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Review

Organotypic cell cultures and two-photon imaging: Tools for in vitro and in vivo assessment of percutaneous drug delivery and skin toxicity

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ABSTRACT

The outermost protective layer of the skin, the stratum corneum, is responsible for skin impermeability toward external medications and potentially harmful chemicals. Stratum corneum is the target for physical and chemical approaches to enhance drug permeation. These approaches are commonly investigated in the field of drug delivery, but the drug absorption enhancement is often linked with local toxicity. In this review we are discussing two emerging technologies for drug and chemical studies in the skin: organotypic cell cultures and non-invasive two-photon microscopic imaging. Even though several cell culture based 'skin equivalents' have been introduced and validated for skin irritation testing, they are usually leaky and inadequately characterized in terms of permeation. Rat epidermal culture model (ROC) has been thoroughly characterized and it shows comparable barrier properties with the human skin thereby being useful in drug permeation and toxicity studies. In vitro and in vivo visualizations of permeants and skin structures are now feasible due to the rapid development of two-photon microscopy that allows improved depth scanning and direct in vivo visualization of the permeating compounds and adverse reactions in the skin structures. In summary, the new tools in percutaneous drug delivery studies will provide new insights to the permeation process and local toxicity. These tools may facilitate development of effective and safe transdermal drug delivery methods.

Contents

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1. Introduction

Skin diseases are frequently medicated by topical drug formulations. They are more convenient than oral formulations, because a smaller dose of drug is required for the local effect and the systemic side effects can be minimized. In addition to the localized dermal treatments, the drugs are often delivered across the skin for the treatment of systemic diseases. Transdermal drug delivery permits sustained drug delivery and results in more stable drug concentration in plasma compared to the situation after oral drug administration. However, drug administration through the skin is hampered by the barrier properties of the skin and by local adverse effects. Usually, only small lipophilic compounds that require daily dose of 10 mg or less can be used effectively using the transdermal route [1]. Despite major research efforts, the success in the transdermal delivery of bio-drugs (oligonucleotides, proteins, DNA) has been only modest.

The low permeability of the skin is due to the unique structure of the outermost layer of the skin, stratum corneum [2]. In order to widen the spectrum of molecules that can be administered dermally and transdermally, the skin barrier should be overcome safely and reversibly. Several chemical, electrical and mechanical methods have been used to enhance skin permeability by evoking structural alterations in the stratum corneum [3]. These methods include iontophoresis, electroporation, ultrasound, microneedles and chemical enhancement of stratum corneum permeability.

Excised specimens of animal and human skins have been widely used to test drug permeation in the skin. It is generally recognized that the most commonly used animal skin, the nude mouse skin, is too leaky to be a reliable substitute of the human skin. On the other hand, the availability, variability and quality of the human skin samples cause practical problems. Furthermore, the excised pieces of human skin represent dead tissue that is not suitable for toxicity evaluation of drug delivery systems. Alternative methods are needed also for the safety evaluations of chemicals.

The EU Regulation for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [4], requires in vitro testing for eye and skin irritations for substances marketed in volumes between 1 and 10 t per year. EC documents also require and advice to validate in vitro assays as full or partial replacements of the animal test [4–6]. Validated in vitro tests accepted by the regulatory agencies allow full replacement of the animal tests for skin corrosion and irritation [7–9]. The current status of in vitro test methods for skin corrosion and irritation, and their regulatory applicability are presented in Eskes et al. [10].

Three-dimensional reconstructed models of skin epidermis are promising alternative to the animal experiments and ex vivo human skin samples [11]. Unlike excised skin samples, the cell models are viable and they can be used to study specific biological responses to the treatments. Before they can receive official recognition as an alternative to human skin, the cell models need to be validated. The validated methods for skin corrosion testing have been adopted in the EU legislation since 2000 (methods B.40, B.40bis, B.46), and at OECD level since 2004 (guidelines 430, 431, 435, 439). Currently, ECVAM has considered three validated test methods for skin irritation (EpiSkin™ SIT, EpiDerm™ EPI-200 SIT, SkinEthic™ RhE SIT42bis) to be satisfactory to be included in the EU CLP (GHS) classification system [12].

Overall, several cell culture models have been described in the literature. They are based on isolated primary cells, immortalized cell lines or on stem cells. Validity of such cell models, in terms of permeability and toxicity evaluation, depends on the properties of the cultivated stratum corneum barrier. In fact, the level of documentation and barrier functions of various models are quite variable, and oftentimes inadequate.

Imaging techniques have been improving tremendously during the last decade. For example, radioimaging (SPECT, PET), fluorescence and luminescence based in vivo imaging, and cell based imaging methods (like high content screening) have progressed significantly. The aforementioned imaging methods are not particularly suitable for skin permeation studies, because the skin is a non-transparent 3D cellular system. In the past, confocal microscopy has been successfully used to study permeation routes of fluorescent probes in the presence of iontophoresis and to study the liposomal drug delivery in the human ex vivo skin [13]. Traditional confocal microscopy, however, does not allow direct in depth scanning of intact skin samples or monitoring permeability in vivo in humans. Interestingly, these aspects may be applicable to the new two-photon microscope methods. These methods might then allow direct monitoring of drug and delivery system permeation in the skin, even in human skin in vivo.

Cell cultures and imaging are rapidly evolving methods that may become very useful in dermal and transdermal drug delivery researches. They are potentially useful in the evaluation of both permeation and toxicity, the main pillars of transdermal drug delivery. This review summarizes the current state and future challenges of organotypic cell culture and two-photon microscopy as related to percutaneous drug delivery and chemical irritation testing.

2. Structure of the skin

Skin is divided structurally and functionally into three distinct layers, the outer epidermis, the inner dermis and subcutaneous fat tissue. The epidermis constitutes a dynamic system in which the major cell type, keratinocytes, differentiate during the passage from the proliferating basal keratinocytes to the surface of the horny layer where the dead keratinocytes, referred to as corneocytes, are eventually discarded [14]. This process takes about 2–3 weeks.

The stratum corneum (SC) consists of terminally differentiated flattened and keratin filled corneocytes embedded in a matrix of lipid bilayers [15,16]. The cellular remodeling takes place in the upper granular cell layer, and simultaneously with cell terminal keratinization, the lipid stacks and enzymes of lamellar bodies are released into the intercellular space in the SC [17,18]. The cornified cell envelope replaces the plasma membrane in corneocytes as the endpoint of epidermal differentiation and cell death. In the cornified envelope, several proteins (keratin, loricrin, filaggrin, involucrine) are highly cross-linked by transglutaminase [19]. Covalently bound lipid envelope is assumed to function as an anchor between the cornified protein envelope and intercellular lipids. This is crucial for the proper orientation of SC lipid lamellae.

The SC lipid organization is considered to be very important for the skin barrier function The total lipid content of human SC is about 14% of the dry mass [20]. A unique mixture of SC lipids is formed when polar lipid precursors are converted into non-polar lipids, such as ceramides (30–41%; at least 9 different classes), cholesterol (20–32%) and free fatty acids (9–25%), and a small amount of cholesterol sulfate (2–5%) [16,21,22]. In contrast to all other lipid membranes in the body, phospholipids are largely absent from the