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Compound and metabolite distribution measured by MALDI mass spectrometric imaging in whole-body tissue sections

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Abstract

The determination of the compound distribution in laboratory animal tissue in early development is a standard process in pharmaceutical research. While this information is traditionally obtained by means of whole-body autoradiography using radiolabeled compounds, this technology does not distinguish between metabolites and parent compound. The technique described in this article, termed matrix-assisted laser desorption/ionization (MALDI) mass spectrometric imaging, can fill this gap by simultaneously measuring compound and multiple metabolites distributed in whole-body tissue sections, using non-labeled compounds.

Keywords: Mass spectrometry; MALDI; Drug; Imaging; Metabolites

1. Introduction

Mass spectrometric imaging (MSI), also termed imaging mass spectrometry (IMS or i-MS), has gained new momentum in the last couple of years with the development of matrix-assisted laser desorption/ionization (MALDI) MSI [1]. Among its advantages over secondary ion MSI is the extended mass range, but even more important is the significantly better sensitivity. First applications of MALDI MSI focused on protein and peptide imaging, enabled by the large mass range of the time-of-flight mass spectrometers. These analyzers are ideally suited for the detection of peptides and proteins and are therefore typically applied in a proteomics setting. The potential value of this technology for imaging applications in biomedical research was recognized early on by laboratories in academia and industry [2–4], with first efforts focusing on mapping of potential drug targets and disease biomarkers. As the pharmaceutical industry became active in the field, it was logical to also explore the lower mass range where most current drugs are situated. However, the detection of compound on a bare metal sample plate by MALDI MS is a quite simple task compared to the complexity of compound detection on biological tissue sections. Suitable sample preparation methods were developed which allowed direct detection of compound from animal tissue sections [5] by MALDI mass spectrometry [6]. While the spectral information from the compounds can also be obtained by analyzing tissue extracts, it is the spatial distributions which are the most relevant information for drug discovery. Conventionally, this was achieved by combining compound distribution data obtained through whole-body autoradioluminography [7] (WBAL) and metabolite data obtained by analyzing tissues extracts with LC/MS. In WBAL, radioactive labeled compound is administered to animals, which are sacrificed after different post-dose time points. Whole-body sections of these animals are then exposed to a radioactivity detector which allows visualizing the spatial radioactivity distribution. There are many benefits of WBAL including parallel sample processing, standardized procedures and high sensitivity, with the strongest one being applicable quantification [8]. Against these advantages, there are also two major limitations: The compound of interest must be radiolabeled and the technology does not distinguish between compounds and their metabolites. While the first leads merely to more complexity and increased cost for each experiment, the last cannot be overcome by this technology. These two issues led

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to the investigation of MALDI MSI as applied to whole-body sections, for this technology allows a specific and simultaneous detection of multiple analytes based on their molecular weights and the fragmentation patterns (Fig. 1) [9,10]. By acquiring full mass spectra from each image point, the information content of the experiment is greatly enhanced. One can extract the specific distributions of the administered compound and its analytes, but beyond that there are hundreds of signals from endogenous metabolites which are simultaneously acquired. This allows not only following the compound metabolism, but also potentially to gain information on the organism's response to drug treatment. The measurement of the molecular weight with medium accuracy alone does not unambiguously identify a specific analyte, as multiple analytes might contribute to the same measured signal. The confidence in the identification can be improved by increasing the mass accuracy or by measuring fragmentation patterns. A suitable mass accuracy of better than 1 ppm required to get a sum formula in the low mass range can be obtained with a ICR mass analyzer, but there is currently no commercial instrument available which couples a high sensitivity MALDI source with this type of analyzer. Therefore, identification is done by fragmenting the desorbed ions in the mass spectrometer and matching the fragment masses with the molecular structure. There are a number of instruments available which offer MS/MS capability in combination with a MALDI source.

The major challenge to this application of MALDI MSI was the interfacing of the tissue sections to the mass spectrometer. In the most common WBAL application, the whole-body tissue sections are attached to polymer film and slowly freeze–dried over 1–2 days. In contrast, MALDI MSI requires the sample to be attached to a metal plate with specific dimensions and to be coated with matrix. The transfer of sections to metal plates by means of charged paper (omitting the polymer film) was shown to be feasible [10], but it is extremely difficult to achieve and, therefore, not well suited for routine application of obtaining whole-body sections of large animals. Therefore, a robust protocol was developed which allows processing of sections mounted on standard polymer tape. In brief, the sections are kept frozen until just before processing, fast freeze–dried, attached to metal plates, spray-coated with MALDI matrix and sputter-coated with a thin film of gold. The sections prepared in this manner are introduced into the mass spectrometer, in our case equipped with a time-of-flight analyzer, where the laser is used to raster over the tissue section, while acquiring a mass spectrum and/or fragmentation spectra from each laser spot. Out of this wealth of data specific ion images are extracted for every analyte of interest. Although MSI does not depend on radioactive labeled compound, the method was tested and optimized in conjunction with WBAL. This comparison allowed for a validation of the technology on real drug compounds and identification of the strengths of both technologies.

