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Real time and *in vivo* pharmaceutical and environmental studies with SpiderMass instrument

B. Fatou[a], P. Saudemont[a], M. Duhamel[a], M. Ziskind[b], C. Focsa[b], M. Salzet[a,*], I. Fournier[a,*]

[a] Université de Lille 1, INSERM, U1192 – Laboratoire Protéomique, Réponse Inflammatoire et Spectrométrie de Masse (PRISM), F-59000, Lille, France
[b] Université Lille, CNRS, UMR 8523 – PhLAM – Physique des Lasers Atomes et Molécules, F-59000, Lille, France

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ABSTRACT

Remote Infrared Matrix Assisted Laser Desorption Ionization (Remote IR MALDI) system (SpiderMass) with endogenous water as matrix allows to perform real-time DMPK *in vivo*. In this work, SpiderMass was used to analyze the impact on metabolite production or release of invalidated pro-protein PC1/3 macrophages by Short RNA (shRNA) *versus* scramble shRNA with Paclitaxel. Time course *in vivo* experiments were then performed on the inner and outer faces of patients' forearms or comedo treated with Melascreen (Ducray) containing ascorbyl glucoside. Finally, the impact of car pollution (emitted soot) on skin was also investigated. Taken together, we demonstrate that the SpiderMass instrument opens the door to clinical, pharmaceutical and environmental domains for real-time, *in vivo* pharmacokinetic (Drug Metabolism and PharmacoKinetics, DMPK) analysis.

1. Introduction

Since its introduction, MALDI-MSI has been demonstrated as one major technique that can revolutionize pharmacokinetics by providing a label-free imaging alternative for the detection and localization of drugs and drug metabolites (Flinders et al., 2017; Goodwin et al., 2011; Prideaux et al., 2010; Swales et al., 2016; Tsubata et al., 2017). However, this technique does not offer the possibility to have real-time information. Moreover, a clearly unmet challenge that cannot be addressed by MALDI MSI is the *in vivo* DMPK/ADME (Absorption, Distribution, Metabolism, and Excretion) experiments. Only ambient mass spectrometry techniques like desorption electrospray ionization (DESI) can be used for *ex vivo* tissue surface analysis of drugs (Bailey et al., 2015; Cardoso-Palacios and Lanekoff, 2016; Olsen et al., 2016; Sero et al., 2015; Siebenhaar et al., 2015; Stojanovska et al., 2015; Swales et al., 2014). However, no *in vivo* data have been provided yet by any ambient mass spectrometry technique. In this context, we demonstrate the possibility to perform time course studies for the detection of drugs or soot components on skin, as well as the direct analyses of invalidated knockdown macrophages for the proprotein PC1/3 themselves or their secreted metabolites directly on cell culture.

2. Experimental procedures

2.1. Reagents

The rat alveolar macrophage NR8383 cell line (CRL-2192) were obtained from ATCC (USA). Paclitaxel (Taxol) was obtained from Sigma.

3. Culture of the NR8383 PC/3 KD or NT cell line

The rat alveolar NR8383 wild type (WT) macrophages were cultured with Ham's F12 K medium supplemented with 15% fetal bovine serum. NR8383 PC1/3 knockdown (KD) and NR8383 non-target (NT) shRNA cell lines were cultured in Ham's F12 K medium supplemented with 15% fetal bovine serum and 12 µg/ml puromycin at 37 °C in a humidified atmosphere (5% CO_2). NR8383 PC1/3 knockdown was performed using lentivirus transduction, as described previously (Gagnon et al., 2013). PC1/3-KD and NT cells with or without Taxol (Placitaxel) pre-activation were centrifuged for 15 min at 5000*g* (Duhamel et al., 2018). The collected pellet was pipetted (10 µl) and placed onto glass slides and dried using a vacuum dryer before analysis. For the secreted metabolites, the culture media from NT or PC1/3-KD cells were collected before being concentrated with a speed-vac and deposited on glass slides for analyses.

* Correponding authors at: Réponse Inflammatoire et Spectrométrie de Masse (PRISM), INSERM, U1192 – Université Lille 1, Bât SN3, 1er étage, Cité Scientifique, F-59655, Villeneuve d'Ascq Cedex, France.
E-mail addresses: michel.salzet@univ-lille1.fr, michel.salzet@inserm.fr (M. Salzet), isabelle.fournier@univ-lille1.fr (I. Fournier).

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4. Human volunteers

A cohort of ten healthy volunteers including 5 men and 5 women was selected for the experiments without any discrimination according to age. Before the experiments the volunteers signed an informed consent, authorization form describing the experimental protocol and instrument and exposure to the hazards. No personal information such as the name of the individuals were used in these experiments. A randomized number was assigned to each individual.

5. *In-vivo* skin analysis

Three distinct experiments were performed according to the same sequence for each individual. The volunteers were asked to place the inner or outer faces of their forearms at the adequate position under the laser beam. Spectra were acquired prior to and after turning on the laser and when it was switched on for a 30-s irradiation period. The MS spectra were averaged over the 30-s irradiation period and extracted from the TIC. For the first experiment a pharmacological product Melascreen (Ducray) was tested in time course from 10 min to 6 h30 after a single deposition. In the second experiment, the patient forearms were placed in contact with nettles. To test for the effect of soot, the volunteers were asked to place fingers with or without gloves close to the car exhaust pipe during 1 min.

