ACTIVITY OF VEHICLES AND DIFFUSION THROUGH THE HORNY LAYER

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Synopsis

The interest in transdermal delivery for extracutaneous treatments has stimulated a better understanding of percutaneous absorption, mainly in trying to increase skin permeability and controlling the release of drugs or their precursors. Permeation can be increased by various types of enhancers such as fatty alcohols and acids, their esters, Azone, monoglycerides, pyrrolidone derivatives, cyclic monoterpenes, methyl sulfoxides and urea. There is evidence that they act by different modes of action: disruption of the intercellular lipid structures, altering their composition or the cohesiveness between cells, effects on the cell membrane or on intracellular components. Enhancement by these agents does not correlate with their irritancy, and it is not identical between man and a number of animal species. Additional vehicle effects are caused by modifying the solubility and partitioning of the active compounds. Liposomes and niosomes are just examples. The ratio between the interfollicular and the follicular penetration pathways can also be altered. Solutions seem to favour the former, particles the latter. On the other hand, there is also an increasing need for penetration blockers in view of the risk of toxic and allergic reactions to the chemicals used in our private and occupational life. Whether for enhancement, or blocking of penetration, it has to be taken into consideration that cosmetic and dermatological topical applications act differently on diseased skin from normal skin.

Riassunto

L'incrementato interesse per i trattamenti topici ha provocato una migliore conoscenza sull'assorbimento percutaneo. Si è cercato così di incrementare la permeabilità cutanea controllando meglio la cessione sia dei farmaci che dei loro precursori. Il grado di permeazione può essere incrementato da vari tipi di acceleranti l'assorbimento quali gli alcool e gli acidi grassi, i loro esteri, l'azone, i monogliceridi, i derivati del pirrolidone, i monoterpeni ciclici, i metil sulfoxidi e l'urea. È stato dimostrato che queste sostanze agiscono con diversi meccanismi di azione: scompaginamento delle sostanze lipidiche intercellulari, alterazione della lo-
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ro composizione o della coesione delle cellule, effetti sulle membrane cellulari e sui componenti intracellulari. L’accelerazione nel grado di assorbimento di questi agenti non è correlato con il loro potere irritativo e non è uguale tra l’uomo e le diverse specie animali. Inoltre, modificando il grado di solubilità e il coefficiente di ripartizione dei composti attivi si possono ottenere dai veicoli ulteriori effetti. Ne sono alcuni esempi i liposomi ed i niosomi.

Può anche essere alterato il rapporto tra penetrazione interfollicolare e penetrazione follicolare. Le soluzioni sembrano favorire la prima via di penetrazione mentre le particelle (emulsioni) favoriscono la seconda.

D’altra parte si sente anche la necessità di incrementare lo studio sulle sostanze che riducono o impediscono l’assorbimento percutaneo soprattutto ridurre il rischio di reazioni tossiche o allergiche provocate da inquinanti ambientali con cui si viene in contatto durante la vita di relazione o nell’ambiente di lavoro.

Nell’usare accelleranti o bloccanti l’assorbimento bisogna tener anche conto che la cute sana e la cute patologica reagiscono in modo diverso all’applicazione topica di farmaci dermatologici o di cosmetici.
Introduction

This paper will focus on some aspects of the horny layer in relation to its barrier function and modifications induced by vehicles including additives such as penetration enhancers. The following reviews give more details: Brandau & Lipold, 1981; Bronaugh & Maibach, 1985; Elias, 1983; Landmann, 1988; Maltoltsy, 1986; Schaefer et al. 1982; Schalla, 1989; Schalla & Schaefer, 1987; Scheuplein, 1978; Scott & Dugard 1989; Shroot & Schaefer, 1987.

The reader should bear in mind that vehicles also influence other features of the horny layer, such as smoothness, appearance, subjective feelings etc., which are not within the scope of this article.

Composition of the Horny Layer

It is generally agreed that the horny layer is the main barrier against diffusion. This layer is composed of cells which die during their terminal differentiation, with consequent reduction of the water content which in turn leads to a tightly packed structure of filaments and matrix resistant to many liquids and solvents. This cellular content is encased in an even more resistant cornified envelope. The intercellular space between these flattened cells is composed of lamellar structures formed by multiple bilayers of the hydrophobic and lipophilic regions of fatty acid esters, ceramides, cholesterol sulphate and others (reviewed by Elias, 1983; Elias et al. 1987; Landmann, 1988; Matoltsy, 1986). The lamellae tightly pack the structures and completely fill the intercellular space in the innermost horny layer, whereas they form lacunae in between the now non interrupted lamellae in the mid-portion and outer stratum corneum (Hou et al. 1991).

There is an inverse linear correlation between the horny layer thickness and the transepidermal water loss (TEWL) as a simple measure of overall skin permeability for hydrophilic compounds (Leveque, 1989; fig. 1).