2. Methods

2.1. Tissue sections

The aim of our work was the development of a robust protocol to be used in a routine environment where studies involve WBAL and MALDI MSI measurements from the same animals. Therefore, the whole-body sectioning process previously optimized for WBAL was not further modified for MALDI MSI. In such a combined study, animals (rats and mice) were dosed with ^{14}C labeled compound and sacrificed at different time points post-dose (Fig. 1). Immediately afterward, they were deep-frozen

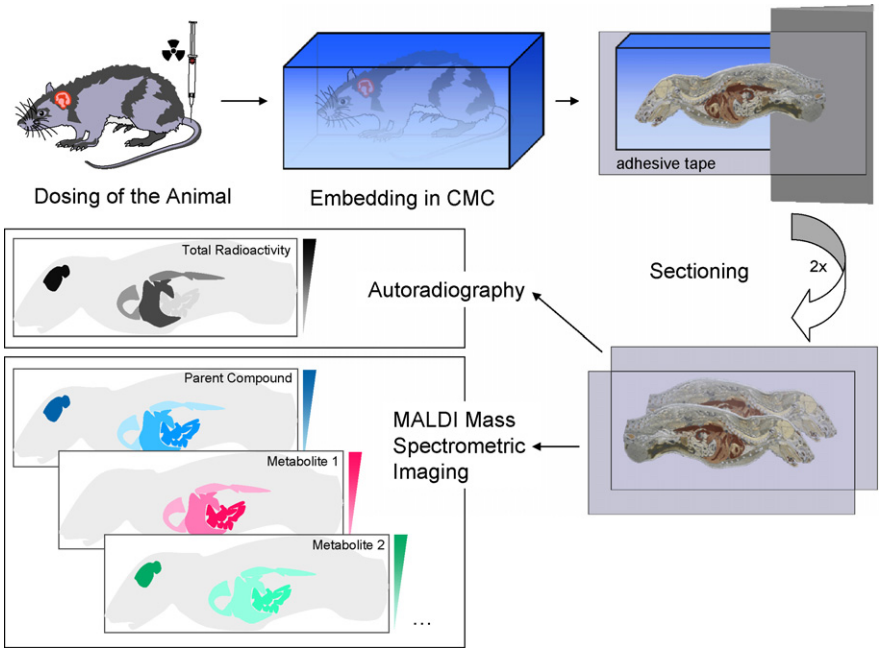

Fig. 1. MALDI MS imaging process for whole-body tissue sections. The animal is dosed, sacrificed after a defined period, frozen and embedded into a polymer block. Sections for WBAL and MALDI MSI are mounted on adhesive tape. While WBAL gives information of the total radioactivity, MSI allows distinguishing between compound and its metabolites.

Table 1
Summary of optimized sample preparation for WB sections

Process	Equipment/materials	Parameter	Time
Embedding	Sodium carboxymethylcellulose (CMC)	−75 °C	20 min
Sectioning	CM 3600 cryomicrotome (Leica)	40 μm at −20 °C	1 h
Drying	Desiccator with membrane pump	−80 to 20 °C	2 h
Mounting	OptiPlates (Applied Biosystems), Adhesive Tape (TESA™, #56170-00004)	2 per mouse, 4 per rat	10 min
Optical scan	HP6300C	254 dpi (10 μm)	2 min
Matrix deposition	Sat. CHCA in 50:50 (v/v) ACN/0.1% TFA	20 cycles	1 h
Sputtering	Sputter coater SC7680 with FT7690 Film, Thickness Monitor (Quorum Technologies)	5 nm Au	30 min

by immersion into a mixture of dry ice and hexane at −75 °C, rapidly shaved and embedded in a 2% pre-cooled semi-liquid gel of sodium carboxymethylcellulose (CMC). Sagittal sections of 40 μm thickness were obtained at −20 °C in a CM 3600 cryomicrotome (Leica Microsystems GmbH, D-Nussloch) according to the method of Ullberg [11], except that the sections to be used for MALDI MSI were not dehydrated but sealed and stored at −80 °C until being processed for further analysis.

