Corneoxenometry: A Bioassay Exploring Skin Barrier Breaching

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17.1 Introduction

One of the outmost functions of the epidermis is to guarantee a continuous permeability barrier preventing the body from the ingress of potentially noxious xenobiotics. The toxic, irritant and caustic compounds are various. The skin permeability barrier function (SPBF) is therefore essential for maintaining a regulated and constant internal milieu. In recent decades, much research was undertaken to modulate or keep intact the SPBF (Notman et al. 2013) which is located in the stratum corneum (SC). The concerns are multifaceted. On the one hand, some formulations are designed for protecting or restoring the SPBF (Xhauflaire-Uhoda et al. 2008a). On the other hand, chemical penetration enhancers, also named absorption enhancers or accelerants, are offered for overcoming the genuine SPBF in order to increase specific drug penetration through the SC. In fact, penetration enhancers act in a number of distinct ways to induce a temporary and reversible failure in the SPBF (Woodford and Barry 1986; Hadgraft and Walters 1994; Keerthi et al. 2012; Seto et al. 2012). Some of these compounds alter or disrupt the epidermal lipids in their solubility properties and ordered structure (Notman et al. 2013). Other penetration enhancers impede the corneocyte's cohesiveness and the tidy SC structure.

The desirable attributes for penetration enhancers are varied (Woodford and Barry 1986;

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Hadgraft and Walters 1994). The compounds must be pharmacologically inert without any activity at cell receptor sites. In addition, the penetration enhancer must be compatible, both chemically and physically, with the drugs and vehicles in the relevant dosages. Its onset of action has to be rapid with a predictable duration of activity. The effects are expected to be completely and rapidly reversible upon removal of the material or formulation from the skin. Furthermore, the effects should ideally be unidirectional, allowing only the ingress of specific xenobiotics without any loss of endogenous compounds from the internal tissues. Penetration enhancers should be cosmetically acceptable, odorless, inexpensive, tasteless, colorless and spreading smoothly over the skin with a suitable "feel". The risk for irritation, allergy and systemic toxicity must be minimal or absent. Despite the diversity of penetration enhancers, none of them combines all of the desirable above-mentioned attributes.

Some penetration enhancers are chemicals specifically designed for this purpose. An example is given by the 1-dodecylazacycloheptan-2-one (laurocapram, Azone®). Other compounds, such as surfactants and solvents, are more regular constituents of any topical formulation (Som et al. 2012). The efficacy of penetration enhancers toward various drugs were thoroughly explored and compared (Williams and Barry 1992). Synergistic effects were reached after combining different classes of penetration enhancers such as solvents and lipid fluidizers (Wotton et al. 1985; Ward and Du Reau 1991). Some binary and ternary mixtures were reported to be more active than single penetration enhancers (Rojas et al. 1991). In complex formulations, each component possibly acts in many different ways, precluding the determination of the actual operative interactions.

17.2 SPBF Modulation and Corneoxenometry

There is a need for accurate assessments of the alterations in the SPBF because any effect quantification of penetration enhancers should allow to design safe, reliable and effective formulations (Diembeck et al. 1999). SPBF is hardly explored with confidence on most in vitro models using reconstructed epidermis or whole skin. Usage of excised human skin is subject to ethical concerns and necessitates both a surgical setting and an experienced laboratory. Skin of animals is often irrelevant due to prominent interspecies differences.

In vivo testing with penetration enhancers was claimed to be performed safely by some investigators in contrast to others who reported severe cell damage in the epidermis and even skin necrosis (Lavrijsen et al. 1994). Such potential hazards call for ex vivo predictive bioassays on human SC (Abrams et al. 1993; Goffin et al. 1997a; Welss et al. 2004; Kandàrovà et al. 2009; Macfarlane et al. 2009; Engelbrecht et al. 2012; Kojima et al. 2012; Ochalek et al. 2012). This chapter focuses on the value of corneoxenometry and corneosurfametry in predicting the value of chemical penetration enhancers. The corneoxenometry bioassay named after corneocyte, xenobiotic and metry was introduced as a convenient and simple approach to explore the effect of some xenobiotics on human SC (Goffin et al. 1997a). It is a variant of corneosurfametry which was specifically designed for testing neat or diluted surfactants (Piérard et al. 1994; Goffin et al. 1995, 1996; Piérard and Piérard-Franchimont 1996; Uhoda et al. 2003).