![Fig. 1 - Inverse linear correlation between transepidermal water loss and horny layer thickness (Leveque, 1989)](image-url)

The horny layer thickness in vitro and in vivo depends on the relative humidity (RH). Up to 85% RH, the thickness is increased to 125% relative to 0% RH. A steep increase was calculated for RH > 98% leading to 65µm thickness at 100% RH compared to 20 µm in vivo at 0% RH (Blank, 1984).

Most of the fascinating contributions in the last two decades have focussed on the essential role of the intercellular domain for percutaneous absorption, i.e. the intercellular pathway through the horny layer. Nonetheless, other routes such as the transcellular pathway should not be ruled out. The latter route is much shorter. 'Vertical' gates in the direction of the viable epidermal layers can hardly be seen between neighboured corneocytes in fig. 2 - not surprisingly, since the mean diameter of these hexagonal cells is in the range of 30 µm whereas their thickness is less than 1 µm. The distance for a molecule to diffuse by the intercel-
lular pathway is therefore extremely long relative to the transcellular route.

Moreover, detergent-induced cell dissociation and desquamation can be stimulated by EDTA and inhibited by aprotinin, a proteinase inhibitor, underlining the importance of proteinaeous structures for the morphological changes observed during terminal differentiation within this barrier layer (Egelrud & Lundstrom, 1990). The corneocyte attachment was found to be correlated with the number and distribution of corneosomes (modified desmosomes in the horny layer). The inner stratum compactum was tightly packed and corneosomes were numerous, whereas cohesion were mainly peripheral in the stratum disjunction. In this superficial part of the horny layer, corneosomes were restricted to corneocyte edges (Chapman & Walsh, 1990). On the other hand, extracellular lipids derived from membrane-coating granules seem to play a significant anti-cohesive role preventing apposition of corneocytes, indicating that lipid envelopes are unlikely to mediate cohesion in normal stratum corneum (Chapman et al. 1991).

The follicular route was considered to be important only in the early phase of penetration, whereas the major part of any given compound permeates later on via the interfollicular epidermis. Based on a few experiments this phenomenon was explained by a reduced barrier of the follicular epithelium on the one hand and the approximately 100 fold larger surface area of the interfollicular epidermis on the other hand. This point of view needs to be modified in the light of recent results (see the article “Advances in Percutaneous Absorption” in this monograph). As Brian Barry (1987) pointed out: “We can postulate that for most penetrants of intermediate polarity, a variety of routes will come into play dependent on skin condition and diffusional time, but for simplicity investigators often consider only the predominant route for any particular drug. However it is important not to concentrate solely on one theoretical pathway and to ignore other possibilities”.

The interest in transdermal delivery for extracutaneous treatments has further stimulated the efforts to better understand percutaneous absorption, mainly in trying to increase skin permeability and controlling the release of drugs or prodrugs.

**Penetration Enhancers**

Permeation can be increased by various types of enhancers such as fatty alcohols and acids, their esters, in particular monoglycerides, Azone, pyrrolidine derivatives, cyclic monoterpenes, methyl sulfoxides, salicylic acid and urea. There is evidence that their modes of action differ.
Disruption of the intercellular lipid structures seems to be one of the most common mechanisms for enhancers, including Azone, oleic acid and dimethyl sulphoxide. The enhancers could be put into three main categories: those which promoted permeation through both polar and lipid routes of the intercellular space, accelerants which preferentially affected the lipid route, and enhancers which mainly modified polar pathways (fig. 3) (Barry, 1987).

**Fig. 3 - A composite diagram representing postulated sites for penetration enhancers to act in the intercellular domain.** The figure illustrates the change from relative order to relative disorder after the insertion of accelerants. Small circles represent polar accelerants such as the pyrrolidones, propylene glycol, ethanol and aprotic solvents; linear chain molecules represent Azone and bent molecules correspond to cis-unsaturated long-chain compounds such as oleic acid and oleyl alcohol (Barry, 1987).

Other modes of action of penetration enhancers involve altering the composition of the intercellular space and/or the cohesiveness between cells as well as effects on the cell membrane or intracellular components. The enhancement by these agents does not correlate with their irritancy, and it is not identical between man and a number of animal species.

**Vehicles as Carriers of Active Ingredients**

Other components of vehicles do not interact as much as the penetration enhancers with horny layer structures. Rather, they modify the solubility and partitioning of active compounds in a given vehicle and thereby the permeation.

Liposomes and niosomes are newer developments in this direction. Such bilayered and multilayered vesicles to some extent mimic the intercellular lamellar structure in the stratum corneum and can serve as carriers for active ingredients. In this respect, they probably act more as an additional reservoir rather than as a penetration enhancer. Results suggesting that liposome entrapped molecules have increased skin permeability have hampered by that fact that the vehicles used were far from optimal for the given agents. In other words: it is easy to improve skin permeability by every method if a poorly releasing vehicle is used as reference. We could for example not enhance the penetration of hydrocortisone by incorporating it into niosomes when we used for comparison a vehicle which we had found to release the drug very well to the skin.