2.2. Section processing for MALDI MSI

The process of preparing WB sections for MALDI MSI analysis is crucial to the success of the technology and was optimized over many iterations not presented here, but the optimized procedure is summarized in Table 1. Speed is a critical factor in the process as the chemical state of the tissues must be preserved and degenerative processes upon contact with ambient air or at room temperature need to be minimized. The sample preparation was initiated by placing the sections on a copper block (2 cm × 6 cm × 20 cm) which was kept at −80 °C and transferred to a desiccator simultaneously with the sections for dehydration. The temperature of the metal block rises slowly to room temperature, which lead to fast freeze–drying of the section in contact. This process was typically complete after 1–2 h, depending on the tissue. The sections were removed from the vacuum and each section was mounted on adjacent metal plates (two for mice, four for rats) using double-sided adhesive tape. Great care was taken to ensure uniform mounting without air bubbles and contamination of sample. After separation of the plates with the tissue, each of them was optically scanned with a flatbed scanner (HP6300C) at a resolution of 254 dots per inch. This resolution was chosen to allow simple superimposition of the optical picture with the molecular images generated by MALDI MSI, which were acquired in a metric raster.

Matrix coating is obviously a critical step in sample processing and this is currently one of the aspects of significant research activity in both academia and industry. The optimal technique strongly depends on the tissue and compound being investigated, with a spray deposition currently resulting in best quality of the images [6,9,10]. The spray solution contains some percentage of organic solvent, acid, and one or multiple matrices at different concentrations. For optimal performance, this system should be optimized for every compound separately. The approach we have chosen is to use a generic spray solution which results in good quality data for most of the compounds we have tested. The protocol was only changed for substances which could not be detected using the standard procedure. This was not necessary for the data shown here.

Standard matrix coating involved spraying matrix solution (saturated CHCA in 50:50, v/v, ACN/0.1% TFA) with a pneumatic TLC sprayer. The selection of CHCA as matrix was based on the positive effect thereof in the fragmentation experiment (see below). Fifty percent organic was found sufficient to dissolve the compounds and metabolites out of the tissues while the water content allowed a slow crystallization processes optimal for high sensitivity in MALDI. Approximately 2 ml of matrix solution were deposited on each plate with a low air pressure of 0.7 bar and over a time of 30 min. Special care was taken to spray enough matrix solution to keep the surface moist, but not wet. After complete drying of the last matrix layer, a 5 nm layer of gold was sputter-deposited on each plate (SC7680 Sputter Coater, FT7690 Film Thickness Monitor; Quorum Technologies, Newhaven, UK). This particular treatment has been found to dramatically improve the intensity of the mass spectrum signal in the low mass range [12,13]. The plates were either immediately loaded in the mass spectrometer for MSI analysis, or alternatively, they were stored in the dark, at room temperature in gas-tight sleeves until they were loaded in the mass spectrometer for further MS analysis. Storage longer than 7 days was however found to be detrimental in terms of obtainable signal intensity.

2.3. MSI acquisition and data processing

Once dried, the plates were analyzed in a 4700 Proteomics Analyzer TOF-TOF™ mass spectrometer (Applied Biosystems/MD Sciex, Foster City, CA). The instrument is equipped with an autoloader for 24 plates, which allows 6 sagittal rat sections (corresponding to 4 plates for each section) or 12 mice sections to be analyzed in one run. The instrument parameters were optimized for best performance in the mass range corresponding to the compound and its metabolites, in this case 300–1000 *m/z*. Parent ion spectra were acquired in reflector mode, with a time bin of 1 ns and a sensitivity of 50 mV/div, and averaging over 50 laser shots. The laser intensity for these experiments was adjusted for an optimal signal-to-noise ratio of the compound peak without saturation. This empirically determined intensity was kept constant during the course of the experiments. Once the optimal conditions were found, the '4700 Imaging Tool' software [4] written in our labs was used to acquire the image data

of the plates. Image coordinates were set to the plate boundaries and 500 μm was selected as image raster step, resulting in a compromise between information content and acquisition time. Typically 1.2 h was required per plate with 88 times 88 image points (11 h for MS/MS imaging with 1000 laser shots per position). The image acquisition software also controls the autosampler, so that complete data from 6 rat sections could be measured over a weekend.

Due to the limitation of the small sample plates, the data files of multiple plates from the same sections must be assembled in one data file for convenient image analysis. Custom made software ('MSI File Tool') was used for this purpose. Besides combining data sets, this software was used to resize the data and to extract specific dimensions or mass ranges. This function is useful to reduce data sets bigger than 1 GByte or to contact the spectral dimension to values below the limitation of the data analysis software (32,768 points).

Data were processed and visualized using the software 'BioMap', which is freely available from the author's web site (http://www.maldi-msi.org). A user tutorial can also be found on this site and the following description outlines just the specific functions used for the results shown here. Intensity distribution maps were calculated by selecting the mass range corresponding to the peak boundaries of the analyte of interest. These boundaries were determined by using the pixel analysis function displaying the mass spectrum of a pixel showing the compound signal. This allowed an accurate selection adequate to the mass resolution and minor mass shifts over the plates. The intensity map calculation was performed with the 'Mean with BC' function of BioMap. This function averages all the intensity values of the mass spectrum inside the selected boundaries and subtracts the mean value of the boundary intensities.