Corneoxenometry is used for investigating the effects of chemicals potentially harmful to the SC (Goffin et al. 1997a, b, 1998, 2000; Xhauflaire-Uhoda et al. 2008b).The bioassay entails a collection of cyanoacrylate skin surface strippings (CSSS) from normal human skin. The harvested SC sheet which is uniform in thickness is subjected to the ex vivo action of the selected xenobiotics. CSSS covered in excess with each chemical are kept for 2 h at room temperature in a close environment in order to limit any evaporation from the test solution. Samples are thereafter thoroughly rinsed under running tap water, air dried and stained for 3 min with a toluidine blue-basic fuchsine solution at pH 3.45. Any lipid disruption and protein denaturation is responsible for an increased dye binding on corneocytes (Fig. 17.1). Harsh compounds to the skin considerably increase the staining intensity of the CSSS (Goffin et al. 1997a, b, 1998, 2000; Uhoda et al.

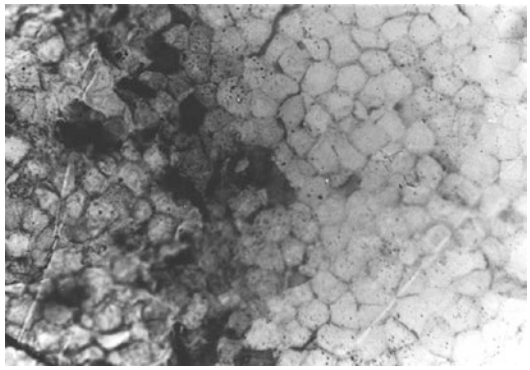

Fig. 17.1 Corneoxenometry. Aspect of a cyanoacrylate skin surface stripping stained by a toluidine blue-basic fuchsine after contact with a penetration enhancer. The staining of corneocytes is uneven and indicates where the damages take place

2003; Welss et al. 2004; Kandàrovà et al. 2009; Macfarlane et al. 2009; Engelbrecht et al. 2012). After placing the samples on a white reference tile, reflectance colorimetry (Chroma Meter CR400 Minolta, Osaka, Japan) is used to derive the L* and Chroma C* values. Colorimetry is used to quantify the corneoxenometry reactivity. The colorimetric index of mildness (CIM) is calculated (Abrams et al. 1993; Goffin et al. 1996, 1997b; Piérard and Piérard-Franchimont 1996; Uhoda et al. 2003) as follows: CIM = L* − Chroma C*. The relative index of irritancy (RII) is calculated as follows: [RII = 1 − [(CIM product) (CIM water)$^{-1}$]. Obviously, RII is not a direct measure of any SPBF breaching. However, it correlates with clinical signs of irritancy, and with increased transepidermal water loss (Piérard et al. 1995). In fact, the bioassay explores the combined effects of (a) lipid removal and disorganization, and (b) protein denaturation as well. Hence, any RII increase is a clue for SC damage responsible for SPBF impairment.

17.3 Dose-Response Corneoxenometry with Chemical Penetration Enhancers

Data from both corneosurfametry and corneoxenometry are reproducible and sensitive enough to frequently disclose significant CIM and RII differences between formulations (Piérard et al. 1995). A dose-response effect was searched for ethanol and laurocapram using the corneoxenometry bioassay (Goffin et al. 2000). In the same study, other assessments were performed using a gel formulation (propylene carbonate, hydroxypropyl cellulose, butylhydroxytoluene, ethanol, glycerol) containing 10 % propylene glycol and a combination of three other enhancers, namely, N-acetyl-L-cysteine (NAC), urea and salicylic acid (SA). The three latter penetration enhancers were present in various proportions with keeping their global concentrations at the 20 % level.

