
Does the drug candidate reach amyloid plaques or does a repeat dose of drug candidate decrease the amyloid plaques formation? Is my drug candidate molecule reaching specific region of the brain and what are the drug and metabolite concentrations at different time points?

Introduction

To answer these questions, Imabiotec has developed different approaches to look at your drug candidate and to evaluate its distribution, its quantification, as well as for its drug metabolites [1] and biomarker in a single experiment, providing a fast and effective way to assess efficacy or potential toxicity of your molecules. Mass Spectrometry Imaging (MSI) is widely used to study the brain and consequently all central nervous system processes or dysfunctions. Numbers of pathologies target the brain and the central nervous system have been studied using MSI such as Alzheimer’s [2], Parkinson’s [3], Gaucher’s [4], Fabry’s [5] diseases, Migraine, Pain, Psychiatric Diseases, Sleep Disorders…In this context, specific molecular variation closely related to pathological development also known as disease biomarkers can be assessed by this emerging molecular imaging technique. In preclinical drug development, MSI provides pharmacokinetics and pharmacodynamics information about a drug targeting specific anatomical regions of the brain using high definition images.

Drug and its metabolites can be localized and can be discriminated on the same molecular image, as well as some biomarkers of toxicity, inflammation or disease state. Several applications will illustrate the benefits given by MSI in CNS studies and are listed below:

1. **Amyloid Beta (Aβ) peptide localization in Alzheimer’s brain model:** Study of efficacy by Mass Spectrometry Imaging

2. **Distribution & quantification of a Alzheimer’s drug candidate and its metabolites in brain substructures:** Quantitative mass spectrometry application (QMSI)

3. **Molecular histology of the brain substructures with biological implications:** High lateral Resolution Mass Spectrometry Imaging (HR-MSI)
1. Amyloid Beta (Aβ) peptide localization in Alzheimer brain model: study of efficacy by Mass Spectrometry Imaging

Distribution amyloid beta (Aβ) peptides in transgenic brain coronal sections; zoom on lateral ventricle region for optic and molecular images showing co-localization of amyloids plaques (black arrows) and Aβ peptides 1-42. Mass accuracy measurement are reported for each Aβ forms in the table, differences between experiment and theoretical values are expressed in ppm (part per million).

Figure 1

Experimental Section

Animal: Brain from transgenic mouse model (Tg2576, 7 months) was removed; snap frozen and stored at -80°C.

Sectioning: Brain was sectioned following the coronal plan (10 μm of thickness) using Microm HM560 cryostat (Thermo Scientific, Germany) at -20°C and mounted on ITO conductive glass slides (Bruker Daltonik, Germany). Prior to analyzes, washing step protocol [6] was applied on tissue section to increase detection of peptides. HE Staining was performed after MSI acquisition for better visualization of histological regions and amyloid plaques.

Matrix: SA (30 mg/ml AcN/W+TFA 0.2% 1:1; v:v) was chosen and deposited manually using TLC sprayer (Sigma, France).

Mass spectrometry imaging: Solarix 7.0T FTICR (Bruker Daltonik, Germany) with SmartBeam II laser. Full scan Positive mode (800-6000 Da) at 100 μm spatial resolution.

Software: FlexImaging 3.0 (Bruker Daltonik, Germany) & Quantinetix 1.4 (ImaBiotech, France).

Results & Discussion

- Amyloid beta peptides are the main component of amyloid plaques which are found in the brains of patients with Alzheimer's disease. Aβ could therefore be hypothesized associated to Alzheimer disease state biomarkers or pathology' consequences. The accurate localization of Ab may be useful to monitor the efficiency of a drug targeted specific Alzheimer’s disease regions in the brain.

- Amyloid beta peptides were observed with sporadic (hot spots) distribution in transgenic brain section at the level of cortex and hippocampus region as shown in figure 1. Several Aβ forms including (1-37, 1-38, 1-39, 1-40 and 1-42) have been detected on mass spectra and followed on molecular images from figure 1. Aβ was also detected on sagittal brain section from transgenic mouse (data not shown).

- High degree of confidence in the identification of amyloid beta peptides was achieved thanks to high mass accuracy measurement capacity of FTICR mass spectrometer.

