Biological and Pharmacological Tests for the Exploration of Skin Barrier Function

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skin • cutaneous absorption • skin barrier • nicotinic acid esters • corticosteroids

An investigation of the skin barrier function may be conducted following different approaches in vitro (Franz 1975) or in vivo (Feldmann and Maibach 1965; Rougier et al. 1983) by measuring the amount of a given compound permeating the different skin layers. On the other hand, quantification of a cutaneous (Oestmann et al. 1993) or systemic biological reaction may also be used for studying the skin barrier function. We will only describe cutaneous biological reactions in man after application of pharmacologically active compounds.

1 Skin Barrier Function

1.1 Physiological Basis of Skin Barrier

Although the skin remains a very complex organ, two main layers are characterized from a macroscopic point of view: an external epithelium called epidermis, and a vascularized stroma, the dermis, physiologically supporting the epidermis. The genuine barrier against xenobiotics is located within the epidermis, which itself comprises two main layers, the viable epidermis and the horny layer. The biological reactions that will be

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measured are mainly vascular responses arising from the dermal microcirculation.

1.2 Pharmacologically Active Molecules

Numerous compounds affect the dermal microcirculation after topical application and influence skin color to be more white or red. For example, nicotinic acid esters are vasodilators inducing a clear erythema. Methyl- (NM, MW = 137.1), ethyl- (NE, MW = 151.2), butyl- (NB, MW = 179.2) and hexyl-nicotinate (NH, MW = 207.3), here quoted by increasing order of lipophilicity, are the most used. Usual concentrations amount to 0.5–10 mM after solubilization in appropriate solvents. These molecules, after crossing the epidermis more or less easily depending on the state of the cutaneous barrier, will dilate the capillary loops in the dermis, then the small arteries of the superficial vascular plexus, and finally the blood vessels of the lower dermal plexus. Thus, the measurement of skin blood flow or of skin color will allow an estimation of the skin barrier function. When considering nicotinic acid esters, the presence or the absence of an erythematous reaction may be an indication of skin barrier function.

Corticosteroids induce a decrease of dermal blood flow (a vasoconstriction). The mechanism (s) of this reaction are poorly understood. The resulting skin whitening, or blanching reaction, may be used for the investigation of skin penetration of the corresponding compounds (Mac Kenzie and Stoughton 1962; Mac Kenzie 1962). However, due to the great differences in pharmacological activity between different corticosteroid molecules (their anti-inflammatory potency is related to their corticosteroid classification), the whitening reaction often does not really reflect percutaneous absorption (also see ▶ Chap. 133, "Assessment of Erythema and Pallor" Sect. 10). Nevertheless, the measurement of skin color remains of value for cutaneous bioequivalence studies where the same molecule is applied onto the skin in different formulations (Caron et al. 1990; Pershing et al. 1991; Pershing et al. 1992).

2 Skin Color Changes after Nicotinic Acid Ester Application

2.1 Cutaneous Application

Methyl nicotinate is dissolved in water, while the other esters are solubilized using, among others, propylene glycol, isopropanol, or liquid paraffin (Treffel and Gabard 1993; Issachar et al. 1998). Small filter paper discs (diameter <1 cm) are soaked in these different solutions (an aqueous solution of NM 5 mM is often used) and brought into contact with the skin for a short time, usually 15–30 s. The internal (or ventral) side of the forearm is the place of choice for application in humans. Alternatively, the aqueous NM solution may be applied with a micropipette (10–50 µl).

The cutaneous reaction may be followed visually using color charts or precisely and reproducibly measured with a suitable instrument. The measurements are done every 10 min until 40 min, and then at 60 and 90 min. By that time, the vascular reaction generally has vanished and the reddening has disappeared.

2.2 Instruments

Two instruments are commonly used (see ▶ Chap. 133, "Assessment of Erythema and Pallor"). The Chromameter (Minolta, Japan) allows the precise evaluation of skin color (Wilhem and Maibach 1989) and the laser Doppler velocimeter (Perimed, Sweden and Moor, UK) is used for determination of cutaneous blood flow (Bircher et al. 1994).

