Corneosurfametry and Corneoxenometry

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23.1 Stratum Corneum and Chemical Xenobiotics

The interaction between surfactants and the stratum corneum (SC) has many-sided aspects. Lipid removal, protein denaturation, corneocyte swelling, impaired SC barrier function, and SC roughness induction are among the best recognized effects. Testing skin compatibility of surface-active agents usually relies on a large panel of volunteers. Such a procedure is costly and time consuming. As a result, several alternative methods were designed both in vivo and in vitro.

Skin washing with a cleanser is occasionally responsible for both sensorial and chemical irritations. Most individuals complaining with sensorial or chemical irritation find it necessary to select skin cleansers using a trial-and-error approach. However, a number of in vivo methods and in vitro tests were described for the prediction of potential surfactant irritancy. These include the so-called pH rise of bovine serum albumin, the corneocyte swelling, the collagen swelling, and the zein solubilization tests [1]. Under some experimental conditions, however, data gained by these regular tests failed to correlate with in vivo observations [2]. Such pitfalls suggested that other relevant in vitro methods would be welcome.

23.2 Corneosurfametry

The interaction between the SC and various chemical xenobiotics is conveniently assessed on cyanoacrylate skin surface strippings (CSSS). Corneosurfametry (CSM) was coined after corneocyte, surfactant, and metry. It refers to effects of surfactants and wash solutions on the SC [3–8]. For this purpose, CSSS are harvested from the inner forearms of healthy volunteers. A solution of the test product or its neat formulation is sprayed over a series of CSSS which are placed in plastic trays covered by lids. After a given period of incubation at controlled temperature, the samples are thoroughly but gently rinsed in running tap water, air-dried, and stained for 3 min in a toluidine blue-basic fuchsin solution. Thereafter, the samples are copiously rinsed with water and dried prior to perform color quantification using reflectance colorimetry. Indeed, surfactants remove lipids and denaturate corneocyte proteins, thus disclosing chemical sites available for staining reactivity. A combined dotted and rimmed pattern is visible on corneocytes at the microscopic examination.

Using quantitative reflectance colorimetry (Chroma Meter CR400, Minolta, Osaka, Japan), the mean values of luminancy (L^*) and Chroma C^* are calculated from measurements made on

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three sites from each sample placed on a white reference tile. Mild surfactants exhibiting little denaturating effect on corneocytes give a combination of high L^* values and low Chroma C^* values. Typically, L^* decreases and Chroma C^* increases with the irritancy potential of the product. The difference between the L^* and Chroma C^* values of each sample represents the colorimetric index of mildness (CIM). The CSM index (CSMI) of a given test product corresponds to the difference in color between water-treated control samples and those samples exposed to the test product. It is conveniently calculated according to the following formula:

$$\mathrm{CSMI} = \Delta L^* 2 + \Delta C^* 20.5$$

Increasing the temperature at which CSM is run increases the SC reactivity [9]. The microwave CSM variant is a more rapid procedure [10]. In this procedure, CSSS are immersed in a flask containing the test surfactant solution. Samples are then placed in a microwave oven containing a 500-ml water load. Microwave CSM is conveniently run at 750 W for 30 s. The next steps are identical to the regular CSM procedure.

Responsive CSM is a variant method where skin has been preconditioned before CSSS sampling [11]. For instance, the method is based on repeat subclinical injuries by surfactants monitored in a controlled forearm immersion test. At completion of the preliminary in vivo preconditioning procedure, CSSS are harvested for a regular or microwave CSM bioassay using the same surfactant as in the preconditioning in vivo procedure. Preconditioning the skin by this way increases CSM sensitivity helping to discriminate among mild surfactants [11]. In this context, subjects with atopic dermatitis commonly show increased CSM reactivity [12]. Similarly, some individuals with sensorial irritation exhibit increased CSM reactivity [13].

Shielded CSM was designed for testing the so-called skin protection products [14]. Such products claiming for a barrier effect should theoretically shield against noxious agents. In shielded CSM, regular CSSS are first covered by the test skin protection product ahead from performing CSM using a reference surfactant. Comparative screenings of skin protection products are conveniently performed using shielded CSM without exposing volunteers to any potential hazards linked to in vivo testing.

