/4cm] is transdermal electrical conductivity for 4 cm distance of electrodes in the diffusion cell system (DCS) with a membrane, TEC1 is the same re-calculated for 1 cm distance of electrodes. Our previous studies (Hojerová et al., 2011) have shown that the TEC1 value in DCS at 32 ± 1 °C across the intact skin membrane should be equal to or less 1.00 mS/cm. Therefore, each skin membrane, which did not fulfil this value, was excluded as damaged.

Skin membrane exposure to sodium lauryl sulphate

In the first set of experiments, we compared the effect of various concentrations of SLS on the TEC1 value across the skin membrane in DCS. A dose 100 μL of one from three SLS solutions (5, 10 and 15 % in distilled water) was added on the intact skin membrane surface. Each solution was evaluated in seven replicates. Diffusion was followed for 1 h (an infinite dose) under non-occlusive conditions. Then surface of skin membrane was rinsed with 1 mL of PBS in four replicates and receptor chamber was recompensed with 5.5 mL of fresh PBS. The same fluid (0.6 mL) was added into the donor chamber and the TEC1 value after skin exposure to SLS was measured.

Dermal permeation of indigotine through intact and damaged skin membrane

In the second set of experiments, 0.5 % solution of indigotine in distilled water was used as a chemical hydrophilic marker for a control of skin barrier integrity or skin damage. A dose of 100 μL of indigotine solution was applied for 20 h at 32 ± 1 °C on intact skin membrane just as on skin membrane after SLS damage. The samples of indigotine in RF were taken for absorbance measurements using a two-beam UV/Vis spectrophotometer Shimadzu UV-1800 with UV-Probe PC software for the statistical analysis of the calibration graphs (Shimadzu, Kyoto, Japan), detection at 610 nm.

Results and Discussion

Two main strategies are described for monitoring the potential of chemicals to reduce the ability of the skin to serve as a barrier: (1) using physical methods and (2) using a chemical marker. Considering the first strategy, two methods are the most widely used: method of transepidermal water loss (TEWL) and method of electrical resistance (ER). Assessment of the skin barrier to TEWL method is based on the measurement of water loss from the skin, which is the higher, the more skin barrier is damaged (Heylings et al., 2001; OECD, 2004a). ER is the method, where the skin barrier function is assessed by measuring the passage of an electrical current across the skin membrane (Davies et al., 2004) using a special resistivity-meter. Considering the second strategy, the most common marker for skin integrity test is tritiated water (T_2O), where the permeability of the membrane to T_2O is determined and the permeability coefficient (Kp) for T_2O is calculated over a number of hours. This method is time consuming and the use of radioactivity is costly and has safety implications (Davies et al., 2004). As tracers, some chemical compounds, mainly dyes, were verified.

In this study the traditional ER method was replaced with a method of evaluating skin integrity using a simple transdermal electrical conductivity-meter. The TEC_1 value across the receptor fluid in DCS without any membrane at 32 ± 1 °C (10.20 mS/cm) was measured in the same way as the skin membrane integrity. The method of TEC is based on the fact that a membrane mounted into DCS reduces the transfer of ions; the membrane with intact skin barrier more markedly than the membrane with damaged skin barrier.

The results of the first series of experiments with SLS are given in Tables 1, 2, and 3. While the TEC_1 values of intact skin were on average 0.68, after skin treatment with 5, 10, and 15 % SLS, the TEC_1 values increased on average 2.77 ± 0.53, 3.65 ± 0.66, and 4.99 ± 0.23 respectively. The comparison of the TEC_1 data in DCS across the skin damaged with 5, 10 and 15 % SLS and the TEC_1 data in DCS across the intact skin clearly revealed that throughput of ions was 3.92, 5.79, and 7.29-fold higher, respectively, proportionally to the concentration of SLS (Figure 1).

Given that the skin metabolic activity in the *ex-vivo* model does not occur naturally, as in the *in vivo* human skin does, natural repairing of the skin barrier cannot be expected. Therefore, it is impossible to determine whether the SLS solutions in concentrations used in this study have performed as irritative (a temporary loss of barrier function) or corrosive (a permanent loss of barrier function). Despite this, we consider that the method of TEC allows obtaining valuable information on the effect of chemical substances to the skin.