<table>
<thead>
<tr>
<th>Aβ forms</th>
<th>m/z exp.</th>
<th>m/z theo.</th>
<th>Δm/ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ 1-37</td>
<td>4075.0003</td>
<td>4075.0032</td>
<td>0.7</td>
</tr>
<tr>
<td>Aβ 1-38</td>
<td>4132.0244</td>
<td>4132.0144</td>
<td>2.4</td>
</tr>
<tr>
<td>Aβ 1-39</td>
<td>4231.0928</td>
<td>4231.1089</td>
<td>3.8</td>
</tr>
<tr>
<td>Aβ 1-40</td>
<td>4330.1612</td>
<td>4330.1502</td>
<td>2.5</td>
</tr>
<tr>
<td>Aβ 1-42</td>
<td>4514.2824</td>
<td>4514.2844</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Mass measurement accuracy was calculated at low ppm level (between 0.4 and 3.8) on 5 Aβ forms and reported in table from figure 1.

- Co-localization between Aβ 1-42 peptides and amyloid plaques was observed on optical and molecular images (black arrows on HE staining indicate amyloid plaques). This result highlights two MSI capabilities: the complementarity of MS imaging and histological (or immunohistochemistry) imaging techniques for disease biomarkers hunting and capacity of drug distribution with biomarkers of efficacy.

- In combination with classical LC-MSMS, MS imaging provides a fast answer to your questions of drug distribution, biomarker distribution and quantification at a high resolution. ImaBiotech has developed a dedicated service to assess drug efficacy or toxicity by combining drug distribution and biomarkers directly from an in vivo study.
2. Distribution & quantification of a Alzheimer’s drug candidate and its metabolites in brain substructures: Quantitative mass spectrometry application (QMSI)

Animal: C57 Black6 wild type mice were dosed with the drug and brains were removed, snap frozen and stored at -80°C.

Sectioning: Brains were sectioned following the sagittal plan (14 µm of thickness) using Microm HM560 cryostat (Thermo Scientific, Germany) at -20°C and mounted on ITO conductive glass slides (Bruker Daltonik, Germany). Washing of sections with chloroform was performed (30 seconds) for better visualization of histological regions.

Matrix: 2.5 DHB (40mg/ml Methanol/TFA 0.1% 1:1; v:v) was chosen and deposited using SunCollect device (Sunchrom, Germany).

Results & Discussion

- The second MSI application is focused on a candidate drug against Alzheimer’s disease targeting specific regions of the brain. This study is closely related to the previous example (n°1) on Amyloid beta peptides localization; in fact, it is possible to evaluate the action of a drug on its environment or on potential disease state biomarkers such as Aβs. Thereby MSI may play a significant role in understanding ‘target engagement’ in early phases of drug discovery. This example deals with a sponsor candidate drug study, which is still under development and therefore requires confidentiality.

- The candidate drug shows a specific distribution in the hippocampus, the 3rd and 4th ventricles, the septum, the plexus choroid and in the medio-ventral hypothalamus. The drug identification was validated by on tissue mass spectrometric fragmentation experiment (MS2) highlighted attended mass fragments detection. These histological regions of the brain could be involved in Alzheimer’s disease development and consequently could demonstrate the efficiency of drug targeting in CNS.

- MSI provides a global approach: gives information not only on drug distribution as previously explained, but also provides their metabolites. Based on preliminary microsomal assays of drug metabolism, we are able to discover some mass to charge ratio (m/z) related to potential metabolites of our drug, directly from the original MSI data set (no additional experiment needed). Therefore, a drug metabolite was detected in the choroid plexus and the medio-ventral hypothalamus, which closely co-localized with its parent compound. This example shows MSI benefits compared to classical molecular imaging techniques such as QWBA, which cannot discriminate the parent drug and its metabolite.

- Finally, using quantitative mass spectrometry imaging [1] and Quantinetix software, we are able to determine the amount of the drug directly in small histological structures of the brain. In this example, the drug is quantified in the medio-ventral hypothalamus and in the plexus choroid at some µg/g of tissue level whereas other histological regions have weak concentration. As for example the hippocampus, the amount of drug was calculated at 119 pmol/g of tissue. This value was cross validated by LC-MS quantitative measurement on corresponding hippocampus homogenate of the same animal (139 pmol/g of tissue).

TEC Determination & Calibration: Dilution range of drug between 0.02 – 10 µM. 1 µL of standards deposited near dosed section on the slide. Drug concentration for TEC experiment was set at 10 µM and deposited on control section. TEC was calculated according methodology from Hamm et al [1].

Mass spectrometry imaging: Solarix 7.0T FTICR (Bruker Daltonik, Germany) with SmartBeam II laser. Positive CASI mode (50 Da Window) at 80 µm spatial resolution.

