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Application News

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AXIMA Performance MALDI-TOF

Tissue Imaging MALDI-TOF Mass Spectrometry - Localizing Clozapine in Rat Brain Tissue

- Analysis of animal tissue by MALDI-TOF MS
- MS and MS/MS detection of drug directly from the tissue sample
- Imaging software used to localize distribution of drug and endogeneous compounds

Tissue Imaging MALDI-TOF Mass Spectrometry - Localizing Clozapine in Rat Brain Tissue

Introduction

The analysis of biological material by MALDI-TOF MS instruments has become an integral part of analytical research over the last 20 years. Biological samples normally include blood, serum and tissue samples, all requiring complex separation procedures prior to analysis. More recently, researchers have begun to analyze tissue samples directly by MALDI with minimal sample preparation. A technique known as matrix assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) now enables the direct in-vitro analysis of a tissue section. In this technique, a tissue sample is frozen in liquid nitrogen and cryosectioned onto an electrically conductive slide. MALDI matrix is deposited onto the sample to assist ionization of the compound of interest. The sample is then loaded into the MALDI-TOF MS instrument; the laser fires on a known set of coordinates and data is recorded within a set mass range, measured by time of flight (TOF). Each spectrum relating to where the laser has fired can then be compared to identify relative differences in intensity of a set mass/charge (m/z) value, or m/z range. Developments in MSI software have now enabled these results to be displayed visually so ion intensities can be represented as a colorimetric heat map image of the sample. In addition to developments in MSI, positive identification of proteins has been achieved following direct on tissue digestion and MS/MS analysis of resulting peptides.

The strength of MALDI MSI as a technique can be seen when searching for a drug, metabolite or endogenous compound distribution in a tissue sample. Traditionally established techniques, such as the use of radiolabelled drug compounds or immunohistochemistry, do not necessarily provide information about what reaction might have happened after drug administration. Radiolabelled experiments provide information on the distribution of all labelled chemistries whilst immunohistochemistry follows a set number of protein epitopes and therefore modification of the protein may not be realized. An ability to spatially map an array of specific metabolic changes within a sample can provide vital additional information that may not otherwise be discovered.

In this study, rats were dosed orally with the anti-schizophrenic drug clozapine. The aim of the experiment was to determine the distribution of clozapine and its metabolites within a tissue sample. This approach was investigated as a possible alternative to existing methods of identifying metabolites in non-radiolabelled pharmacodynamic studies. The advantage of this methodology is that it may help identify the distribution of a specific drug and related metabolites in non-labelled studies.

Methods

Male CDI rats (250-300 g, Charles River) were dosed orally with 100 mg/kg clozapine with a pre-treatment time of 60 minutes. 12 µm thickness cryosections of brain tissue were thaw mounted onto electrically conductive MALDI Fleximass slides. Samples were stored at -20°C until time for analysis.

Slides were thawed and given two water washes. This involved 2 x 1 min incubations in 80°C Milli-Q water with mild shaking. This enabled removal of endogenous salts that can interfere with sample analysis. Slides were vacuum desiccated for 30 minutes in order to dry. MALDI matrix (α-cyano 4-hydroxycinnamic acid; 5 mg/mL dissolved in 75% methanol) was printed on to the sample using a non contact method, by the Chemical Inkjet Printer™ (CHIP-1000). 100 pL drops were delivered in 5 drop rounds until 7.5 nL per print position were deposited. Print positions were spaced at 250 µm centre to centre.

Samples were analyzed using an AXIMA Performance™ MALDI-TOF-TOF MS instrument (Shimadzu Corp) in positive-ion reflectron mode. The pulsed extraction (P.Ext) value was set to 600 i.e. delay time optimized for mass m/z 600. To ensure good quality data, acquisition was performed using the CHIP Imaging Experiment software and Laser Roaming was enabled to give optimal spectra per print position.

Data analysis was performed using proprietary imaging software from the MALDI-MS Launchpad suite (Shimadzu Corp). BioMap software (www.maldi-msi.org) was also used to enable additional analysis of MSI data.

Results

The MALDI analysis of the tissue sample successfully detected clozapine throughout the sample. In order to check that clozapine had been detected it was also analyzed as a purified compound in MS mode at a concentration comparable to that dosed in the animals. Clozapine ionized very effectively as a purified compound and was also detected when analyzed from the tissue sample (see Figure 1). As expected many endogenous compounds were also detected from the tissue sample. For additional confirmation, MS/MS analysis was performed directly from the tissue sample on the m/z 327 peak (see Figure 2). Likely fragmentation pathways have been shown from fragment peaks (see Figure 3).

MALDI data from the tissue sample was analyzed using MSI software, BioMap. This revealed the relative intensities of the drug compound within the tissue sample (see Figure 4d). Drug distribution appears throughout the sample, but greatest in the cerebrum region of the brain. In addition to the drug compound there were many ions that were found distributed through the tissue sample.