3. Results and discussion

3.1. Compound distribution

An example was taken from an actual pharmacokinetic study where Long–Evans rats were intra-tracheally (i.t.) dosed with 0.5 mg/kg of a ^{14}C labeled compound. This is a typical dose for this compound and it was not selected for the sake of MALDI MSI method development, but to gain information on the compound distribution. In this study, sections were cut for WBAL and MSI from the same animals, allowing a direct comparison between the technologies. It needs to be noted that sections were not adjacent and therefore a direct pixel-to-pixel correlation is not possible. Fig. 2 combines WBAL images and MS images from the dosed animals. The top panel and third panel show the tissue distribution of radioactivity, as determined by WBAL in 5 min and 1 h post-dose animals with the intensity represented with a gray scale (white equals zero). The MSI distribution maps shown in panel two and four were calculated by integrating over the $^{14}C_2$ isotope signal of the protonated compound. The gray intensity scale represents values from zero (black) to 5000 (white) measured in normalized peak area.

In the MS images of the protonated compound of the 5 min animal (2nd panel from top), strong signals were observed in the trachea, caused by the i.t. administration of the compound, and in the stomach, in line with the WBAL results. The distribution measured by MSI at 1 h post-dose extends to the nasal turbinates and downstream to the esophagus, and a weak signal was detected in the intestine and blood. These locations were also pointed out by WBAL, which however indicates higher levels of radioactivity in the intestine. The discrepancies of the measured intensity in the intestine between WBAL and MSI are attributed to the tissue-dependent ionization efficiency of MSI (see below).

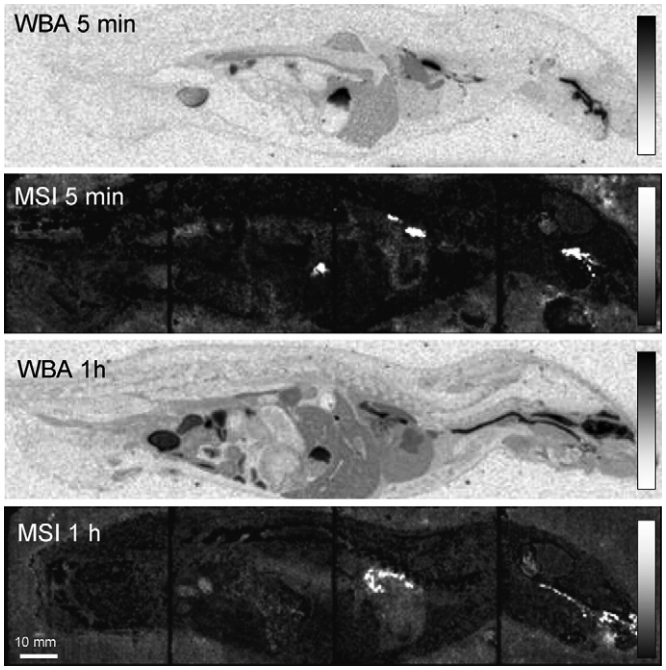

Fig. 2. Comparison of MSI with WBAL using whole-body sections after intratracheal administration of a compound (0.5 mg/kg) to rats. The two corresponding sections are from the same animal, but from different positions. The comparison of the methods shows remarkable similarity in the results: High levels are detected in the trachea, the lung and the stomach and lower levels in blood.

3.2. Quantification and reproducibility

If quantitative information is to be obtained from MALDI MSI, there are three difficulties to take into account: substance specific ionization yield in MALDI, tissue specific ion suppression and dependency of detected signal on the amount/property of the deposited matrix. These factors add to the complexity of quantifications and it is therefore essential to carefully design experiments which allow nevertheless a quantitative statement.

Signal suppression is very specific, depending not only on each tissue, but also on the substance. Therefore, each substance needs to be calibrated in a separate experiment by spiking each tissue and measuring the resulting MALDI signal. The complexity of these experiments can be reduced by directly and homogeneously spiking a whole-body tissue section and acquiring MS images.