Both the nature and concentration of the respective penetration enhancers affected the RII values. For each formulation, the interindividual variability was reasonably low. Linear dose-effect responses were obtained with ethanol in the range 0–100 %, and laurocapram in the range 0–5 %. The 10 % propylene glycol-based gel exhibited a wide range in RII values when supplemented with NAC, urea and SA. In the bioassay, NAC exhibited a moderate effect on the SC. RII values raised with increasing amounts of urea replacing NAC. The RII worsening was more striking with SA supplementation replacing urea. The combination of SA and urea always proved to be more active than SA alone.

17.4 Corneoxenometry and Organic Solvents

The effects of organic solvents were studied on many occasions (Peck et al. 1994; Garcia et al. 2000). In particular, they were compared using corneoxenometry (Ochalek et al. 2012). Series of CSSS were immersed for 1, 5, 10, 30, 60 or 120 min in vials containing deionized water or an organic solvent including chloroform, ethanol, hexane, methanol, chloroform:methanol (2:1, v/v), hexane:ethanol (2:3, v/v) and hexane:methanol (2:3, v/v). After contact with the selected solvent(s) for the predetermined time, CSSS were thoroughly rinsed for 20 s under running tap water, air-dried and stained for 3 min with toluidine blue-basic fuchsine dyes.

The CIM ranking from the least to the most aggressive product was as follows: hexane

(40.7), ethanol (26.5), methanol (23.5), hexane-ethanol (23.3), chloroform (20.8), chloroform-methanol (15.5) and hexane-methanol (7.8). CIM values showed that the effect of hexane-methanol on SC was significantly higher ($p < 0.01$) than those of all other solvents with the exception of chloroform-methanol. There was no significant difference between ethanol, methanol and hexane-ethanol, but each of them was significantly ($p < 0.05$) more aggressive than hexane.

The influence of exposure time of solvents with the SC showed some inter-product differences. However, all correlations reached significance ($p < 0.01$) and best fitted as logarithmic relationships. For each solvent, most of the CIM changes were reached within 10 min.

The organic solvents under consideration are known to extract lipids (Bligh and Dyer 1959; Scheuplein and Ross 1970; Deffond et al. 1986; Imokawa et al. 1986; Abrams et al. 1993; Lavrijsen et al. 1994). In addition, SC alterations other than lipid extraction are likely (Abrams et al. 1993). Large interindividual CIM differences were found for each solvent or mixture (Goffin et al. 1997b) reflecting the variability in the overall lipid extraction by these solvents (Diembeck et al. 1999). The induced alterations on normal human SC by solvents (corneoxenometry bioassay) were indeed reported to be more variable than those induced by diluted surfactants (corneosurfametry bioassay) (Goffin et al. 1998; Xhauflaire-Uhoda et al. 2008b). Despite interindividual inconsistencies in corneocyte alterations, significant differences were reported among solvents using the corneoxenometry bioassay (Goffin et al. 1997b). Hexane-methanol and chloroform-methanol were the mixtures strongly altering the SC structure. Chloroform-methanol is indeed considered to be the most potent extraction mixture for lipids in biologic samples. However, it did not reach the top ranking at the corneoxenometry bioassay (Goffin et al. 1997b). Such a finding further illustrated the fact that organic solvents alter other biologic components (Diembeck et al. 1999), which in turn affect the corneoxenometry data.

The corneoxenometry bioassay allows to assess the influence of the contact time between solvents and the SC. In previous studies (Goffin et al. 1997b), the time range between 1 and 120 min was selected following available information about the kinetics of lipid extraction from human SC [13]. The corneoxenometry data were in line with previous experiments using other methodological approaches (Deffond et al. 1986; Imokawa et al. 1986; Abrams et al. 1993; Lavrijsen et al. 1994). However, it does not explore the effects of solvents on the living epidermis and on the nature and intensity of inflammation that is present in irritant dermatitis.

Conclusion

Corneoxenometry appears as a relevant and predictive bioassay for assessing the overall effect of single and combined penetration enhancers. It is cheap, rapid, minimally invasive and relevant to human skin. In addition, the reproducibility, specificity and sensibility are reasonably high. Corneoxenometry is therefore a valuable screening test proposed as an alternative to animal and in vitro testings.

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