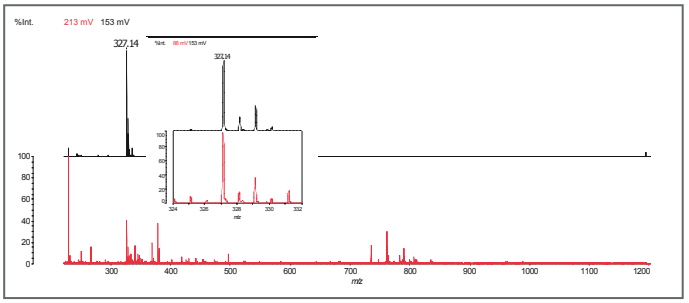


Figure 1: MS analysis of clozapine (Empirical formula of clozapine: $C_{18}H_{19}ClN_4$ – predicted protonated monoisotopic accurate mass, m/z 327.1371). Purified compound (black spectrum) matched the expected isotopic pattern of clozapine. This ionized with stronger intensity to that directly from tissue (red spectrum) which also contains other endogenous compounds such as lipids and metabolites.

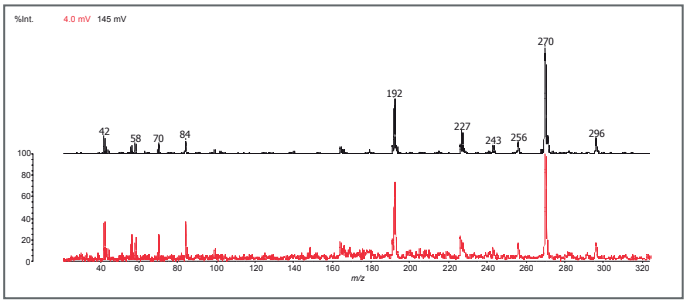


Figure 2: MS/MS analysis of m/z 327.14 peak. Purified compound (black spectrum) ionizes with stronger intensity to that directly from tissue (red spectrum).

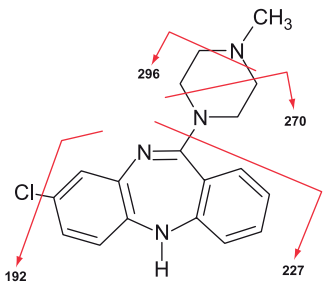
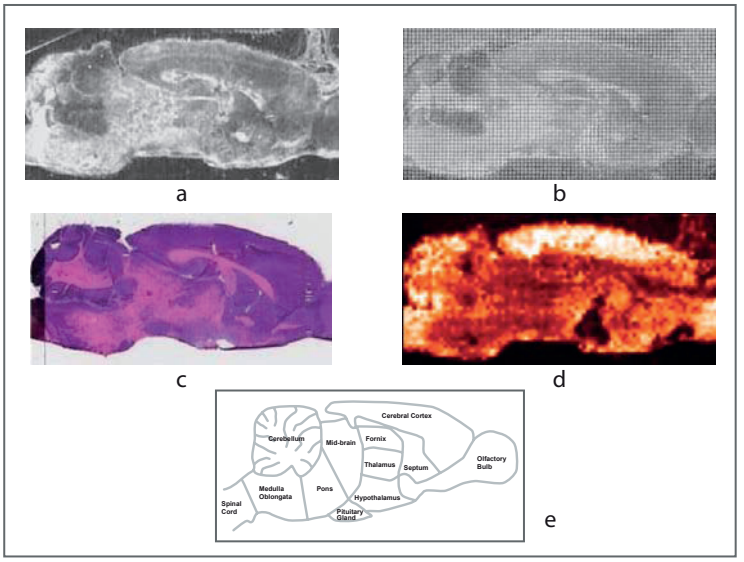


Figure 3: Fragmentation pathways of clozapine



[Ion intensity scale: 0%  *100%]*

Figure 4: Image generated from scanner inside CHIP-1000 before (a) and after (b) printing. H&E stained serial section (c). MALDI-MSI image: clozapine distribution m/z 327 (d). Rat brain anatomy (e).

One ion of special interest was at m/z 313. This mass was consistent with expected mass of de-methylated metabolite of clozapine (see Figure 5). The presence of a chlorine gives a characteristic isotopic pattern in the theoretical spectrum of de-methylated clozapine. This was also reflected in the isotopic pattern of the peak detected in the tissue sample at m/z 313 Da. The distribution of the m/z 313 peak also exhibited a similar distribution pattern to that of clozapine although some regions showed relatively higher levels to that of the parent compound distribution (see Figure 6). In addition to detection of clozapine and the primary de-methylated metabolite, a number of endogenous compounds were detected in the mass range analyzed (m/z 1-1200). The dominant ion signals may correspond to distributions of lipid molecules (see Figure 7).

These experiments illustrate the importance of endogenous compounds when investigating drug distributions in tissue samples. Targeting the right tissue is of critical importance when designing drug candidates. MALDI tissue imaging can provide extra information that other techniques cannot offer.