The ratio between the interfollicular and the follicular penetration pathway can also be altered. Solvents seem to favour the former, penetration as particles, washing off the remaining excess of a topical from the skin surface and probably also entrapping into liposomes or niosomes, the latter.

**Reduction of Penetration**

The most common approach used for reducing the activity is dilution of the active agent in the preparation, e.g. by mixing a drug containing topical with its vehicle without any drug. However, it was observed that some such diluted preparations were as active as the undiluted ones (Gibson et al. 1984). One reason for this phenomenon is that quite often suspensions are used containing solubilized as well as unsolubilized drug. Only the former can immediately be liberated to the skin. Crystals have first to be dissolved within the vehicle or at least within the stratum disjunctum of the horny layer before they can penetrate into the skin. Dilution of such su-
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spensions diminished the unsolubilized portion, but not the solubilized concentration because crystals will again dissolve up to drug saturation in the given vehicle.

Another point to be stressed in respect to decreased penetration is the following:

Reduction of the active agent at the target site is not automatically followed by a decreased activity, as holds true for the opposite, i.e. enhanced penetration may not always improve the efficacy of cosmetic, dermatological or transdermal treatment. It has to be born in mind that the dose response relationship is usually saturable and that there is a maximal response which cannot be further augmented by increasing the concentration. The need for use of penetration enhancers (or reducers) has therefore to be checked individually.

There is also an increasing need for broad penetration blockers in view of the risk of toxic and allergic reactions from the chemicals used in our domestic and occupational lives. The barrier creams, including topicals containing ion exchangers, silicone, tanning agents etc., are at present far from being ideal for most of their practical uses. Therefore, the best protection still remains the avoidance of direct contact with such chemicals by instrumental aids or by wearing gloves.

Altered Horny Layer Structures

The stratum corneum is modified by environmental factors, skin diseases and their treatment and so on. Acute sunburn is followed by a sharp, short-lasting increase in TEWL (see "Advances in Percutaneous Absorption" in this monograph); chronic UV exposure induces a thickening of the horny layer. Thickening of the horny layer is not automatically accompanied by a stronger barrier function since it depends on the type: ortho-hyperkeratosis such as in callosities strengthens the barrier, pathological hyperkeratoses in most ichthyotic conditions diminish the barrier function, as in parakeratotic diseases such as psoriasis and acute and subacute eczema (fig. 4) (review in Schalla, 1987, 1989).

![Fig. 4 - Time course of the transepidermal water loss (TEWL) of involved and uninvolved psoriatic skin under PUVA therapy.](image)

Treatments may modify the horny layer in several ways:

Occlusion is followed by maximal hydration of the horny layer. As already pointed out, the thickness increases by a factor of about 2.5. The barrier function is usually reduced by occlusion as shown in a number of studies (reviewed in Brandau & Lippold, 1982; Bronaugh & Maibach, 1985; Schaefer et al. 1982). The increased skin permeability is usually accompanied by an increase in the topical as well as the systemic bioavailability. We have found as an exception of this rule that occlusion only augmented the systemic bioavailability (Schalla et al. 1987).

Bathing also induces a maximal hydration of the horny layer, but it is of shorter duration. Interestingly, the reservoir function of the horny layer seems to be reduced by bath application of active agents. Fischer (1976) compared the time courses of photosensitivity by a trioxsalen bath to those from trioxsalen painting and methoxypsoralen tablets (fig. 5).
The photosensitivity dropped immediately after termination of a bath for 15 min, a far shorter time than that after painting which did not reach its maximum within the first hour. We were able to confirm this and in addition found no detectable serum levels, in contrast to painting, indicating that the kinetics after bathing are completely different from those of topical application. The kinetics are in fact superimposable on those for iontophoresis, but at a lower level (fig. 6).

In either of those particular applications, the horny layer reservoir is diminished and effecti-ve drug concentrations in the skin are reached earlier. If short application periods (rough estimate < 30 min) and higher drug concentrations in the same vehicle are used the topical bioavailability is often higher than that by conventional application, but the systemic bioavailability is lower. Hence, the benefit/risk ratio is better when such short applications are used.

Corticoids cause skin atrophy and thinning of the horny layer (Wendt & Frosch, 1982). One can assume that the barrier function is reduced and that this in turn may reinforce any side effects.

Retinoids also alter the structure of the horny layer. Serum levels normally reach a steady state after 1-2 weeks of repeated dosing to the intact horny layer. As shown in fig. 7, a plateau is, indeed, achieved within the 2nd week, but this is followed by another increase. This indicates that the barrier function is disturbed by chronic exposure to retinoids which thus after 2 weeks facilitate their own penetration.
References