To determine if the increase in ionic permeability is due to physical destruction of the stratum corneum, a dye-binding step was incorporated into the test

Tab. 1. Skin barrier abilities before and after exposure to 5 % aqueous SLS solution determined using a) the TEC value as a physical marker, b) the indigotine amount as a chemical marker

n	TEC [mS/cm]		Indigotine permeability into receptor fluid [μg/cm²]	
	before exposure	after exposure to 5 % SLS	through skin after exposure to 5 % SLS	through intact skin*
1	0.74	2.39	1.77	0.92*
2	0.61	2.37	1.32	0.54*
3	0.78	3.36	1.93	0.97*
4	0.68	2.41	1.45	0.52*
5	0.75	2.73	1.75	0.85*
6	0.62	2.48	1.54	0.51*
7	0.81	3.67	2.54	1.03*
Mean ± SD	**0.71 ± 0.07**	**2.77 ± 0.53**	**1.75 ± 0.40**	**0.76 ± 0.23***

TEC: transcutaneous electrical conductivity, SD: standard deviation, n: number of the diffusion cells.
*An independent evaluation of the other diffusion cell.

Tab. 2. Skin barrier abilities before and after exposure to 10 % aqueous SLS solution determined using a) the TEC value as a physical marker, b) the indigotine amount as a chemical marker

n	TEC [mS/cm]		Indigotine permeability into receptor fluid [μg/cm²]	
	before exposure	after exposure to 10 % SLS	through skin after exposure to 10 % SLS	through intact skin*
1	0.62	3.09	2.11	
2	0.50	3.25	2.52	
3	0.58	3.21	2.52	
4	0.94	4.78	4.47	
5	0.56	4.10	2.94	
6	0.55	3.17	2.47	
7	0.85	4.16	3.36	
Mean ± SD	**0.65 ± 0, 17**	**3.65 ± 0, 66**	**2.91 ± 0, 79**	**0.76 ± 0.23***

TEC: transcutaneous electrical conductivity, SD: standard deviation, n: number of diffusion cells.
*Data from Table 1 — an independent evaluation of the other diffusion cell

Tab. 3. Skin barrier abilities before and after exposure to 15 % aqueous SLS solution determined using a) the TEC value as a physical marker, b) the indigotine amount as a chemical marker

n	TEC [mS/cm]		Indigotine permeability into receptor fluid [μg/cm²]	
	before exposure	after exposure to 15 % SLS	through skin after exposure to 15 % SLS	through intact skin*
1	0.61	4.88	3.30	
2	0.58	4.89	4.03	
3	0.82	4.56	3.58	
4	0.67	5.18	4.97	
5	0.68	5.11	3.88	
6	0.87	5.19	5.54	
7	0.65	5.13	4.16	
Mean ± SD	**0.69 ± 0.11**	**4.99 ± 0.23**	**4.21 ± 0.79**	**0.76 ± 0.23***

TEC: transcutaneous electrical conductivity, SD: standard deviation, n: number of diffusion cells.
*Data from Table 1 — an independent evaluation of the other diffusion cell

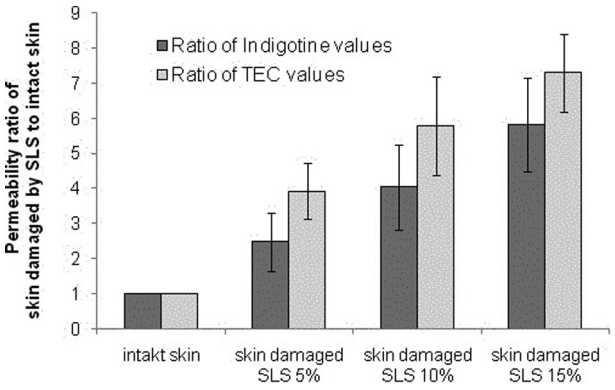

Fig. 1. A ratio of permeability of skin damaged by SLS to intact skin, determined using a) the TEC value as a physical marker, b) indigotine amount as a chemical marker.

procedure. In the second series of experiments, the amount of indigotine was quantified as a chemical marker after passage through the intact skin as well as skin damaged by SLS. The results available in the Table 1, 2 and 3 were confirmed by growing up the indigotine transfer through SLS damage skin, proportionately with increasing SLS concentration, and increasing TEC_1 value. The amount of indigotine permeating to RF through the skin damaged with 5, 10 and 15 % SLS were 2.48, 4.04, and 5.81-fold higher, respectively, than the measured amount of indigotine permeating through the intact skin (Figure 1).

Conclusion

Due to ethical reasons, studies of the potential damage to the skin by chemicals are not allowed to be performed *in vivo*, and, therefore, the development of appropriate *in vitro* methods is essential. The present study demonstrates proportionality between the concentration of SLS solutions exposition of skin membranes and the damage of skin barrier properties. Increasing degree of skin damage is the cause of increasing the TEC values as well as the permeability of a hydrophilic chemical marker increased with comparable results. We consider that the measurement of TEC, especially, in combination with a suitable chemical marker, can be simple, quick, safe and cost effective *in vitro* method for prediction the skin barrier damage not only by surfactants, but also for hazard and risk evaluation of other chemical compounds exposition to human skin.

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