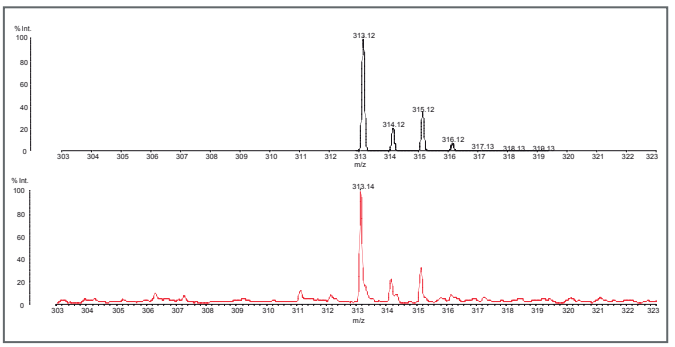


Figure 5: Theoretical spectrum of protonated de-methylated clozapine (black). Spectrum from tissue sample at m/z 313.14 (red). Although the intensity of the ion signal is low, the peak observed in the tissue sample is consistent with the de-methylated metabolite.

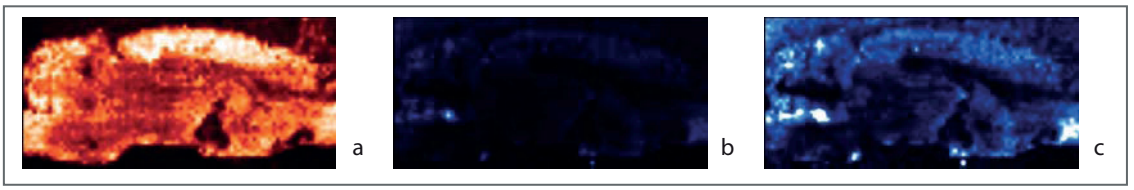


Figure 6: Distribution of clozapine (a), and peak at m/z 313 (b), peak at m/z 313 enhanced brightness (c). Different colour schemes were used to illustrate that the peak at m/z 313 is distributed in a similar pattern to the drug, however some regions of the brain appear to have relatively higher levels compared to that of the parent compound distribution.

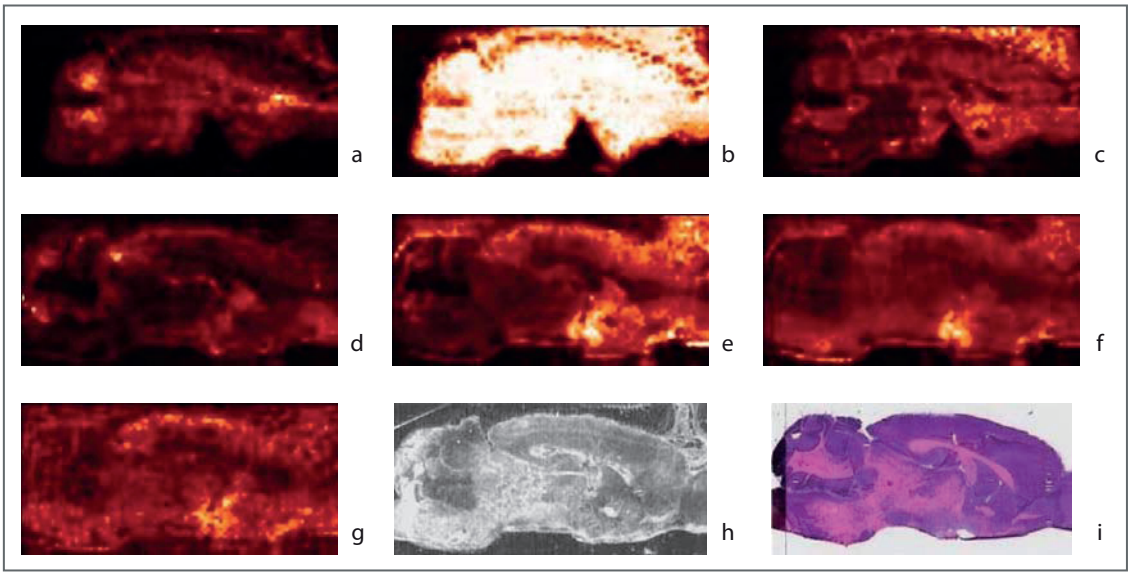


Figure 7: Endogenous molecules detected in the tissue sample. These have been highlighted due to their interesting distribution. m/z 236.72(a), m/z 266.00 (b), m/z 491.26 (c), m/z 496.31 (d), m/z 734.45 (e), m/z 760.50 (f), m/z 994.23 (g), CHIP-1000 scanner image (h), H&E serial section (i)

Conclusion

- MALDI-MSI is a valuable tool for the analysis of tissue samples
- Imaging software can be used to identify distributions of drug molecules and possibly also their metabolites, increasing the chances of discovering dangerous drug metabolites before reaching clinical trials
- Endogenous molecules can also be ionized without difficulty and provide further useful information regarding the environment that the drug target is situated in

Acknowledgements

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*Pfizer, UK

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