2.3 Characteristic Parameters

After cutaneous application of a nicotinic acid ester, several parameters may be evaluated: latency or time lag before appearance of the erythemal reaction, time to peak or maximal value, duration of the erythemal reaction, and area under the erythemal intensity versus time curve. Although both measuring techniques (skin color and skin blood flow) seem

complementary, skin color is more appropriate for the evaluation of nicotinic acid ester penetration. Indeed, the superficial dermal plexus is responsible for the reddening of the skin measured by chromametry. The laser Doppler data also include values from the lower dermal plexus featuring a more important blood flow that may be modified by external factors such as temperature and stress.

2.4 Nicotinic Acid Esters for the Investigation of Skin Barrier Function

The vasodilator properties of NM had already been used by 1962 for the investigation of skin barrier function differences depending on the anatomical site (Cronin and Stoughton 1962). Areas showing a strong or weaker barrier were characterized. These results were later validated using other techniques (Feldmann and Maibach 1967).

Stripping of the horny layer or tearing away the stratum corneum with an adhesive tape diminishes latency and accelerates the appearance of skin reddening (Guessant et al. 1993). Using the same phenomena, racial differences between Caucasian, black and Asian subjects were shown (Guy et al. 1985; Kompaore and Tsuruta 1993). Age may also be a factor influencing the cutaneous response to nicotinic acid ester application (Roskos et al. 1990).

Elsner and Maibach (1991) have shown that the erythemal reaction to NM application was less intense and of shorter duration on vulval skin than on forearm skin. The lower sensitivity of this anatomical region compared to the forearm is one of the factors discussed by the authors to explain this surprising result. Indeed, in this particular case, a skin area with a known reduced barrier function shows a diminished erythemal reaction. The pharmacodynamic aspect of the reaction then becomes important, because percutaneous absorption is not the only factor determining the skin response. Also, circadian rhythms have been shown to influence the cutaneous nicotinic acid ester reaction (Reinberg et al. 1995). These results show that pharmacodynamics must be considered when evaluating skin changes.

3 Skin Color Changes after Corticosteroid Application

3.1 Cutaneous Application

The described method is based on the original work of Mac Kenzie (Mac Kenzie and Stoughton 1962). The different products to be tested (dermatological formulations containing corticosteroids) are applied under occlusion on the inner side of the forearm at the dose of 2–10 mg/cm^2. The duration of application may vary between 0.25 and 6 h. For each application time, the kinetics of the blanching reaction are followed at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h on each tested area after cleaning the skin and removing the unpenetrated product.

3.2 Instruments

Although some authors advocate the use of laser Doppler measurements (Anja et al. 1998), visual scoring of skin whitening or measurement of skin color using the Minolta Chromameter seems to be more sensitive and widely used (Mac Kenzie and Stoughton 1962; Mac Kenzie 1962; Caron et al. 1990; Pershing et al. 1991; Pershing et al. 1992; see ▶ Chap. 133, "Assessment of Erythema and Pallor").

3.3 Characteristic Parameters

For each application duration, the relevant parameters for quantifying the blanching reaction are: time to maximal value (reaction peak), maximal value, and the area under the time curve. These different parameters may be compared and used for the appreciation of cutaneous bioavailability of different products, but are less suitable for evaluation of percutaneous skin absorption.

4 Conclusion

Skin barrier function may be qualitatively appreciated by the evaluation of biological or pharmacological reactions using nicotinic acid esters or

corticosteroids. However, the skin response represents the sum of complex and intricate phenomena. Indeed, skin barrier state, cutaneous blood vessel sensitivity to the applied compound, corticosteroid potency class, and the level of blood flow in a given skin area, all these factors simultaneously contribute to the measured biological response. Therefore, the quantitative extrapolation of the obtained results as to percutaneous absorption must be done carefully.

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