6. Metabolite profiling using the SpiderMass instrument

The design of the instrument setup is already described in a previous study (Fatou et al., 2016). Briefly, the first part represents a tunable wavelength laser system that can operate between 2.8 µm–3.1 µm (Radiant version 1.0.1, OPOTEK Inc., Carlsbad, USA), connected to a 10-ns pulse duration Nd:YAG laser pump (Quantel, Les Ulis, France). A biocompatible laser fiber (450 µm inner diameter, length of 1 m, Infrared Fiber Systems, Silver Spring, USA) is connected at the laser system output and a 4 cm focusing lens is attached to the extremity of the laser fiber. The irradiation time was set at 10 s and at 4 mJ per laser pulse. The second part is composed of a Tygon tubing (2.4 mm inner diameter, 4 mm outer diameter, Akron, USA), directly connected to the mass spectrometer (Synapt G2s, Waters, Manchester, UK) by an atmospheric pressure interface described elsewhere (Balog et al., 2015). The SpiderMass prototype system used for these experiments is presented (Fig. 1A–B) Spectral acquisition was performed in negative resolution mode with a scan time of 1 s. MS/MS spectra were recorded under real-time conditions using the same instrument, with the precursor ions subjected to collision-induced dissociation in the transfer cell. Special care was taken when working with infrared laser beams. The laser used for this study belongs to safety class IV, which requires wearing of specific laser safety goggles throughout all experiments. The MS spectra were then exported into a prototype classification software (Offline Model Builder version 1.1.773.0, Waters Research Centre, Budapest, HU) to perform principal component analyses (PCA) and extract the loading plots.

7. Results

7.1. Taxol modifies lipid & metabolite profiles in both NT and PC1/3-KD cells

Lipid profiles of non-targeted (NT) and PC1/3-KD cells were collected with the SpiderMass instrument at 1 h, 3 h, 6 h and 24 h after Taxol treatment (Suppl. Figs. 1A, B). In parallel, their secreted metabolites were also screened (Suppl. Figs. 2A, B). Plotting of the scores of the first three principal components (Fig. 2) shows separation of the acquired spectra according to treatment. Wild type, NT Cells and their secretome are clearly separated from each other (Fig. 2). After Taxol treatment, metabolite changes are observed between the two cell types and their secretome as well. Taken together, PC1/3 inactivation leads to modifications in the nature of the KD cells' metabolites constituents and secreted metabolites compared to NT cells. Moreover; Taxol treatment impacts the nature of metabolites produced by these cells (Suppl. Figs. 1 & 2) and their biological function as we recently published at the proteomic level (Duhamel et al., 2018).

7.2. Time course Real time pharmacokinetic of Melascreen cream on skin

Time course analysis of a photo-protector for brown spots and photo-aging (Melascreen) was performed on the skin of human volunteers. Patients' forearms were analysed in positive and negative mode with the SpiderMass (Fig. 3). Relative abundant fatty acids, diglycerides, phospholipids and triglycerides have been identified as was previously identified on patient fingers (Fatou et al., 2016). In negative mode, zooming at *m/z* 90–500 Da shows the detection of L-pyrolidone ascorbic acid and metabolites of arachidonic acid as previously published (Fatou et al., 2016) (Fig. 3, Zoom). Gender comparison (Fig. 4) in negative mode reveals discriminant ions *i.e.*, ions at *m/z* 154.01, 174.02, 227.12 and 383.23 Da in men and 267.07, 293.09 and 517.31 Da in women. Comparison tests performed on the outer and inner forearms showed the presence of *m/z* 554.3 Da, corresponding to a dihydroceramide, only in the former (Fig. 5). Application of the photo-protector on the skin gave a single peak at *m/z* 336.9427 Da on a high-resolution instrument, corresponding to the Melascreen principal component (Fig. 6A). A zoom at *m/z* 310–370 Da confirmed the

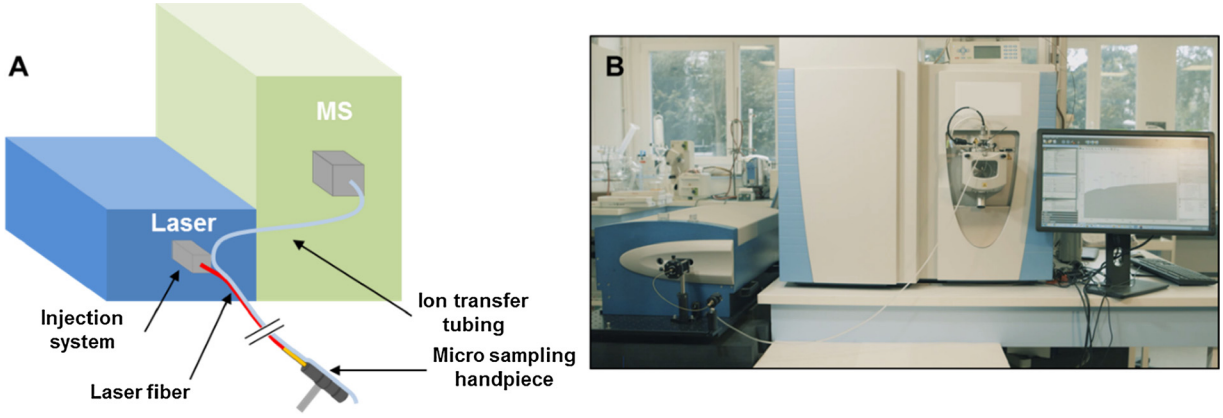

Fig. 1. A) Schematic representation of the SpiderMass system showing the connection between the laser, the mass spectrometer and sampling probe. B) Picture of one system with the laser system on the left with the fiber and the sampling probe in front of it, the mass spectrometer and its computer on the right and the transfer tubing linking both parts.