Following toxicity and efficacy markers in skin substructures using Mass Spectrometry Imaging (MSI)

The better understanding of drug penetration and effects through the various layers of the skin may play a key role in the drug development process especially for pharmaceutical, cosmetic and dermatological products. In this context, mass spectrometry imaging (MSI) provides useful and precise information about exogenous compounds’ targeting in the skin substructures (at the micrometer scale), their amount (using quantitative MSI), their impact on endogenous molecules of the skin tissue (small endogenous metabolites, lipids, peptides or proteins) and their potential adverse effects (inflammatory or toxicity response).

Introduction

Skin is a highly complex organ made of several layers (stratum corneum, epidermis, dermis, hypodermis...) with specific properties and functions. In addition, there are different types of glands (sebaceous, sweat...), the dermal papillae, the sweat pores, the sensory nerves, the blood vessels; the follicle ducts, etc.... The skin acts as a barrier protecting from its environment and is the first line of defense of the human body. As a living organ, the skin could be affected by various disorders such as, psoriasis, rosacea onychomycosis atopic, Dandruff and Seborrheic Dermatitis (D/SD), acne or skin senescence... The knowing of specific biomarkers of each pathology gives access to an early diagnosis of diseases while the efficacy, toxicity or inflammatory markers permits to evaluate the action of dermatological drugs. For example, in D/SD disease, the up-regulation of cytokine (especially interleukins) levels in diseased skin provides an early indication of disease proliferation, as well as histamine, which acts as inflammatory biomarkers in this pathology. The down-regulation of ceramides represents, meanwhile, an alteration of the skin barrier integrity and consequently is an important proof of disease evolution.

Molecular data on drugs and skin’ biomarkers are generally obtained by Liquid chromatography coupled with mass spectrometry (molecular profile of skin homogenate) or using Raman spectroscopy (depth profiling experiment). MSI combines the main advantages of these two techniques. MSI generates two dimensional virtual images of a sample, a tissue section corresponding to a specific signal on generated mass spectra i.e. a specific molecule from the tissue. MSI and more specifically MALDI Imaging is used to obtain a global view of the molecular composition of the skin and of its different substructures. Another advantage of MSI is to analyze the skin at high spatial resolution. The laser beam diameter can reach 20 µm and allows having access to small histological structures of the skin. In order to evaluate the action of dermatological drugs related to these pathologies, MSI provides the precise localization and profiling of these exogenous molecules after a topical administration. MSI helps to understand their efficacy and pharmacokinetics (PK)/pharmacodynamics (PD) by following specific endogenous biomarkers.
MSI was already used to study the distribution of endogenous species [1] in skin such as cholesterol [2] or proteins such as decorin, lumican or keratin [3] and especially lipids [4] for example, glycosphingolipids (globotriasylceramide and the galabiosylceramide) involved in Fabry’s disease mechanism [5]. Furthermore, it was also used to follow penetration of exogenous compounds in the skin [2] such as the anti-inflammatory drug, ketoconazole [6]. Vaccines can also be studied using MSI to evaluate the response of the skin itself to the treatment by following inflammatory or toxicity biomarkers and estimate changes in endogenous metabolites levels. Several examples of endogenous and exogenous compounds analysis in skin substructures will be illustrated in the following paragraph. MSI was used in order to follow biochemical changes at the micrometer level and to support the understanding of PK/PD and potential toxicity.

**Experimental**

Human skin biopsies were sectioned using a cryostat (Microm) at 10 µm. The cutting plane was chosen in order to see all dermal layers of the tissue. Sections were transferred to ITO-coated glass slides. Then, a cryodrying step is performed in the cryostat chamber followed by a drying step under vacuum. The matrix 2,5-DHB (at 40 mg/mL in MeOH/Water+0.01% TFA) was used and deposited using an automatic sprayer device, the SunCollect (Sunchrom). Optimized deposition parameters were chosen to obtain better detection, sensitivity and matrix homogeneity. MALDI-MS and MSI measurement was conducted using two mass spectrometers; an Autoflex Speed TOF (Bruker Daltonics) and a Solarix 7.0T FTICR (Bruker Daltonics) both equipped with SmartBeam II Laser. The positive detection mode was used with optimized parameters (mass range, voltage, transient length…) for each instrument. Regarding the MSI experiments, various spatial resolutions were selected between 20 and 60 µm depending on analyzed tissue area. Image acquisition and visualization was achieved with FlexImaging 3.0 (Bruker Daltonics). After removal of the matrix by MeOH washing, HE staining was performed on skin section according to ImaBiotech’s SOP to observe dermal layers and correlate with MS imaging data.