Animal CSM can be performed in a way similar to human CSM [15]. The method is available for safety testing of cleansing products specifically designed for some animal species. In addition, any interspecies differences in skin reactivity to surfactants are conveniently assessed [15].

23.3 Corneoxenometry

The corneoxenometry (CXM) bioassay was named after corneocyte, xenobiotic, and metry. It was introduced as a convenient approach to explore the effect of some chemical xenobiotics other than surfactants on human SC [16, 17]. The basic procedure is similar to CSM and its variants. The main CXM indication resides in testing skin irritation while avoiding any in vivo hazards. Another indication concerns the comparative assessment of penetration enhancers commonly used in topical formulations [18]. Still another CXM indication deals with the determination of a dose-effect relationship for agents active on the SC structure and function [19].

CXM was used for testing a series of chemicals harmful to the SC [16–20]. The bioassay entails collection of CSSS from normal human skin. The harvested SC sheet, uniform in thickness, is subjected to the ex vivo action of the selected xenobiotics. Series of CSSS covered in excess by their respective chemicals are kept for 2 h at room temperature in a closed environment, for instance an oven, to prevent evaporation of the test solution. Samples are then thoroughly rinsed under running tap water, air-dried, and stained with a toluidine blue-basic fuchsin solution at pH 3.45 for 3 min. Any lipid removal and protein denaturation induce increased dye binding on corneocytes. It has been shown that harsh compounds to the skin considerably increase the intensity of staining of the CSSS [1, 4, 11, 13, 16, 17, 20, 21]. After placing the samples on a white reference tile, reflectance colorimetry (Chroma Meter CR400 Minolta) is used to derive the L^* and Chroma C^* values. Colorimetric data objectively quantify the CXM bioassay.

The colorimetric index of mildness (CIM) is calculated as previously defined [11, 16, 17, 20, 21] following CIM = L^* − Chroma C^*. The relative irritancy index (RII) is calculated following [RII = 1 − [(CIM product) (CIM water)$^{-1}$]. Obviously, RII is not a direct measure of the barrier function. However, it correlates with clinical signs of irritancy. In fact the bioassay explores the combined effects of lipid removal and disorganization and of protein denaturation as well. Hence, any rise in RII is a clue for SC damage responsible for barrier function impairment.

23.4 Penetration Enhancer Testing

One of the upmost important functions of the epidermis is the formation of a well-structured barrier between the body and the ingress of potentially noxious xenobiotics. The latter compounds correspond to environmental contaminants, chemical irritants, toxins, and still others. The barrier function is vital to keep constant the internal living tissues. Much research was performed to understand the skin barrier function of the SC. In some instances, however, chemical penetration enhancers (absorption enhancers or accelerants) represent an attractive potential overcoming the barrier efficacy and increasing drug penetration through the SC. Penetration enhancers typically induce a temporary and reversible decrease in the skin barrier properties. They act in a number of ways, some of which altering the solubility properties or disrupting the ordered nature of the epidermal lipids [22]. Other molecules alter the intercorneocyte cohesiveness.

The desirable attributes for penetration enhancers are varied [22–24]. The compound should be pharmacologically inert without any effect at receptor biologic sites. The risk for irritation, allergy, and toxicity should be minimal. The enhancer should be compatible, both chemically and physically, with drugs and vehicles in the dosage form. It should possess a rapid onset of action with a predictable duration of activity. In addition, the effects should be completely and rapidly reversible upon the product removal from the skin. Furthermore, the effects should ideally be unidirectional, allowing only the ingress of specific xenobiotics without loss of any endogenous component from the body. The penetration enhancer should be cosmetically acceptable, odorless, inexpensive, tasteless, and colorless.

Despite the wide range of purported penetration enhancers, there is no chemical combining all of the desirable attributes. Some chemical enhancers are specifically designed for this purpose such as 1-dodecylazacycloheptan-2-one (laurocapram or Azone®). Other compounds are more common constituents of topical formulations such as surfactants and solvents. The relative efficacies of enhancers towards distinct drugs have been largely explored and compared [25].