For this purpose, 4 ml of compound solution (0.25 mg/ml in 50:50, v/v, ACN/H_2O) were sprayed over a control rat sec-

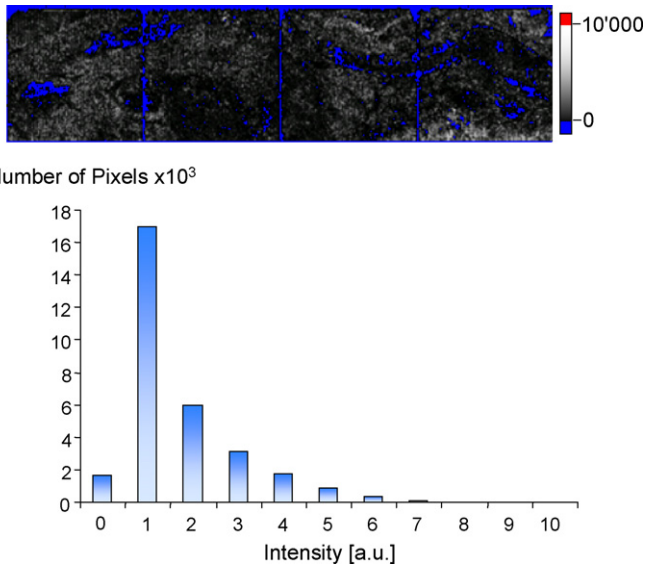

Fig. 3. Measured intensity distribution of the compound homogeneously deposited over the sample. Tissue specific suppression of signal is observed, illustrating the requirement for tissue specific ionization efficiency calibration. Some areas show no detectable signal (blue color). The histogram shows that these affected areas make up for only 5% of the image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

tion using the pneumatic nebulizer described above. With this amount of solvent, the section was completely wet and it was assumed that the compound solution soaked into the tissue to its full thickness. After drying, the section was coated and analyzed using the method described above.

The resulting molecular image of the compound is shown in Fig. 3 (top panel). As expected, the anatomy of the animal is visible. The color scale was chosen to highlight the pixel values below zero in blue. The histogram (bottom panel) of this molecular image shows that over 95% of the pixels exhibit intensities higher than 1000, i.e., only 5% (pixels in blue) of the whole area shows evidence of strong ion suppression. This is an encouraging first result, which will serve as a basis for further studies. It shows clearly that this control experiment is required to gain reliable information from MALDI MSI. If this data is not available, the lack of signal in a MALDI MS image cannot be read as indication for no analyte being present at that particular location, as suppression effects might prevent detectable signals [13].

To obtain a baseline, relative quantification by WBAL and MSI was compared by analyzing WB tissue sections from the same animal. For each technique, data from three sections was acquired using the method described above from a 1 h post-dose animal. The data was loaded into BioMap, where regions-of-interest (ROIs) were drawn based on the optical scan to cover the stomach, lung and trachea of the animal. These ROIs were copied to the co-registered WBAL and MSI data sets. Using the histogram function with 200 bins, the ROI data was reduced and exported to Excel. Empty pixels were removed by setting a suitable threshold above the image noise. The remaining pixel intensities were integrated and normalized by the pixel count. In the case of the MSI data, the values were divided by the tissue-specific ionization efficiency factors obtained from the previously described spiking experiment: stomach 4.6, lung 9.3, and trachea 4.5. These factors were obtained by drawing ROIs over the corresponding tissues and averaging signal intensities. The result of this relative quantification by MALDI MSI is shown in Fig. 4, the WBAL data is shown in Fig. 5. The bars depict the relative intensities for the specific tissues with the error bars resulting from the three sections (only two for the MSI of the stomach, sample A does not contain this region).

This comparison shows good correlation between both techniques. Both MSI and WBAL measurements exhibit a relative standard deviation of better than 10%. The relative concentrations match well: the value measured by WBAL in the lung is 78% of the trachea concentration, the corresponding fraction 69% when measured by MSI. The relative concentrations of stomach compared to trachea are 90% for WBAL and 73% for MSI. The MSI ratios would be dramatically different if the tissue specific ionization efficiency factors were not applied. The same concentration results in more than twice the signal intensity of the compound desorbed from the lung compared to the trachea. The spatial calibration experiment could successfully compensate for this effect.

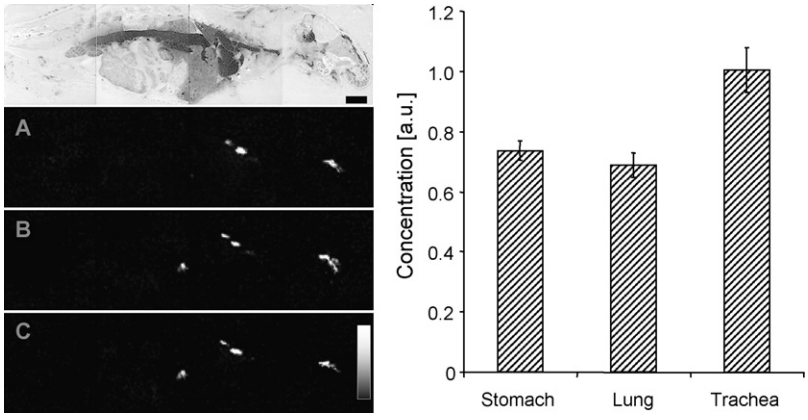

Fig. 4. Relative quantification by MSI. Left: optical image (top), and MSI images from three different sections from same animal (A–C). Data evaluation using BioMap by averaging the pixel intensities over the respective tissues. Error bars shown for the three samples (right).

