Would you evaluate the candidate drug efficacy by following companion biomarkers related to glomerulonephritis and the drug itself in fine histological kidney regions such as glomeruli? Is there a biomarker associated with kidney small substructures (tubules, ducts...), kidney dysfunction (inflammation, necrosis...) due to a disease, stress or drug toxicity?

**Introduction**

The kidney serves several essential regulatory roles in the human body and the dysregulation of renal physiological properties can induce serious pathologies. Moreover, it could be a major site of organ damage caused by drug toxicity (i.e. nephrotoxicity). The kidney can be separated into three major parts; the cortex, the medulla and the pelvis with functional significance. These regions have several small subregions of a few micrometers scale, such as for example the renal corpuscles (glomeruli), the tubules, the loops of Henle or the collecting ducts. There are different classes of lipids with specific role in the kidney cell proliferation, the cellular signaling or the inflammation process. For instance, dysfunction of sphingolipids (SL) and glycerophospholipids (GSL) metabolism induces the accumulation of these molecules in kidney substructures (e.g. glomeruli) which could result in different kidney diseases [1], such as polycystic [2], cancer, diabetic nephropathy, Fabry disease [3], glomerulosclerosis or nephritis.

Classical mass spectrometric analysis coupled with liquid chromatography (LC-MS) uses tissue homogenates, cannot provide spatial data. However, Mass Spectrometry Imaging (MSI) permits simultaneous detection and quantification of a wide range of molecules without labelling (drugs, exogenous and endogenous metabolites, lipids, peptides or proteins) while keeping their spatial information at the low micrometer level. So, knowing the distribution and the quantification of these molecules, such as the specific lipids in small histological kidney substructures, will be useful in drug development to improve understanding of pharmacodynamics (physiologic kidney changes induced by the drug on its local environment) and of toxicity (drug-induced kidney abnormalities). In this Application Note, several lipid biomarkers with key roles in renal diseases and with precise localization in the kidney substructures will be illustrated.
Experimental Section

Animal: Kidneys from wild type (male wistar strain) rat were removed; snap frozen and stored at -80°C.

Sectioning: Kidneys were sectioned following transversal plan (10 μm of thickness) using Microm HM560 cryostat (Thermo Scientific, Germany) at -20°C and mounted on ITO conductive glass slides (Delta Technology USA).

Matrix: 9AA (9-Aminoaacidine) powder (100 mg) was used and vaporized on tissue sample using home-built sublimation apparatus (200°C, 12 min, 2.10-3 mbar).

Mass spectrometry imaging: Solarix 7.0T FTICR (Bruker Daltonik, Germany) with SmartBeam II laser. Positive mode (600-1800 Da) at 60 μm spatial resolution.

Software: FlexImaging 4.0 (Bruker Daltonik, Germany) & Quantinetix 1.4 (ImaBiotech, France).

Lipid distribution in kidney transversal sections from wild type rat: zoom on renal cortex region for optic and molecular images showing co-localization of glomeruli (black arrows) and GM3 related Species. Mass accuracy measurement are reported for each lipid shown in the table, differences between experiment and theoretical values are expressed in ppm (part per million).
Results & discussion

Gangliosides (GL) are molecules composed of a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids (e.g. n-acetylneuraminic acid, NANA) linked on the sugar residue. GL are considered as biomarkers in numerous diseases targeting especially the brain such as Tay-Sachs, Gaucher or Sandhoff diseases. In these diseases, GL levels increase because of alteration or degradation of enzyme function [7]. One therapy could be to repair the enzymatic function [8]. NANA-Gal-Glc-ceramide abbreviated as GM3 (G for ganglioside, M for monosialic acid with only two sugar moieties) is detected in kidney section using MSI. As presented in the insert of the figure, GM3 related species are highly co-localized with glomeruli, which are a network of capillaries that performs the first step of blood filtering. Some dysfunction of glomeruli properties may induce renal pathology such as glomerulo- nephritis or sclerosis [9]. Three forms of GM3 species have been detected in glomeruli with specific fatty acid chain lengths (18:0, 20:0 and 22:0). All these chemical structures have been confirmed by MS/MS measurement and by high mass accuracy measurement (below ppm level).

Conclusions & summary

High spatial resolution MSI allows the detection and quantification of a wide range of lipids in specific histological kidney substructures unlike classical LC-MS. Some of the histological related lipids observed using MSI, such as gangliosides (glomeruli), cardiolipins (cortex) or sulfatides (medulla) are considered as reliable biomarkers of disease state. They permit the evaluation of the efficiency or the potential toxicity of a treatment. The detection of these markers within tissue section, in combination with the precise distribution of the drug candidate in these different kidney substructures provides huge amount of information in support of PK/PD studies.

ImaBiotech provides crucial information about the efficiency or potential toxicity of your drug candidate, thanks to the detection of specific renal disease biomarkers which are localized in fine histological kidney substructures.
Benefits

- Characterization of kidney disease
- Lipid companion biomarkers evaluation at the level of drug localization
- Renal disease state markers discovery in specific kidney tissue substructures
- High spatial and high spectral resolution imaging
- Molecular histology combines with classical histology techniques

Keywords

- Companion biomarkers
- Mass spectrometry imaging
- Renal disease
- Nephropathy
- Gangliosides
- Cardiolipins
- Sulfatides
- Sphingolipids
- Glomerulus

References


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