In vivo testing with penetration enhancers was performed safely by some researchers in contrast to others who reported severe cell damage in the epidermis and even skin necrosis [26]. Such hazards called for ex vivo predictive bioassays on human skin or SC [26, 27].

The two classes of penetration enhancers, namely, the solvent type and the lipid fluidizer type, are conveniently combined to reach synergistic effects [28, 29]. In complex formulations, each component possibly acts in distinct ways, precluding any determination of specific interactions. Binary and ternary mixtures were reported to be more active than single-penetration enhancers [30]. However, the ideal combination activity of the chemicals is difficult to predict unless a more precise knowledge of the involved mechanisms has been deciphered. In sum, there is a need for accurate assessments of the SC permeability alterations in order to design safe, reliable, and effective formulations [31]. CXM has shown its predictive value in this field.

23.5 Corneoxenometry and Dose–Response Effect of Chemical Penetration Enhancers

A dose–response effect was searched for ethanol and laurocapram using the CXM bioassay [19]. 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. The three latter penetration enhancers were incorporated in various proportions with keeping the sum of their respective concentrations at the 20 % level.

Data from CXM appeared reproducible and sensitive enough to disclose significant differences between formulations [19]. Both the nature and concentration of penetration enhancers governed the RII values. For each test 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 salicylic acid. NAC exhibited a moderate effect on CXM. RII rose with increasing amounts of urea replacing NAC. The RII rise was stronger when using salicylic acid instead of urea. The combination of salicylic acid and urea proved to be more active than salicylic acid alone.

23.6 Corneoxenometry and Organic Solvents

The effects of organic solvents were studied in many instances [32, 33]. In particular, they were compared using CXM [16]. 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 for one of the predetermined duration, CSSS were thoroughly rinsed under running tap water for 20 s, air-dried, and stained for 3 min with toluidine blue-basic fuchsin dyes.

The ranking from the least to the most aggressive solvent according to the mean CIM 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) [16]. CIM values showed that the effect of hexane-methanol on SC was significantly higher ($p<0.01$) than those of any other solvent with the exception of chloroform-methanol. Chloroform-methanol is well known as the most potent extraction mixture for lipids in biological samples. However, it did not reach the top rank using the CXM bioassay [16]. Such a finding further illustrated the fact that organic solvents may alter other biological components which in turn affect the CXM data. No significant difference was yielded between ethanol, methanol, and hexane-ethanol, but each of them was significantly ($p<0.05$) more active than hexane. The influence of exposure time between SC and solvents showed some solvent-related differences. However, all correlation were significant ($p<0.01$) and best fitted a logarithmic relationship. It appeared that most of the changes in CIM were reached within 10 min for each solvent.

Despite interindividual inconsistencies in corneocyte alterations, significant differences were reported among solvents using the CXM bioassay [16]. The test organic solvents are recognized to extract lipids [26, 27, 34–37]. In addition, alterations in the SC other than pure lipid extraction are likely [27]. Large interindividual differences in CIM were found for any of the solvents or mixtures [16] suggesting the variability in the overall lipid extraction by these compounds [26]. The alterations induced in the human SC by solvents at the CXM bioassay were indeed reported to be more variable in extent that those induced by diluted surfactants as shown at the CSM bioassay on normal subjects [13, 16].

Conclusion

CSM and its CXM variant appear as relevant and predictive bioassays for assessing the overall effect of single and combined chemical xenobiotics. They are cheap, rapid, minimally invasive, and relevant to human skin reactivity. In addition, the reproducibility, specificity, and sensibility are quite high. These bioassays therefore represent valuable screening tests proposed as an alternative to animal testing and hazardous human testing.

The CSM and CXM bioassays allow to assess the influence of the contact time between

the SC and noxious chemicals. The time range between 1 and 120 min appears relevant following available information about the kinetics of lipid extraction from human SC. The CSM and CXM data are in line with a handful of other procedures. However, they do not explore the effects of xenobiotics on both the living epidermis and the nature and intensity of inflammation that could result in irritant dermatitis.

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