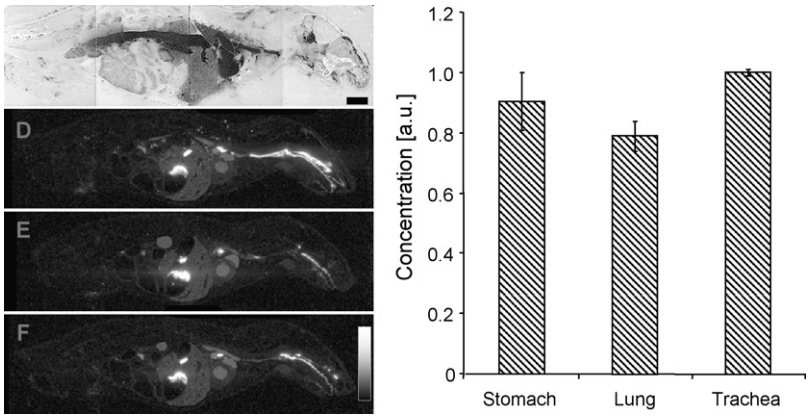

Fig. 5. Relative quantification by WBAL. Optical image (top), and WBAL images (D–F) are shown on the left, quantification is shown on the right. The scale bar represents 10 mm.

3.3. Spatial resolution

The maximal obtainable spatial resolution in MALDI MSI depends on the sample stage accuracy, the laser spot size and sample preparation procedure. The commercial instrument used for these experiments was specified for spatial resolution but not modified. The limiting factor was the laser beam spot size, which was measured using the ablation pattern created on a colored surface. The laser ablated an ellipsoid, of dimensions being approximately 80 μm × 50 μm. Such measurements obviously depend on laser attenuation settings, but they are indicative of the approximate ablated area.

The critical factor process in MSI is always the matrix coating. By applying a wet matrix layer, analytes diffuse laterally which leads to a loss of spatial resolution. To eliminate this effect, the sample preparation protocol described above aimed at minimizing migration by keeping the surface merely moist. Since this diffusion is a major limiting factor, only the analysis of the sample itself can determine its full extent. Typical methods for defining image resolution such as meshes or other fine structures were not found to be adequate in this case.

The method chosen for the evaluation of the displacement was analysis of a well-defined structure of a WB rat section. The intestine is ideally suited for this analysis, as the content typically results in intense signals not present in the surrounding tissue. This can be imaged by MALDI MSI as shown in Fig. 6. The top panel shows the optical image of the whole rat section, with an expanded region in the lower left panel. In the center panel, the MALDI MSI signal of a component present at high concentration in the intestine is shown. A rectangular section over a lobe is evaluated in the profile shown to the right. The profile matches well with the anatomical features shown in the optical reference image. The MSI raster of 200 μm shows good contrast between adjacent pixels in the intestinal wall borders and no measurable quantities of analyte have migrated to the surrounding tissue. Based on this evaluation, the achievable maximal resolution of MALDI MSI on WB sections was determined to be better than 500 μm.

While the image raster of 500 μm applied to the dosing experiments described above results in a good compromise between image content and acquisition time, there are cases where it is desirable to obtain images of a target organ with higher spatial resolution. A MSI was therefore acquired with a spatial resolution of 100 μm on a single sample plate containing a section of the 1 h post-dose rat in the abdominal region including the lung. The MS image of the protonated compound signal is shown as an overlay to the optical image of the same section in Fig. 7. The

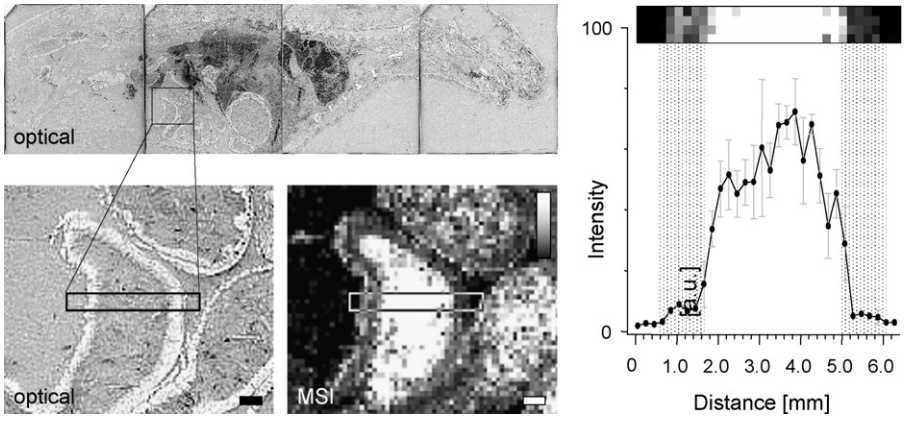

Fig. 6. MSI analysis across an intestinal lobe of a whole-body rat section. The profile shows well-separated compartments of the intestine content, the intestine and the surrounding tissue. Image acquired with a 200 μm raster; displacement is less than 0.5 mm.