**Results**

**Toxicity Biomarkers (necrosis or apoptosis for carnitine):** The first example of application of MSI in skin analysis was the study of the distribution of a small endogenous metabolite, carnitine. Carnitine is a quaternary ammonium compound biosynthesized from the amino acids, lysine and methionine. It plays a key role in necrosis and apoptosis in cells and can be used as biomarkers of theses phenomena in skin studies [7]. In fact, the modulation (up or down regulation) of the carnitine level can give some precious information about potential adverse effects of a drug administered in tissue. We present, in figure 1, the distribution of an ion corresponding to the carnitine molecule ([M+H]+, m/z 162.11247) characterized using high mass accuracy (below ppm level) and on tissue fragmentation. This ion is localized in the epidermis, the follicle ducts and the apocrine sweat glands. It may be explained by the fact that all these tissues, and especially the epidermis, are primarily composed of keratinocytes cells, the most common type of skin cells. The fully cornified keratinocytes that form the outermost layer are constantly shed off and replaced by new cells. This cycle involves some necrosis phenomena which are in agreement with the carnitine detection in this histological area. Furthermore, it is also possible to achieve some molecular histology using this data to localize specific skin substructures such as, the apocrine sweat glands or the follicle ducts, as shown in figure 1. In this case, MSI allows the detection of potential necrosis or apoptosis biomarker, carnitine in skin substructures.

**Figure 1**

Distribution of carnitine in different skin substructures (from human biopsy) using MSI (MALDI-FTICRMS, positive ion mode, 30 µm of lateral resolution)

**Figure 2**

Distribution of some lipids of different classes, sphingomyelin (green), phosphatidylcholine (red) and Triacylglycerol (in blue) obtain using MALDI-TOF mass spectrometer (positive ion mode, 30 µm of spatial resolution). Each of these lipids are related to specific substructures of the skin (see H&E Staining optical image and overlay molecular image)
Inflammatory and disease state biomarkers (Triglycerides for Psoriasis and Ceramides for Eczema): The second example of application is related to the study of the distribution of a class of compounds widely present in the skin, the lipids. Some of the lipids can act as inflammatory markers for the skin [8]. For example, Eicosanoids are a large class of signaling molecules that includes prostaglandins (PGs), thromboxanes, leukotrienes, hydroxy fatty acids or lipoxins. These lipids are easily detected using MSI and can be localized in skin substructures. The figure 2 shows the distribution of different lipids from specific classes; sphingomyelin (SM (34:1), [M+K], m/z 741.48); phosphatydicholine (PC (34:1), [M+K], m/z 782.66) and triacylglycerol (TG (54:3), [M+K], m/z 907.77). Each of these ions has a specific distribution in the tissue and allows the differentiation of the skin substructures (Epidermis, dermis and hypodermis). The epidermis shows higher intensity signal for sphingomyelin, whereas triacylglycerol is more intense in the hypodermis. The overlay distribution of these three lipids gives a nice picture closely related to the skin histology.

Moreover, using high resolution mass spectrometry, we are able to obtain a global lipid profile for each skin substructure as presented in figure 3. Two mass spectra corresponding to specific locations in the dermis and the epidermis are shown. From these mass spectra we can identify several lipids using lipid database search and can separate them in different classes (sphingolipids, glycerophospholipids, glycerolipids, sterol lipids, phenol lipids). We found that almost low molecular mass lipids are concentrated into the epidermis whereas there are higher molecular mass lipids in the dermis; this is the case of sphingolipids, major constituents of the outer layer of the skin.

As shown in the insert of figure 3, a ceramide ion, corresponding to ceramide-phosphate (18:1/18:1) species ([M+Na], m/z 666.4839), is observed at the level of the epidermis. Ceramides are associated with permeability barrier function of the skin and concentrated especially at the level of the stratum corneum. These lipids are important for cosmetology or dermatology because they are modulated in some skin disorders, such as eczema or psoriasis. Thanks to MSI we have observed the fine localization of these different lipids in the skin. It is also possible to evaluate the inflammatory response or other disease state in both the dermal and the epidermal layers.

The third application of MSI in skin analysis is related to the study of skin penetration of pharmaceutical compounds after topical administration. In this example, a mixed solution of three drugs was applied on human skin and after 24 hours punch biopsies were obtained. The figure 4.a displays the mapping of the three compound penetrations through skin layers at a spatial resolution of 50 µm. These molecular images highlight the different behavior of each drug after topical administration. In fact, compounds 1 & 3 are somewhat similar in their localization but with more intensity for the compound 1 in the dermis and hypodermis. However, the compound 2 appears to be even more concentrated in the dermis with no penetration in the hypodermis. The graphic of figure 4.b is a schematic representation of the variation of the signal of each drug through dermal layers; it permits to assess easily the specific penetration of each compound. This example shows the ability of MSI to obtain a quick assessment of drug penetration in the skin substructures.
These examples of applications illustrate the potential of MSI in skin tissue analysis. MSI has the unique ability to generate both molecular and spatial information during a single experiment. It provides a global approach: provides information not only on drug skin penetration, but also provides information on potential impact on toxicity (necrosis or apoptosis) using biomarkers such as carnitine, or inflammatory biomarkers such as eicosanoids, or other disease state biomarkers (endogenous metabolites, lipids, peptides or proteins).

Thanks to MSI, ImaBiotech can provide precise information about drug distribution in the skin substructures, as well as supportive information about efficacy and potential.

**Benefits**

- Quantify drug and metabolites in different
- Unknown metabolite research and identification
- Inflammatory/Toxicity biomarkers monitoring
- Disease state markers assessment
- High spatial (20µm) and spectral (R>500000)
- Molecular histology and segmentation

**References**

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**Keywords**

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