Software: FlexImaging 3.0 (Bruker Daltonik, Germany) & Quantinetix 1.4 (ImaBiotech, France).
3. Molecular histology of the brain substructures with biological implication: High lateral Resolution Mass Spectrometry Imaging (HR-MSI) Distribution of endogenous species from coronal and sagittal brain sections obtained using MALDI-TOF mass spectrometer at low (150 and 300 µm) and high (20 µm) spatial resolution.

Experimental Section

Animal: Brains from control wild type rat were removed; snap frozen and stored at -80°C.

Sectioning: Brains were sectioned following sagittal and coronal 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: 2.5 DHB powder (150 mg) was used and vaporized on tissue sample using home built sublimation apparatus (150°C, 8 min, 2.10-3 mbar).

Figure 3. Molecular histology of the brain substructures with biological implication: High lateral Resolution Mass Spectrometry Imaging (HR-MSI).

Results & Discussion

- Instrumental development for mass spectrometry imaging allows improving spatial resolution of the generated molecular image especially thanks to laser beam spot diameter decreasing and new matrix deposition device. The sublimation process allows achieving a thin matrix layer (1 µm of thickness) with small matrix crystals (less than 1 µm of diameter). These parameters induce a minimized analyte spreading within tissue and combining with high focused laser, creates a high spatial resolution mass spectrometric images.

- Two examples of a coronal and sagittal brain sections images are presented at low spatial resolution, 150 and 300 µm respectively, in figure 3. Some contrast ionic species are used to differentiate simply large histological structures of the brain. From one hand, white (green filter) and grey (red filter) matters are easily discriminated on overlay molecular image from coronal section. From the other hand, cerebellum region is well localized from the rest of the sagittal brain section (red filter). Low spatial resolution images allow observing only large histological regions while high spatial resolution can give higher level of interpretation.

- The figure 3 shows some examples of the benefit given by matrix sublimation method to yield high quality molecular images.

- Mass spectrometry imaging: Autoflex Speed LRF MALDI-TOF (Bruker Daltonik, Germany) with SmartBeam II laser. Positive mode (100-1200 Da) at 150/300 or 20 µm spatial resolution.

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

Thanks to high resolution imaging at 20 µm and matrix sublimation process, fine structures of brain hippocampus and cerebellum can be observed on figure 3. In the hippocampus, fine anatomical structures appear on high definition image such as cells layers from Dental Gyrus or Cornu Ammonis (fine blue line on molecular image). Dental Gyrus consists in three layers of neurons which are involved in neurogenesis (generation of neurons) in adult rat brain. For this reason, the ability to map molecular changes in this specific region of the brain might be useful to better understanding memories targeting diseases. Some studies [7] demonstrates that proteins level modification occur in Cornu ammonis region of brain affected by schizophrenia and bipolar disorders. In the cerebellum region, Purkinje cells or neurons (larger cells of the brain) can be accurately localized at the frontier between molecular cells layer and nerve fibers. It plays an important role in some pathology, for example; their loss can be associated with Alzheimer’s disease development.

- Images obtained from sublimation process highlight especially the lipids class molecules (contrast ions used in figure 3) nevertheless some examples of small [8] or large [9] molecules analyses have been published. Lipids such as gangliosides species (GM3, GM1...) could be detected in negative mode using matrix sublimation. These molecules are involved in Fabry’s disease and can be monitored (down regulation) to follow the efficiency of a treatment.
Showing different applications related to Alzheimer’s disease, we demonstrate the utility of MSI to answer specific CNS questions. The combination candidate drug, metabolites and disease state biomarker (Aβ) study demonstrates the type of reliable information provided by MSI. Moreover, biological processes can be assessed directly on tissue sections, such as the formation of amyloid plaques correlated with Amyloid beta peptides detection. Quantitative measurement of the drug candidate in small histological regions using MSI prevents the challenging and time consuming dissection steps. Finally, high resolution molecular imaging allows following molecules at the cellular level, such as neurons or other cells, which could be related to some specific brain diseases.

Thanks to MSI, ImaBiotech can provide specific information about distribution and efficiency (PK/PD) of your drug candidate by following and quantifying also its metabolites or biomarkers at the micrometer scale in brain substructures.

Benefits

- Quantify drug and metabolites in small histological structures of the brain
- Disease state markers assessment
- High spatial (20µm) and high spectral (R>500000) resolution imaging
- Molecular histology combines with classical histology techniques

References


Keywords

- Imaging
- Amyloid Beta peptides
- CNS
- Biomarkers
- MSI
- Cerebellum
- QMSI
- Alzheimer’s Disease
- Hippocampus
- Pharmacokinetics
- Pharmacodynamics

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