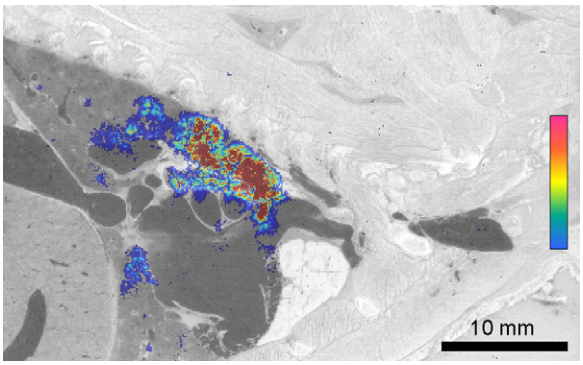

Fig. 7. Imaging at high resolution of 100 μm shows detail in the MS images. The matrix spray deposition procedure leads to minimal horizontal bleeding which does not affect the image resolution.

MSI shows fine details of the compound distributed in the lung, with a uniform coverage indicating that a homogenous matrix coating was achieved. Only a minor lateral diffusion due to the sample preparation and matrix coating process was observed in some areas, the maximal measured displacement being less than 500 μm. This finding supports the results of the intestinal content imaging experiment.

3.4. Compound and metabolite distribution

The strength of MALDI MSI lies without doubt in its molecular specificity and the ability to analyze multiple analytes simultaneously. An example where these unique features provided the relevant information in a compound distribution study is described next.

BT-474 tumor-bearing female HsdNpa:athymic nude mice were dosed with 30 mg/kg of a ^{14}C labeled compound as a single bolus in one of the caudal veins and subjected to WBAL analysis. The distribution patterns showed a rapid biliary excretion of the compound and/or its metabolites through the gastric tract, with the excretion products being observed as early as 30 min post-dose (Fig. 8).

An effort was therefore made to determine the excretion pathway. Frozen animals, still available from the previous WBAL studies, were sectioned again for MALDI MSI. Sample processing was performed as described above. In a first step, sections from the 30 min post-dose animal were subjected to MALDI MSI analysis, measuring a wide mass range from *m/z* 300 to 1000 to cover all potential metabolite masses available from a liver metabolism study. With the first pass analysis of the dataset using BioMap, the compound could be readily detected in the MS images (Fig. 8) but the compound distribution did not account for the high levels found by WBAL in the bile. In a second step, MS images were generated for all the masses of the protonated, sodiated and potassiated known metabolites. An ion signal corresponding to the protonated glucuronide of the compound displayed a distribution correlating with the intestinal content. Glucuronidation is a common mechanism for excretion and therefore, the finding of the glucuronide adduct in the intestinal content made perfect sense. The fact that no compound was detected in the 24 h post-dose animal using MALDI MSI,

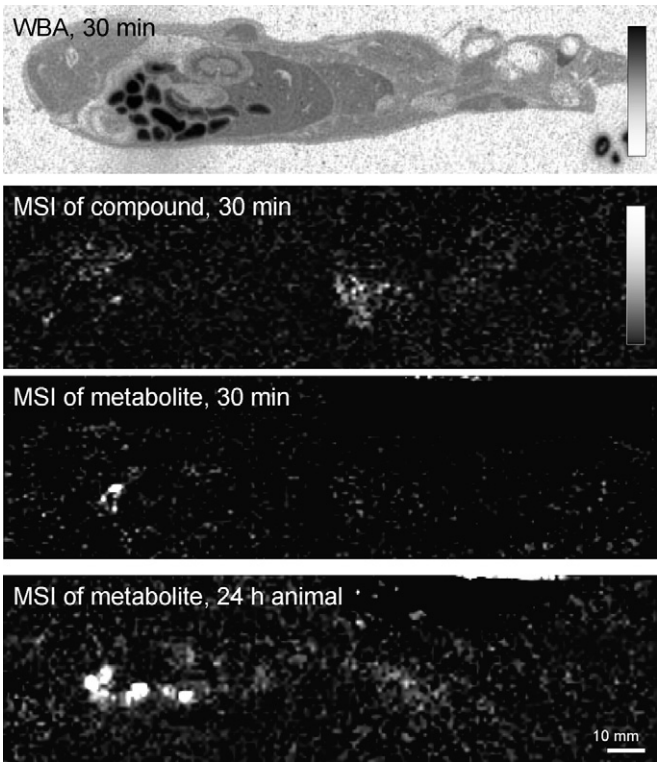

Fig. 8. MSI analysis of WB rat sections after oral administration of a ^{14}C compound. At 30 min post-dose, the compound is detected in the blood, a metabolite is excreted through the bile and detected with MSI on the same section. In the section of the 24 h post-dose animal, this metabolite is detected at high concentration.

but high levels of an analyte matching with the mass to the glucuronide adduct (Fig. 3, bottom panel) supported this finding. A mass alone does not allow identification with high certainty and therefore MS/MS fragmentation was used to confirm this finding.

3.5. Metabolite identification

The compound contains two hydroxyl groups, which are the potential sites for glucuronidation. Even if the mass could be measured with high accuracy (as described above for ICR analyzers), the mass spectrum alone would not distinguish between the two isobaric structures. Therefore, a MS/MS spectrum was acquired directly from the tissue section, fragmenting parent ions with mass of the protonated glucuronide at *m/z* 640.3. Fig. 9 shows, on the top left panel, the parent mass spectrum acquired from the tissues and the MS/MS spectrum of *m/z* 640.3. The MS/MS spectrum consists of 10,000 sub-spectra acquired from a tissue section of a 24 h post-dose animal, selecting a mass window of *m/z* −1 and +3 with respect to the nominal mass. The spectrum contains two specific fragments which could result from both potential glucuronides, therefore the identification of either one was not possible based on this measurement. As a last resort to solve this problem, the two possible analytes were synthesized and analyzed by MS/MS on the same instrument. The spectra shown in the bottom panels of Fig. 9 show as prominent

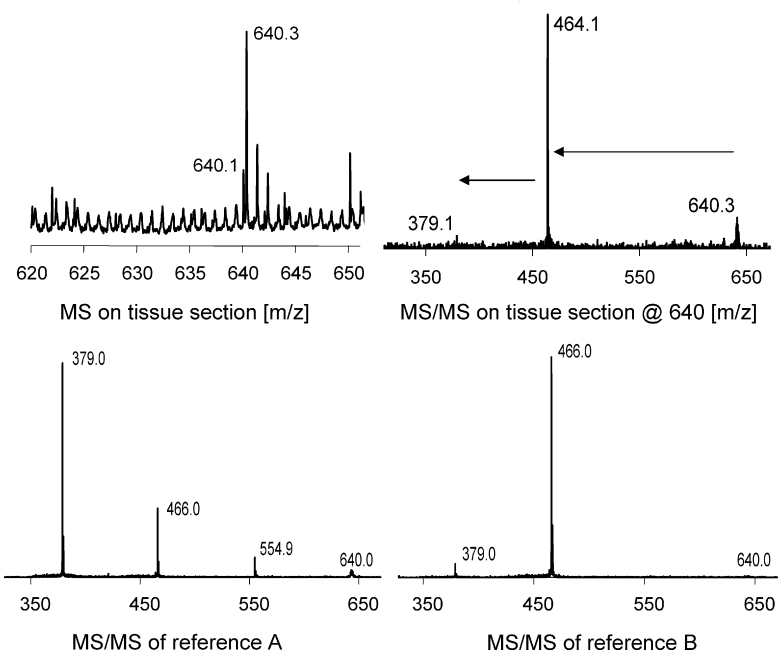

Fig. 9. Identification of the metabolite detected in the rat section shown in Fig. 8. The MS spectrum obtained from a single position on the section shows a good signal-to-noise ratio, but also a large amount of chemical noise. The MS/MS spectrum acquired on the TOF/TOF system shows a clear pattern, which matched the one from the reference substance.

difference the fragment at *m*/*z* 555. The spectrum of reference compound B matched with the spectrum obtained from the tissue section and this match served as evidence for a confirmation of the metabolite structure.

4. Conclusions

The examples shown here clearly demonstrate the benefits of mass spectrometric imaging: On a single tissue section of a dosed animal, the compound and metabolite distributions can be measured and selected metabolites can be unambiguously identified. This information could be obtained within a short period of time, without the requirement for a radioactively labeled compound. Nevertheless, in the course of our experiments we found it extremely helpful to have the WBAL data available. The reason for this is the tissue specific signal suppression observed in MALDI MSI, but to a much lesser extent in WBAL. There results shows here indicate that a tissue specific calibration measurement is a feasible method to circumvent this issue. In comparison to WBAL, it is also obvious that MALDI MSI is not applicable as broad as the former because not all compounds can be ionized equally well. If both methods are to be compared in terms of sensitivity, WBAL has its advantages because prolonged exposure times and higher radioactive doses can be used. In contrast, the advantages of MALDI MSI in the application of compound/metabolite distribution analysis are without doubt specificity and speed. This technology fits ideally into the field of existing technologies and with future developments will expand its application scope.

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